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TITLE: TBI-Related Risk Factors for Alzheimer's Disease:
Early Detection and Prognosis via Multimodal
Brain Imaging and Connectomics

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14. ABSTRACT The purpose of this study is to utilize multimodal imaging to identify AD risk factors specific to the TBI population. The study scope involves (1) finding TBI-affected structural/connectomic features which are significantly associated with AD risk; and (2) imaging biomarkers which are statistically independent from other well-documented AD risk factors and which can assist with early identifying TBI survivors at risk for AD. In this reporting period, we performed automatic identification of AD/TBI pathology, calculated volumetrics, implemented connectomic/topological analyses, found TBI/AD-related connectome differences, published our results and disseminated them. Results indicate that the TBI-affected brains of older adults experience neurodegeneration on a scale which rivals that of age- and sex-matched AD patients. We also found properties of the TBI-affected brain whose characteristics are commensurate with the likelihood of TBI survivors to exhibit accelerated neurodegeneration, which may lead to the identification of early biomarkers of high AD risk.					
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TABLE OF CONTENTS

	<u>Page</u>
1. Introduction	4
2. Keywords	4
3. Accomplishments	4
4. Impact	15
5. Changes/Problems	16
6. Products	17
7. Participants & Other Collaborating Organizations	21
8. Special Reporting Requirements	22
9. Appendices	22

1. INTRODUCTION:

Although traumatic brain injury (TBI) victims are more likely to develop Alzheimer’s disease (AD), it is difficult to estimate AD risk in TBI patients. For this reason, it would be extremely useful to understand how TBI may lead to AD, and to identify new ways to predict how likely it is for a TBI victim to develop AD. The purpose of this scientific study is to utilize sophisticated imaging and connectomic methods to study the brains of older adults who are either healthy, TBI survivors, AD patients, or both. The main hypothesis of the study is that the TBI-affected brain has certain anatomic properties which, if known, could help scientists to predict the likelihood of AD after TBI with high accuracy. The secondary hypothesis of the study is that at least some of these properties are different from those which are already known to be risk factors for AD (e.g. obesity, smoking, a history of high blood pressure, or certain inherited traits). This project thus aims to identify novel AD risk factors specific to the TBI population. The ability to accurately identify TBI victims at high risk for AD could have substantial consequences for the healthcare of such individuals; understanding the relationship between TBI and AD could advance our scientific and clinical knowledge regarding these two conditions.

2. KEYWORDS:

traumatic brain injury, Alzheimer’s disease, risk biomarkers, magnetic resonance imaging, connectomics, network theory.

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The primary aim of this study is to test the hypothesis that the TBI-affected brain has structural and/or connectomic features which are significantly associated, in a statistical sense, with AD risk and which can allow TBI survivors to be stratified based on such risk. Our secondary hypothesis is that some of these biomarker-like features are specific to the TBI-affected brain, i.e. that they are statistically independent from other known and well-documented AD risk factors. The study aims to use multimodal neuroimaging—including magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI)—to understand the differences between four populations: (1) the healthy aging brain, (2) the TBI-affected brain, (3) the AD-affected brain, and (4) the brain affected by both TBI and AD. We aim to use advanced computational neuroanatomy, neural network analysis and connectomics to identify statistically significant differences between these four groups, with the goal of identifying new biomarkers which can be used to reliably estimate AD risk in TBI survivors. We use a 2×2 factorial experimental design (factors: single mild TBI, AD) to identify and quantify the neuroanatomic and connectomic features of older adults (aged 65+) whose existence is modulated by TBI/AD interactions. Expert-validated, TBI/AD-tailored computational analyses of high-quality, multimodal MRI and DTI volumes acquired at high resolution from an adequately powered volunteer sample have allowed us to quantify brain volumetrics and morphometrics in the presence of TBI/AD-related (non-)hemorrhagic lesions. In each volunteer, MRI/DTI volume information is then used to map the macroscale connectome, its topology and to infer its network-theoretic properties, which are subsequently compared across groups.

Milestone	Timeline	Status
Project Run-in		
	Months	
Local IRB Approval	1	Granted by 06/11/19
HRPO Approval	1-2	Granted by 09/15/19
Maintain all IRB approvals	1-3	Approvals stand
Train research staff in all research protocols	1-2	Completed by 12/14/18
Create, revise, distribute research protocol manual	1-2	Completed by 12/14/18
Identification of AD risk factors in TBI survivors		
	Months	
Perform automatic identification of AD/TBI pathology	1-6	Completed by 04/14/19
Calculate volumetrics and morphometrics	1-6	Completed by 04/14/19
Implement connectomic and topological analyses	1-6	Completed by 04/14/19
Find TBI/AD-related connectome differences	7-16	~60% completion
Internal validation of volumetrics/morphometrics	7-10	Completed by 08/14/19
Confirmation by TBI/AD clinicians	11-14, 26-28	~40% completion
Internal validation of connectomic/topological analysis	13-18	Planned for Y2
Implement statistical analysis of results	19-24	Planned for Y2
Reliability testing	23-26	Planned for Y2-Y3
Alternative hypotheses testing as needed	25-32	Planned for Y3
Revisions to methods as needed	22-34	Planned for Y2-Y3
Open software release via NITRC	32-36	Planned for Y3
Product: methodological manuscripts on analysis	6-18	~50% completion
Product: software for TBI/AD connectome analysis	12	Completed by 10/15/19
Product: manuscripts on TBI/AD connectome analysis	18-24	Planned for Y2
Product: manuscripts on statistical analysis	24-36	Planned for Y3
Product: presentations at national meetings	9, 21, 35	~30% completion

What was accomplished under these goals?

(1) Major activities

Throughout this annual reporting period, the following major activities were either completed or are ongoing:

- (A) performance of automatic identification of AD/TBI pathology
- (B) calculation of volumetrics and morphometrics for the subjects in the dataset
- (C) implementation of connectomic and topological analysis for the subjects in the dataset
- (D) find TBI/AD-related connectome differences
- (E) internal validation of volumetrics/morphometrics
- (F) confirmation by TBI/AD clinicians
- (G) production of methodological manuscripts on analysis
- (H) production of software for TBI/AD connectome analysis
- (I) presentation of results at national meetings

(2) *Specific objectives*

The specific objective of this scientific study is to identify novel AD risk factors specific to the TBI population by applying sophisticated imaging and connectomic methods to the MRI/CT brain scans of older adults who are either healthy, TBI survivors, AD patients, or both. At the end of Y1 of this project, the activities needed to meet the objective of the study are ongoing and full completion of the specific objectives is anticipated. Progress toward meeting the objectives has been adequate and in line with the stated milestones and completion deadlines of the project. All completed goals have been met.

(3) *Significant results*

Activity (A). computer-based calculations and analyses were performed to identify tissue deformation in MRI volumes, lesion growth displacing healthy tissue, and lesion infiltration into healthy tissue. The phenomena of TBI structural dynamics were modeled as the composition of background and foreground deformations. Local expansions and contractions were accounted for by means of this approach. We combine 3D brain models extracted from multimodal structural brain imaging (T1/T2 MRI, FLAIR, SWI, GRE T2) with DTI tractography to obtain connectivity information required to generate graph-theoretical representations for network models of the human connectome and to study its differences across groups. Image processing methods tailored for TBI anatomy will be used, including some which we have pioneered. Cortical WM was segmented and the estimated GM-WM border will be tessellated to provide a topographically correct areal representation. We employed a combination of automatic segmentation tools including FreeSurfer (FS) for volumes with minimal pathology deformation in addition to the Atlas Based Classification (ABC) method, to whose development we have contributed extensively.

Activity (B). We computed surface-based features (cortical thickness, curvature, displacement) at each time point and between time points using a distance transform for cortical thickness computation. We used the FS and ABC packages to detect pathology in MRI scans. These segmenters are freely available, automatic and their executions require minimal user supervision. Briefly, the ABC algorithm can perform multimodal registration of MR images, tissue classification of the brain, as well as lesion segmentation based on outlier detection and physical model estimation. This segmenter was used to perform both co-registration of MR volumes and intensity normalization within and between scans. The ABC segmenter possesses the ability to perform co-registration of an arbitrary number of MR volumes acquired using various sequences. This made it suitable to the proposed multimodal imaging paradigm because many distinct sequence types are employed.

Activity (C). Connectivity between brain parcels was determined from DTI volumes acquired longitudinally from each volunteer. Diffusion tractography was used systematically to determine connectivity properties and to compute each subject's weighted connectivity matrix, which specified the graph-theoretic description of each brain network. Connectivity between brain parcels was determined from DTI volumes acquired longitudinally from each volunteer. Due to its ability to allow automated construction of WM fascicles, diffusion

tractography was used systematically to determine connectivity properties and to compute each subject's weighted connectivity matrix, which will specify the graph-theoretic description of each brain network. Connectivity between regions was assessed systematically within each patient using purpose-built workflows involving multimodal co-registration of MRI and DTI volumes followed by the calculation of (i) inter-regional connectivity matrices and (ii) longitudinal changes in connectome topology using network-theoretic descriptors of nodal and network-wide segregation (clustering coefficient, modularity, etc.) and integration (characteristic path length, global efficiency, etc.). For each subject and data time point, additional network-theoretic measures (scale freedom, small-worldness, robustness, centrality, degree distribution, communication efficiency) was computed.

Activity (D). To quantify how WM atrophy translates into network communication cost changes, the latter set of quantities are being mapped over the cortical surface to identify brain regions which become more topologically distant from the rest of the brain because of TBI and/or AD, and whose functions may degrade comparably more. To identify regions with appreciable connectivity loss, the fraction of network edges affected by gross TBI and/or AD pathology are computed. Based on this calculation, the percentage of rich club, feeder and local nodes and connections affected by trauma is also being computed and the results of these calculations will be mapped over the cortex. Changes in the communication cost shared by each type of connection (rich club, feeder, local) will be quantified to determine the extent to which the network communication backbone of each patient has been damaged. Figure 1 shows the results of the statistical comparison between three groups (TBI, AD and healthy control (HC) subjects) to identify brain connections whose connectivity densities differ significantly between each pair of groups. These results are illustrative of our progress to understand connectivity differences and similarities between TBI and AD volunteers relative to HCs. Figures 2 and 3 show the results of the voxel-wise comparison between two groups (TBI, AD) to identify locations in the white matter of the brain whose fractional anisotropies was either significantly different (Figure 2) or significantly equivalent (Figure 3, TOST statistical procedure, see figure caption). Figures 4 and 5 show the results of the vertex-wise comparison between the three groups to identify locations in the gray matter whose cortical thickness was equivalent, to a statistically significant extent (TOST procedure), across the two study groups (TBI and AD). These results are illustrative of our progress to understand connectivity differences and similarities between TBI and AD volunteers relative to HCs.

Activity (E). The procedure for visually identifying pathology associated with cerebral micro-hemorrhages has followed state-of-the-art approaches. SWI was used to identify susceptibility effects which make micro-hemorrhages more obvious in SWI compared to other paradigms. Following standard clinical guidelines, hemorrhagic lesions were defined as hypo-intense foci which are not compatible with vascular, bone, or artifactual structures on conventional GRE. Partially based on funding from this contract, we designed and implemented a novel, automatic algorithm for the identification of cerebral microbleeds (CMBs) from SWI images. Two raters trained in neuroimaging and blinded to the results of the automatic labeling process identified blood vessels and CMBs visually and independently from each other. All identified features of interest were either confirmed or

informed by a third blinded rater. This allowed the sensitivity and precision of the classification algorithm to be calculated. This approach allowed CMBs to be identified with a sensitivity of 97.1% (see Figures 6, 7 and 8).

Activity (F). We have started validating the topology analysis results internally. When assessing the reliability of pathology labeling and segmentation, validation can often be difficult due to frequent lack of reliable ground truth, and manual tracings by trained experts have traditionally been appropriate from this standpoint. In this study, both expert-labeled pathology and TBI scans where pathology has not yet been labeled were used to validate our approach and results. Because TBI anatomy often diverges from healthy anatomy, segmentation errors can occur. When assessing segmentation error extent, it is fair to note that, although often considered to be the gold standard, even manual outlining results in moderate (3-10%) intra- and inter-operator variabilities. To address this, manual review in 3D Slicer by three operators with experience in the identification of neurotrauma was performed to edit and correct errors. Because the brains of aging adults may exhibit micro-hemorrhages, particular care must be taken to identify and take these into account appropriately during analysis. The procedure for visually identifying pathology associated with cerebral micro-hemorrhages followed state-of-the-art approaches pioneered by the PI. Although no preliminary results for this activity are available yet due to it having just been initiated, activities are proceeding according to plan and we anticipate having results to report on by the next progress report deadline.

Activity (G). research based on funding from this award was featured in five journal manuscripts, one of which has been published and four of which are currently under review. We have also contributed a technical book chapter as well as 16 peer-reviewed abstracts at various local, national and international conferences. A complete list of these products is provided in Section 6 of this report.

Activity (H). the research activities conducted as part of this project resulted in the development of Matlab and Python programming code and software for the analysis of imaging data. Currently, this material is available from the PI upon request. However, we anticipate making this material available free of charge to the public at large and to the scientific community on GitHub or using a similar form of dissemination.

Activity (I). We have presented our work at the following events:

Meeting of the Gerontological Society of America (GSA '19)	Austin, TX
International Symposium on Biomedical Engineering (ISBI '19)	Venice, Italy
Meeting of the Organization for Human Brain Mapping (OHBM '19)	Rome, Italy
Alzheimer's Association International Conference (AAIC '19)	Los Angeles, CA
Annual Symposium of the National Neurotrauma Society (NNS '19)	Pittsburgh, PA
USC Undergraduate Symposium for Scholarly & Creative Work	Los Angeles, CA
Meeting of the Gerontological Society of America (GSA '18)	Boston, MA

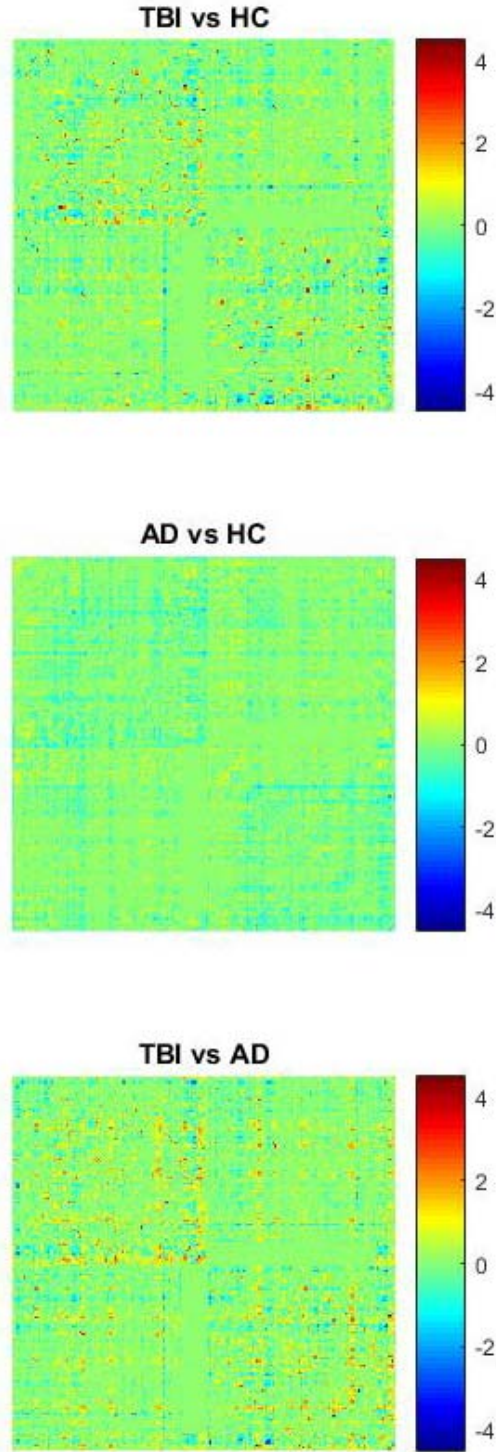


Figure 1. Statistical comparison between groups (TBI, AD and HC subjects) to identify brain connections whose white matter connectivity densities (CDs) differ significantly between groups. Displayed is the connectivity matrix, where each entry $\{i, j\}$ specifies CD for the connection between brain regions i and j . To generate the connectivity matrix, the brain was automatically parceled into 165 regions. Color encodes t statistics associated with testing the null hypothesis of no difference in mean CD between groups. Values of the t statistic whose magnitude exceeds ~ 1.66 are significant at a level of $\alpha < 0.05$. Welch's t test for independent samples with unequal variances was implemented. For each matrix displayed, values shown in shades of red indicate greater CD in the first group listed compared to the second; values shown in shades of blue indicate the reverse.

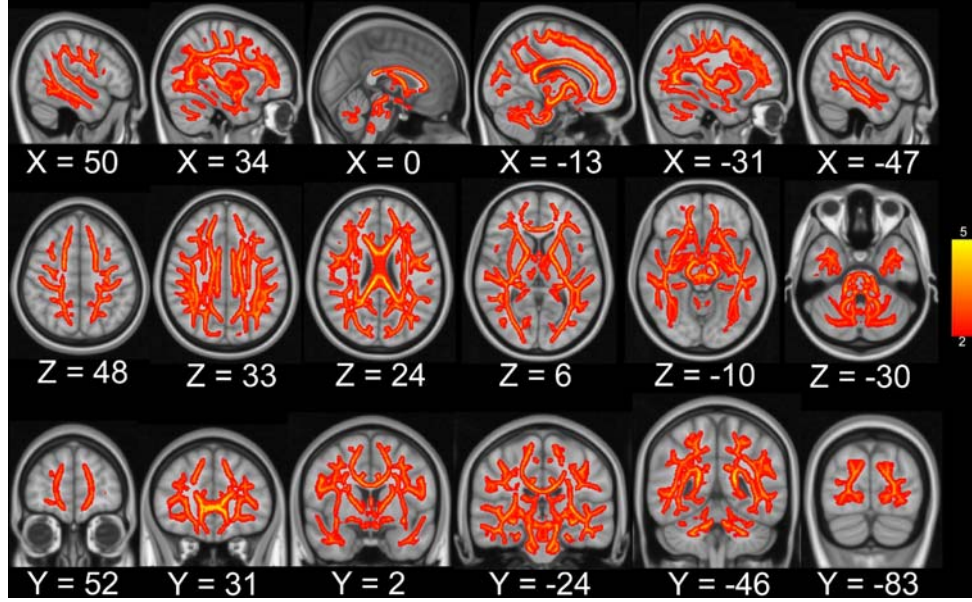


Figure 2. Visual representation of group-level differences in FA between HC participants and mTBI victims at ~6 months after injury. Colors encode $-\log p$, which ranges from 2 (red) to 5 (yellow), corresponding to p -values of 0.05 (red) to ~ 0.0005 (yellow). p -values are displayed using a significance threshold of $\alpha = 0.05$, subject to multiple comparison correction. For convenience, the chosen logarithm base is ~ 4.47 , such that $-\log \alpha \approx 2$. Smaller p -values (brighter yellow) are associated with lower FA values in the mTBI group compared to HC participants, suggesting extensive loss of WM integrity throughout the brain after mTBI. The X, Y or Z coordinate of each slice in MNI₁₅₂ space is provided. Images are displayed in radiologic convention.

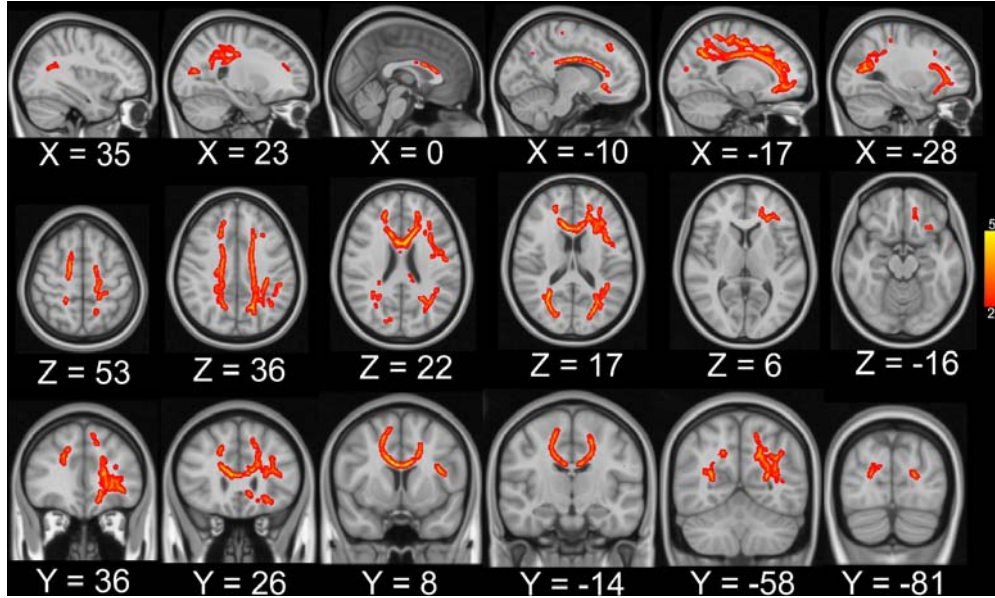


Figure 3. Visual representation of group-level differences in FA between *late middle-age* and *old* mTBI victims. Colors encode $-\log p$, which ranges from 2 (red) to 5 (yellow), corresponding to p -values of 0.05 (red) to ~ 0.0005 (yellow). p -values are displayed using a significance threshold of $\alpha = 0.05$, subject to multiple comparison correction. For convenience, the chosen logarithm base is ~ 4.47 , such that $-\log \alpha \approx 2$. Smaller p -values imply lower FA values in old mTBI victims compared to late middle-age mTBI participants. These results illustrate the effect of age at injury upon WM integrity loss. Images are displayed in radiologic convention. The X, Y or Z coordinate of each slice in MNI₁₅₂ space is provided.

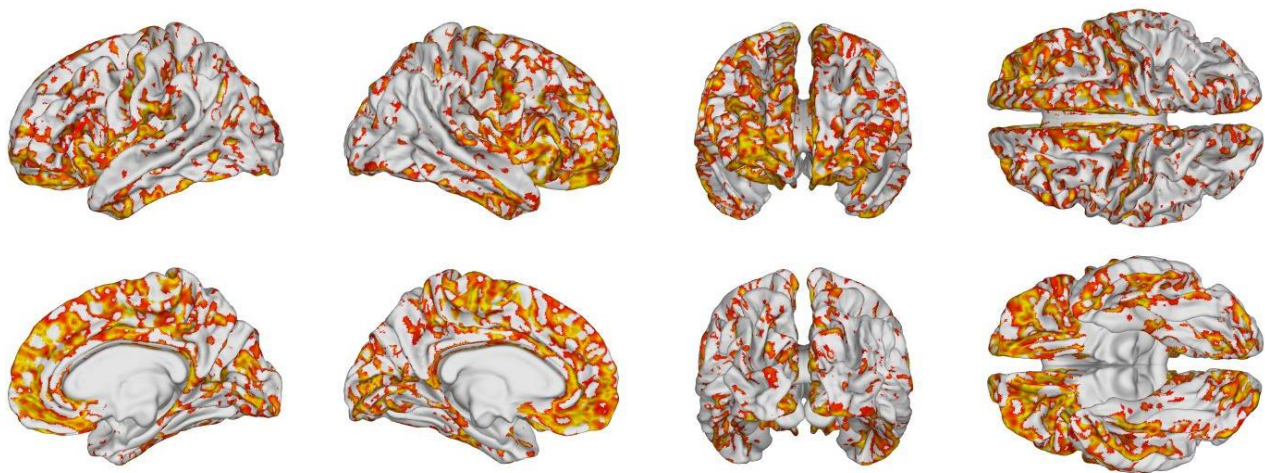


Figure 4. Visual representation of group-level differences in gray matter thickness between AD participants and mTBI victims at ~6 months after injury. Colors encode $-\log p$, which ranges from 2 (red) to 5 (yellow), corresponding to p -values of 0.05 (red) to ~ 0.0005 (yellow). p -values are displayed using a significance threshold of $\alpha = 0.05$, subject to multiple comparison correction. Smaller p -values (lighter yellow) are associated with less significant differences between the TBI and AD groups, suggesting extensive loss of cortical thickness throughout the brain after mTBI in a manner representative of AD.

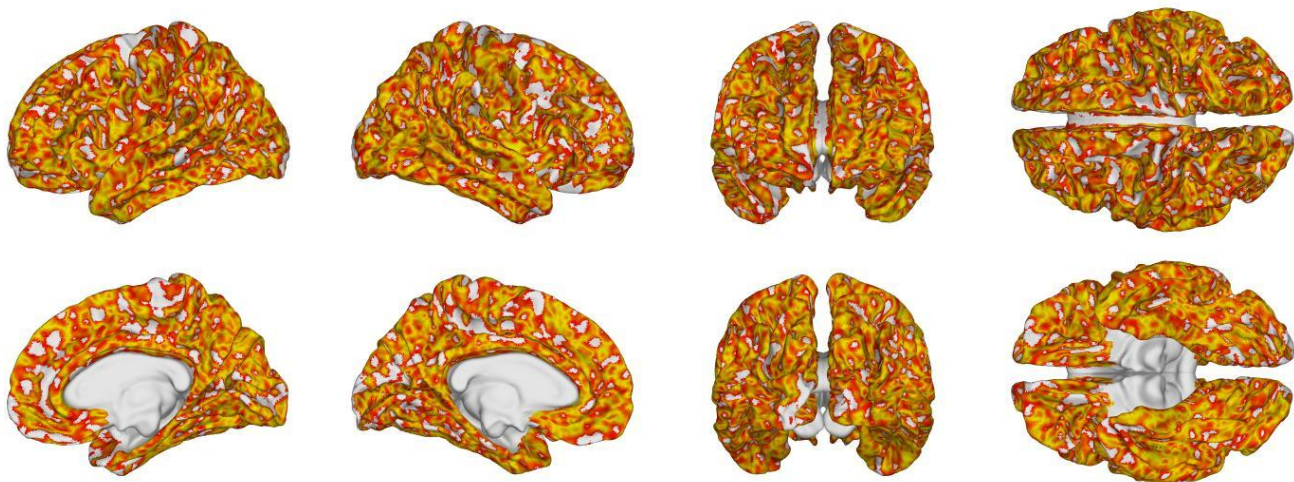


Figure 5. Like Figure 3, for the comparison between TBI and HC. The maps indicate brain-wide loss of gray matter after geriatric TBI compared to the control sample.

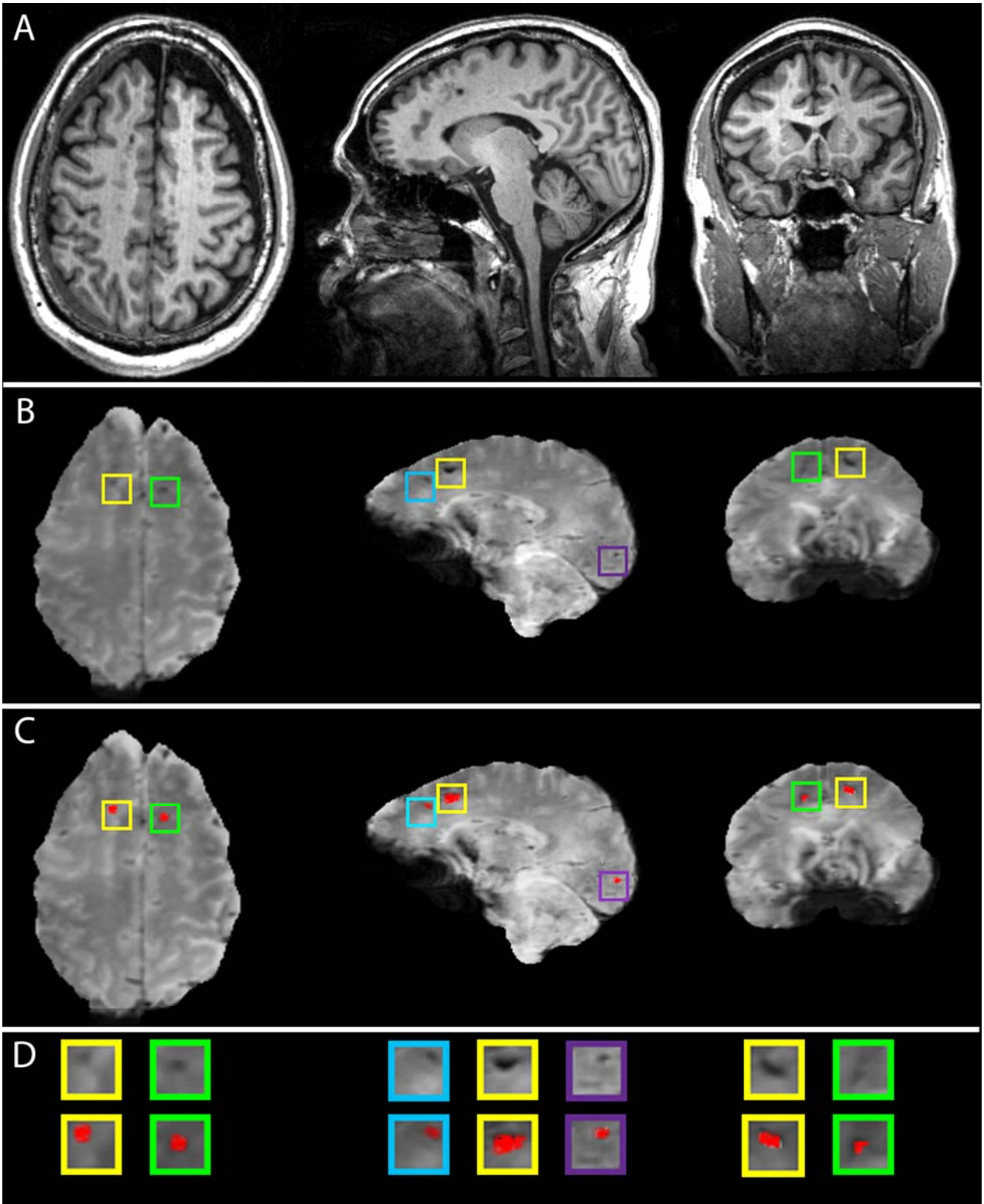


Figure 6. Examples of automatic CMB segmentations in a geriatric mTBI patient. (A) T1-weighted images. (B) SWI images with CMBs highlighted in color-coded boxes. (C) SWI images with superimposed CMB segmentations. (D) Enlarged views of CMBs in (B) and (C). Segmentations like these allow us to quantify hemorrhagic lesion load in each participant.

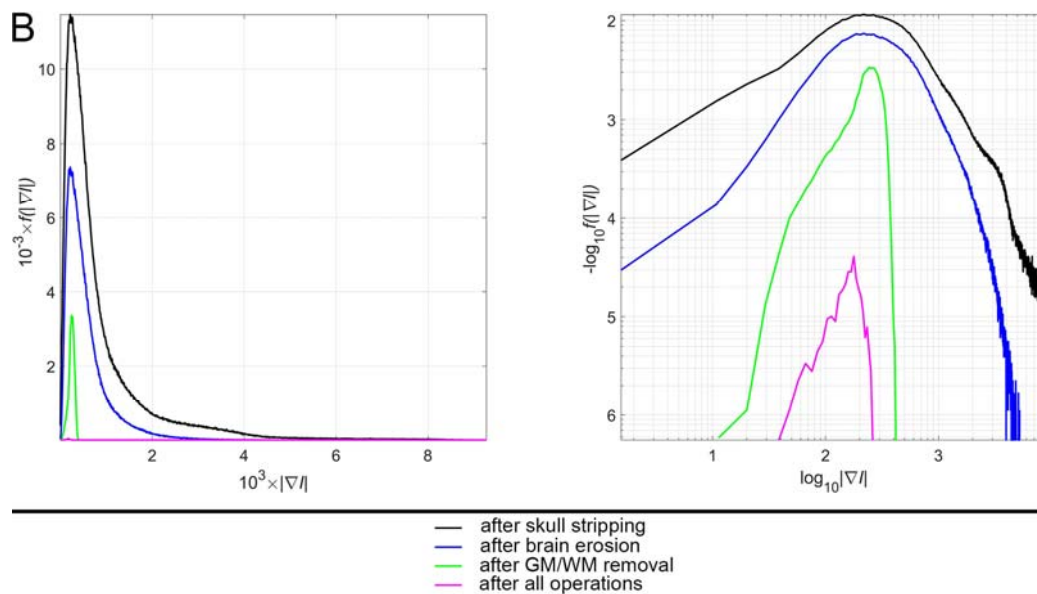


Figure 7. The probability density function (PDF) $f(\cdot)$ for the gradient magnitudes $|\nabla I|$ of SWI intensity I in a sample participant. For convenience, plots are scaled either linearly (first column) or logarithmically (second column) along both axes. Traces depict the corresponding PDF computed over all brain voxels after skull stripping (black), over the subset of voxels remaining after brain erosion (blue), over voxels remnant after the subsequent removal of GM/WM (green) and over CMB voxels alone, after all operations (magenta). In the first column, for visual convenience, values are scaled linearly; for example, $10^3 \times |\nabla I|$ is plotted on the abscissa (rather than $|\nabla I|$). In the second column, the logarithm of the quantity being depicted is shown; thus, the abscissa displays $\log_{10}(|\nabla I|)$ rather than $|\nabla I|$ itself. The left and right columns display the same information, albeit using different scaling, i.e. linear scaling (first column) or logarithmic scaling (second column).

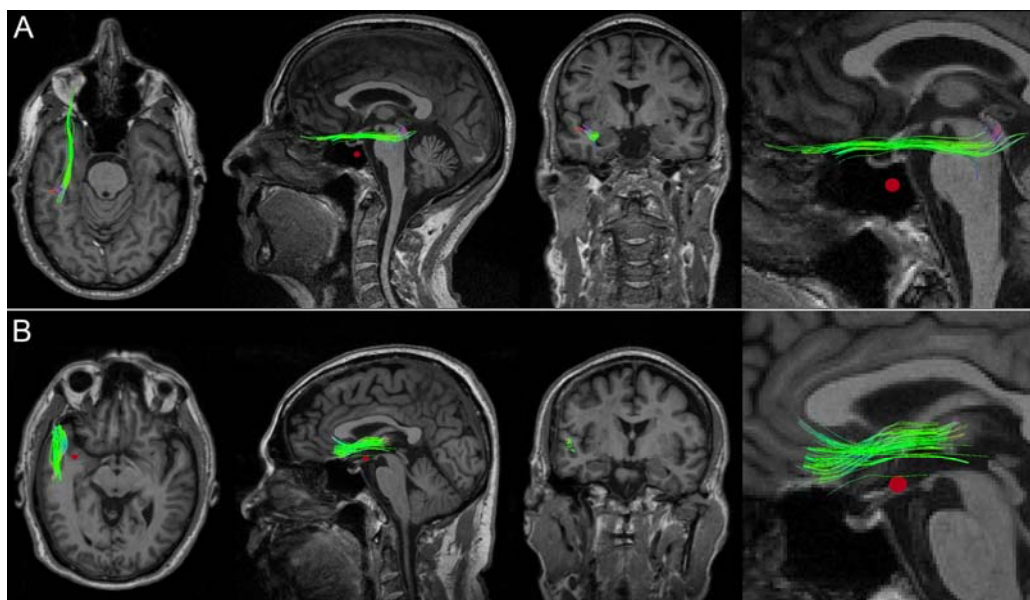


Figure 8. WM streamline clusters in two mTBI victims who exhibit a CMB in the WM of the left anterior temporal lobe. In both (A) and (B), fractional anisotropy (FA) changes at vertices along the fasciculi were significantly correlated with the distances from these points to the nearest CMB (red). Whereas only some of the WM streamlines in each cluster pass through the CMB penumbra, the observed FA changes were found to extend beyond the immediate spatial neighborhood of the vascular lesion. From left to right: axial view, sagittal view, coronal view, and inset.

What opportunities for training and professional development has the project provided?

Although the project was not specifically intended to provide training and professional development, the PI has been able to provide one-on-one mentorship thanks to this award. Specifically, two junior scientists (Maria Calvillo, Sean Mahoney), two graduate students (Di Fan, Nikhil Chaudhari) and three undergraduate students (Kenneth Rostowsky, Alexander Maher, Nahian Chowdhury) benefitted from the learning opportunities provided by this project. The three undergraduate students contributed to methodological efforts which assisted the project as part of a USC initiative which facilitates the process of allowing undergraduate students to undertake scientific research. This is a prestigious program at USC for which only competitive students are selected.

Aside from one-on-one training, the PI has integrated research results from this project into his lectures as part of two courses which he teaches at USC, namely a seminar course for PhD students (GER 592, Multidisciplinary Research Seminar in Gerontology), and a graduate course for master's degree students (GER 508, The Mind-Body Connection Across the Lifespan). The PI also disseminated results from this project as part of guest lectures in two PhD courses in gerontology (GER 600, GER 623), one PhD course in neuroscience and one seminar course in electrical engineering.

How were the results disseminated to communities of interest?

One manuscript describing research undertaken partly using funding from this contract was accepted presented as an oral conference session of the IEEE EMBS Conference in Berlin, Germany, where the PI served as chair of the session. Invitations to present results orally at this conference are made to fewer than 2% of all authors' contributions to this competitive and prestigious conference which is very highly ranked in the field of biomedical engineering.

The PI was also invited to present the results of the study at several conferences, including the American College of Rehabilitation Medicine, where he was keynote speaker at a special symposium and where he also participated in a well-attended "Meet the Experts" session. Only 1% of all conference participants are invited to present as part of a special symposium at this prestigious conference.

The PI undertakes public speaking engagements outside scientific circles on a regular basis. In 2019, he was invited to speak at Pitzer & Claremont McKenna Colleges as part of an invited seminar on the topic of sports concussion, where he featured the results obtained using funds from this award. He also presented his work at Cedars Sinai Medical Center, where he was invited to give a presentation to clinicians and neurorehabilitation professionals.

Last but not least, the PI presented the results of this research to high school students as part of a pre-college summer program at USC. Results were also disseminated to incoming USC freshmen as part of a seminar on brain concussions and their effects upon human health.

What do you plan to do during the next reporting period to accomplish the goals?

Throughout Y2 of the project, we will finalize the task of identifying TBI/AD-related connectome differences using our proposed network theoretic analysis of structural and functional imaging data. Upon completing our internal validation of connectomic/topological analysis, we will proceed toward the statistical analysis of the results, which we anticipate will continue until the end of Y2. We will implement a 2×2 factorial experimental design (factors: single mild TBI, AD) to identify and quantify the neuroanatomic and connectomic features of older adults (aged 65+) whose existence is modulated by TBI/AD interactions. A TBI-adaptive, neuroimaging-based atlas of brain structure will then be utilized to calculate and monitor longitudinal differences in the temporal trajectories of brain structure and connectomics in TBI-/AD- (i.e. healthy control), TBI+/AD-, TBI-/AD+ and TBI+/AD+ volunteers. Multivariate general linear models (GLMs) will be implemented to identify volumetric, morphometric and connectomic signatures present only in the brains of TBI survivors and whose properties are significantly correlated with the severity of their AD-like cognitive impairment and, potentially, with the likelihood of TBI survivors to develop this condition. Last but not least, we will revise our methodology as needed in response to any challenges that arise and continue disseminating our work in the form of manuscripts to be submitted to peer-reviewed journals, conference presentations and other dissemination media.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

The effects of mild TBI (mTBI) upon gross anatomy are typically subtler than those of moderate-to-severe TBI because the latter often changes neuroanatomy and brain circuitry to a dramatic extent. For this reason, the mTBI population studied as part of this project is substantially more homogeneous than that comprising more severe forms of TBI, which implies that mTBI is considerably more amenable to population-level studies and statistical analyses. Additionally, it has already been argued extensively within the TBI and AD research communities that moderate-to-severe TBI predisposes its victims to AD significantly, whereas the extent to which this is the case for mTBI victims is far less clear. For all these reasons, the utility of our study and its relevance are likely greatest for the welfare of mTBI victims. Nevertheless, because ~80% of all TBI victims have mild forms of this condition, this proposed research bears testimony to the substantial potential impact of our study compared to the scenario of studying only moderate-to-severe forms of TBI.

Thus far, our results indicate that the mTBI-affected brain of older adults experiences an amount of connectomic and cortical neurodegeneration which rivals that of AD patients of similar age and of the same sex. This finding is likely to be very impactful because it has also allowed to find properties of the TBI-affected brain whose characteristics are commensurate with the likelihood of TBI survivors to develop a trajectory of neurodegeneration which is more severe than that of other TBI patients, including possibly those who will go on to develop AD. Partly for this reason, this proposed project to identify novel AD risk factors which are specific to the TBI population can be of substantial clinical benefit. Specifically, (1) having the ability to accurately identify TBI victims at high risk for AD could have substantial consequences for the healthcare of such individuals, and (2) understanding the relationship between TBI and AD could advance our scientific and clinical knowledge regarding these two conditions.

What was the impact on other disciplines?

Outside our own discipline, the methodological innovations made as part of this project have made an impact upon the scientific field of image processing. Specifically, because our CMB identification algorithm performs better than any other methods previously proposed (sensitivity: 97%), it is likely that our approach will be useful to other researchers in the field of image processing. The task of finding CMB automatically in the brain using MRI-based analysis is important because over 40% of older adult brains exhibit CMBs, which are frequently associated with conditions like cerebral amyloid angiopathy (CAA) and cerebrovascular disease.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

The results of this project will likely make an impact upon public perceptions of mTBI severity in older adults. Our work indicates that mTBI affects the aging brain substantially more than it affects other age groups, particularly from the standpoint of connectome degradation and cortical thinning. Our finding that AD and mTBI share significant patterns of connectivity losses will likely assist in improving public knowledge of seniors' vulnerability to AD after suffering concussions. The PI will continue to expend effort in the direction of increasing public awareness of these findings so that older adults are granted an adequate amount of attention from public policy makers and from governmental and non-governmental entities seeking to improve the healthcare of our aging population.

5. CHANGES/PROBLEMS:

Nothing to report.

Changes in approach and reasons for change

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to report.

Changes that had a significant impact on expenditures

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Not applicable (no use or care of vertebrate animals).

Significant changes in use of biohazards and/or select agents

Not applicable (no biohazards and/or select agents).

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Avnish Bhattra, **Andrei Irimia** and John D. Van Horn (2019) Neuroimaging and cellular biomarkers of traumatic brain injury in military veterans *Journal of Clinical Neuroscience* (accepted, awaiting publication; *acknowledgment of federal support: yes*)

Di Fan, Nikhil N. Chaudhari, Maria Calvillo, Sean K. Lee, Nahian F. Chowdhury, Kenneth A. Rostowsky, Fan Zhang, Lauren J. O'Donnell and **Andrei Irimia** (2019) Post-traumatic cerebral microhemorrhages and their effects upon white matter connectivity in the aging human brain *IEEE Engineering in Medicine and Biology*, volume 40, pages 198-203. (*acknowledgment of federal support: yes*)

Andrei Irimia (2019) Cross-sectional volumes and trajectories of the human brain, gray matter, white matter and cerebrospinal fluid in 9,473 typically aging adults (*under review; acknowledgment of federal support: yes*)

Andrei Irimia, Di Fan, Nikhil N. Chaudhari, Van Ngo, Fan Zhang, Shantanu H. Joshi and Lauren J. O'Donnell (2019) Mapping cerebral connectivity changes after mild traumatic brain injury in older adults using diffusion tensor imaging and Riemannian matching of elastic curves (*under review; acknowledgment of federal support: yes*)

Alexander Olsen, Talin Babikian, Erin Bigler, Karen Caeyenberghs, Virginia Conde, Kristen Dams-O'Connor, Ekaterina Dobryakova, Helen Genova, Jordan Grafman, Asta K. Häberg, Ingrid Heggland, Torgeir Hellström, Cooper B. Hodges, **Andrei Irimia**, Ruchira M. Jha, Paula K. Johnson, Harvey Levin, Lucia M. Li, Hannah M. Lindsey, Abigail Livny, Marianne Løvstad, John Medaglia, David Menon, Stefania Mondello, Martin M. Monti, Virginia F. J. Newcombe, Agustin Petroni, Jennie Ponsford, David Sharp, Gershon Spitz, Lars T. Westlye, Paul M. Thompson, Emily L. Dennis, David F. Tate, Elisabeth A. Wilde, Frank G. Hillary (2019) Toward a global and reproducible science for brain imaging in

neurotrauma: The ENIGMA adult moderate/severe traumatic brain injury working group (under review; acknowledgment of federal support: yes)

Books or other non-periodical, one-time publications.

Maria Calvillo, Di Fan and **Andrei Irimia** (2019) Multimodal imaging of cerebral microhemorrhages and white matter degradation in geriatric patients with mild traumatic brain injury (book chapter under review; acknowledgment of federal support: yes)

Alexander S. Maher, Nikhil N. Chaudhari, Elliot B. Jacobs, Sean O. Mahoney, Van Ngo and **Andrei Irimia** (2020) Statistical equivalence of resting-state functional correlation patterns in the default mode networks of patients with Alzheimer's disease vs. geriatric victims of mild traumatic brain injury *Fourteenth Symposium of the International Neurotrauma Society (INTS '20)*, March 29-April 2, 2020, Melbourne, Australia (submitted)

Kenneth A. Rostowsky, Nikhil N. Chaudhari and **Andrei Irimia** (2020) Statistically equivalent distribution of cortical thinning and white matter degradation in geriatric patients with mild traumatic brain injury relative to Alzheimer's disease *Fourteenth Symposium of the International Neurotrauma Society (INTS '20)*, March 29-April 2, 2020, Melbourne, Australia (submitted)

Alexander S. Maher, Elliot B. Jacobs, Nikhil N. Chaudhari and **Andrei Irimia** (2020) Default mode network differences between geriatric mild traumatic brain injury and Alzheimer's disease *Fifteenth Annual Conference of the North American Brain Injury Society (ABI '20)*, February 26-29, 2020, New Orleans, Louisiana, USA (accepted)

Andrei Irimia, Kenneth A. Rostowsky, Nikhil N. Chaudhari, Maria Calvillo, Sean K. Lee (2019) Geriatric traumatic brain injury and Alzheimer's disease share similar patterns of white matter degradation *Annual Scientific Meeting of the Gerontological Society of America (GSA '19)*, November 13-17, 2017, Austin, Texas, USA. (accepted)

Kenneth A. Rostowsky, Nikhil N. Chaudhari, Maria Calvillo, Sean K. Lee, Agatha Lenartowicz and **Andrei Irimia** (2019) Similar spatial patterns of white matter degradation in geriatric traumatic brain injury and Alzheimer's disease *Proceedings of the Fifteenth International Symposium on Biomedical Imaging (ISBI '19)*, April 8-11, 2019, Venice, Italy

Di Fan, Nikhil N. Chaudhari, Maria Calvillo, Sean K. Lee, Nahian F. Chowdhury, Kenneth A. Rostowsky, Yu Hu, Lei Cao, Fan Zhang, Lauren J. O'Donnell and **Andrei Irimia** (2019) Cerebral microhemorrhages and their effects upon white matter after geriatric traumatic brain injury *Proceedings of the Fifteenth International Symposium on Biomedical Imaging (ISBI '19)*, April 8-11, 2019, Venice, Italy

Di Fan, Maria Calvillo, Yu Hu, Sean K. Lee, Lei Cao, Fan Zhang, Lauren J. O'Donnell and **Andrei Irimia** (2019) Longitudinal quantification of microhemorrhage effects upon white matter after geriatric concussion *Twenty-Fifth Annual Meeting of the Organization on Human Brain Mapping (OHBM '19)*, June 9-13, 2019, Rome, Italy.

Kenneth A. Rostowsky, Nikhil N. Chaudhari, Maria Calvillo, Sean K. Lee and **Andrei Irimia** (2019) White matter degradation after mild traumatic brain injury revealed by diffusion tensor imaging *Twenty-Fifth Annual Meeting of the Organization on Human Brain Mapping (OHBM '19)*, June 9-13, 2019, Rome, Italy.

Nikhil N. Chaudhari, Maria Calvillo, Sean K. Lee, Nahian F. Chowdhury, Kenneth A. Rostowsky, Lei Cao and **Andrei Irimia** (2019) Intensity gradient segmentation of brain microhemorrhages from susceptibility weighted imaging *Twenty-Fifth Annual Meeting of the Organization on Human Brain Mapping (OHBM '19)*, June 9-13, 2019, Rome, Italy.

Kenneth A. Rostowsky, Nikhil N. Chaudhari, Maria Calvillo, Sean K. Lee and **Andrei Irimia** (2019) Geriatric traumatic brain injury and Alzheimer's disease share patterns of white matter decline *Proceedings of the Alzheimer's Association International Conference (AAIC '19)*, July 14-18, 2019, Los Angeles, California, USA. Also published in *Alzheimer's & Dementia: The Journal of the Alzheimer's Association*, volume 7, pages P1407-P1408.

Kenneth A. Rostowsky, Nikhil N. Chaudhari, Maria Calvillo, Sean K. Lee, Nahian F. Chowdhury, Agatha Lenartowicz and **Andrei Irimia** (2019) Mild traumatic brain injury in older adults and Alzheimer's disease share patterns of white matter degeneration *Thirty-Seventh Annual Symposium of the National Neurotrauma Society (NNS '19)*, June 29-July 3, 2019, Pittsburgh, Pennsylvania, USA

Di Fan, Nikhil N. Chaudhari, Kenneth A. Rostowsky, Maria Calvillo, Sean K. Lee, Nahian F. Chowdhury, Fan Zhang, Lauren J. O'Donnell and **Andrei Irimia** (2019) The effects of cerebral microhemorrhages on white matter connectivity and aging in mild traumatic brain injury *Thirty-Seventh Annual Symposium of the National Neurotrauma Society (NNS '19)*, June 29-July 3, 2019, Pittsburgh, Pennsylvania, USA

Andrei Irimia, Nahian F. Chowdhury and Alexander S. Maher (2018) White matter connectivity changes due to cerebral microhemorrhages in geriatric mild traumatic brain injury *Proceedings of the Annual Scientific Meeting of the Gerontological Society of America (GSA '18)*, November 14-18, 2018, Boston, Massachusetts, USA. Also published in *Innovation in Aging*, volume 2, Supplement 1, p. 105.

Andrei Irimia, Alexander S. Maher, Nahian F. Chowdhury and E. Meng Law (2018) Multimodal study of geriatric mild traumatic brain injury using computed tomography and magnetic resonance imaging *Proceedings of the Annual Scientific Meeting of the Gerontological Society of America (GSA '18)*, November 14-18, 2018, Boston, Massachusetts, USA. Also published in *Innovation in Aging*, volume 2, Supplement 1, p. 300.

Elliot Jacobs, Alexander S. Maher, Nikhil N. Chaudhari, Kenneth A. Rostowsky and **Andrei Irimia** (2019) A novel neuroinformatics workflow for functional connectomic comparison of geriatric traumatic brain injury and Alzheimer's disease *Twenty-First Annual USC*

Undergraduate Symposium for Scholarly & Creative Work, April 10, 2019, Los Angeles, CA, USA

Kenneth A. Rostowsky, Nikhil N. Chaudhari, Maria Calvillo, Sean K. Lee, Agatha Lenartowicz and **Andrei Irimia** (2019) Similar patterns of white matter disruption in Alzheimer's disease and mild traumatic brain injury *Twenty-First Annual USC Undergraduate Symposium for Scholarly & Creative Work*, April 10, 2019, Los Angeles, CA, USA

Other publications, conference papers and presentations.

Andrei Irimia, Alexander S. Maher, Kenneth A. Rostowsky, Nikhil N. Chaudhari (2019) Personalized connectome profiling for the rehabilitation of patients with neural injury and neurodegenerative disorder *Presentation at the Ninety-Sixth Conference of the American Congress of Rehabilitation Medicine (ACRM '19)*, November 3-8, 2019, Chicago, Illinois.

Di Fan, Nikhil N. Chaudhari, Kenneth A. Rostowsky, Maria Calvillo, Sean K. Lee, Nahian F. Chowdhury, Fan Zhang, Lauren J. O'Donnell and **Andrei Irimia** (2019) White matter integrity following post-traumatic cerebral micro-hemorrhages in the aging human brain *Presentation at the Third Annual Conference on the Frontiers of Traumatic Brain Injury (Frontiers of TBI '19)*, September 16, 2019, Institute of Neurology, University College London and Imperial College London, UK.

Di Fan, Fan Zhang, Lauren J. O'Donnell and **Andrei Irimia** (2019) White matter analysis in the aging human brain *Presentation at the Thirty-First Annual Meeting of the National Alliance for Medical Image Computing (NA-MIC '19)*, June 24-28, 2019, Cambridge, Massachusetts, USA

- **Website(s) or other Internet site(s)**

Journal papers, conference publications and main results are disseminated online via the PI's official webpage on the website of the USC Leonard Davis School of Gerontology (www.gero.usc.edu) as well as through the PI's personal website (www.andrei-irimia.com), which is a mirror site of the official university webpage.

- **Technologies or techniques**

A novel algorithm for the automatic segmentation of cerebral microbleeds (CMBs) from SWI MRI was designed as part of this project. The algorithm steps have been published in a peer-reviewed publication.

- **Inventions, patent applications, and/or licenses**

Nothing to report.

- **Other Products**

The research activities resulted in the development of Matlab and Python programming code and software for the analysis of imaging data. This material is available from the PI upon request and will be made available to the public on GitHub or in a similar venue when the software product is mature.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Andrei Irimia, PhD
Project Role: Principal Investigator
Researcher Identifier: 0000-0002-9254-9388
Nearest person month worked: 1
Contribution to Project: Dr. Irimia oversees all aspects of the project and ensures regulatory compliance. He carries out and/or supervises all data analyses, publication efforts and dissemination activities.

Name: Maria Calvillo
Project Role: project scientist
Researcher Identifier: Not available
Nearest person month worked: 3
Contribution to Project: Mrs. Calvillo provided expertise on the assessment of white matter integrity from diffusion tensor imaging data, on quantitative analysis, on the interpretation of results and on manuscript preparation.

Name: Sean Mahoney
Project Role: project scientist
Researcher Identifier: Not available
Nearest person month worked: 3
Contribution to Project: Mr. Mahoney contributed to the assessment of white matter integrity from DTI data, to the quantitative analysis, to the refinement and development of methods for image segmentation and to the interpretation of results. He also assisted with data curation, and with the execution of computer programs.

Name: Di Fan
Project Role: graduate student
Researcher Identifier: Not available
Nearest person month worked: 1
Contribution to Project: Mr. Fan assisted with quantitative analysis, with the refinement and development of methods for image segmentation and with the interpretation of the results.

Name: Kenneth A. Rostowsky
Project Role: undergraduate student
Researcher Identifier: Not available

Nearest person month worked: 5

Contribution to Project: Mr. Rostowsky assisted with data curation, with the execution of computer programs for image segmentation and with the interpretation of the results.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Organization Name: Harvard Medical School

Location of organization: Cambridge, MA

Partner's contribution to the project: Our collaborators at Harvard Medical School provided expertise and advice with our use of their freely available software for image analysis. This expertise was provided at no cost to our team. The Harvard team also contributed to the interpretation of our results and to our publication efforts.

Organization Name: University of California

Location of organization: Los Angeles, CA

Partner's contribution to the project: Our collaborators at UCLA provided expertise and advice with our use of their freely available software for Riemannian curve matching. This expertise was provided at no cost to our team. The UCLA team also contributed to the interpretation of our results and to our publication efforts.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: Not applicable (this is not a collaborative award).

QUAD CHARTS: The updated quad chard is attached to this progress report.

9. APPENDICES:

Two peer-reviewed papers which have been accepted and published are included on the subsequent pages of this progress report. A third paper has been submitted and has not been reviewed yet, however it is included as well because it contains methodological details on the procedures used in the study.



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Review article

Neuroimaging of traumatic brain injury in military personnel: An overview

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ABSTRACT

Background: The incidence of blunt-force traumatic brain injury (TBI) is especially prevalent in the military, where the emergency care admission rate has been reported to be 24.6–41.8 per 10,000 soldier-years. Given substantial advancements in modern neuroimaging techniques over the past decade in terms of structural, functional, and connectomic approaches, this mode of exploration can be viewed as best suited for understanding the underlying pathology and for providing proper intervention at effective time-points.

Approach: Here we survey neuroimaging studies of mild-to-severe TBI in military veterans with the intent to aid the field in the creation of a roadmap for clinicians and researchers whose aim is to understand TBI progression.

Discussion: Recent advancements on the quantification of neurocognitive dysfunction, cellular dysfunction, intracranial pressure, cerebral blood flow, inflammation, post-traumatic neuropathophysiology, on blood serum biomarkers and on their correlation to neuroimaging findings are reviewed to hypothesize how they can be used in conjunction with one another. This may allow clinicians and scientists to comprehensively study TBI in military service members, leading to new treatment strategies for both currently-serving as well as veteran personnel, and to improve the study of TBI more broadly.

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1. Introduction

Interest in traumatic brain injury (TBI) has grown considerably both in the public sphere and among bioscientists over the past decade. Across the world, annually, between 64 and 74 million individuals suffer TBIs from all causes, with around 1.4 million cases being recorded in the U.S. alone [1]. TBI occurs when a force to the head results in temporary or permanent neurological dysfunction. The demographic affected consists predominantly of males, who are twice as likely as females to experience TBI-eliciting events [2]. Aside from primary injuries, TBIs also increase the victims' susceptibility to complications like post-traumatic stress disorder (PTSD), mild cognitive impairment, Alzheimer's disease (AD), Parkinson's disease, chronic traumatic encephalopathy (CTE), and other neurological and psychiatric conditions which can affect mood and fundamental cognitive processes like memory [3,4].

Cases of head injury are markedly prevalent in certain branches of military service, where the emergency care admission rate for TBI is 24.6–41.8% per 10,000 soldier-years, with the hospitalization rate showing an upward trend during deployment [5]. According to U.S. Department of Defense (DoD) estimates, by 2010, there had been 379,519 cases of TBI with varying degrees of severity [6]. Among modern military service members, chronic exposure to repetitive firing of advanced weapon systems near the head is a frequent cause of sub-concussive forces. More acute sources of injury, such as from explosive devices, have lesser incidence but vary more widely on the scale of TBI severity – from mild to severe to fatal. Out of 7–15% of all reported cases, symptoms of concussion can persist for a year after injury, and such victims are frequently diagnosed with post-concussion syndrome (PCS) [7–9], whose effects can be neurological, affective as well as cognitive [10]. Amongst the subclasses of TBI, mild traumatic brain injury (mTBI) is often the most difficult to diagnose, partly due to the potential lack of clinically-measurable outward signs of injury. This has made mTBI pathogenesis a proverbial “black box” where disease progression remains poorly-understood. This, in turn, has

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resulted in ineffective and/or primarily-palliative approaches to addressing the major neurological and cognitive deficits confronting military veterans, particularly as they age. With the advancement of modern neuroimaging techniques – including structural, functional, and connectomic modalities – assessment using brain imaging can frequently be well suited for discerning TBI pathology and for providing insights which can inform the prescription of effective interventions at the most appropriate time points post-injury.

This survey aims to highlight how the application of neuroimaging examination, when coupled with long-term strategies for pharmaceutical interventions, can be leveraged by clinicians and researchers to better understand the progression and treatment of TBI in both active as well as aging, former military personnel. While a compendium surveying the entirety of current TBI research is beyond the scope of this overview, we seek here to feature recent trends in the field and to propose how the insight which they provide could be used in conjunction with available methods for biomarker characterization. Along with battlefield and clinical assessment strategies, recommendations for the additional integration of neuroimaging and pharmacological interventions are provided.

On the broad spectrum of TBIs, concussions are an increasing medically-critical manifestation of this condition. While they do not explicitly exhibit neurological symptoms, these events may still bring about notable psychiatric and neuropsychological deficits. This has been observed especially in instances of military personnel, where 82.4% of reported TBI cases are reported as concussions. The use of improvised explosive devices during the Iraq and Afghanistan Wars resulted in more concussions as compared to earlier military conflicts like the Vietnam War [11]. Moreover, soldiers are as likely to suffer concussions in the line of duty as during training or in the course of military exercises, which is further indicative of military personnel's high susceptibility to this condition. The legendary “toughen up and move forward” sociology in some military circles discourages the reporting these injuries. The repercussions of this can be extremely high because repeat concussions can manifest themselves through serious cognitive or neurological symptoms [11,12]. The potentially-impactful nature of mTBI, both on and off the battlefield, makes its timely identification imperative. When safe to do so, neuroimaging may play an important role in the assessment of brain injury in potentially affected military personnel.

1.1. Neuroimaging identification of neural dysfunction

Beyond computed tomography (CT), magnetic resonance imaging (MRI) has proven to be particularly effective in identifying mTBI-compromised brain regions. Additionally, diffusion imaging of white matter (WM) fiber pathways in blast-exposed military veterans who do not show outwardly-visible symptoms of injury sequelae has proven to be an insightful diagnostic tool [13]. Aside from structural connectivity changes, studies also point towards functional brain network alterations brought about by concussive injuries. Using resting state functional magnetic resonance imaging (fMRI), findings by Nathan and colleagues [14] highlight alterations in the default mode network (DMN) of military personnel victims, where WM connectivity innervating the anterior cingulate cortex (ACC) is found to be higher in mTBI patients. Since functions like executive control and auditory processing have been associated with ACC, changes in its connectivity could be correlated to cognitive alterations experienced by patients [15,16]. Conversely, posterior cingulate cortex (PCC)—along with other posterior brain structures like the cuneus and the calcarine fissure—tends to exhibit reduced functional connectivity within the DMN [17]. This bilateral relationship is also observed in other conditions like

epilepsy, with decreased connectivity in medial prefrontal cortex (PFC) and temporal lobe and increased connectivity in patients' PCC [18]. This highlights the dynamic nature of the DMN and suggests how changes in one region may elicit compensatory responses in other regions to achieve homeostasis of neural information exchange [19]. Together with ACC, the PFC plays a critical role in cognitive control [20]. Witt and colleagues [21], using an auditory oddball task, have demonstrated that the right dorsolateral PFC (DLPFC) of mTBI patients exhibits decreased blood oxygenation level dependent (BOLD) effects in the resting state. Similar results have been observed by Lipton et al. [22], who found that reduced fractional anisotropy (FA) of WM streamlines innervating this structure significantly correlates with unfavorable executive function performance in mTBI patients.

The PFC also serves as part of the pain modulation system and, in a sample of military veterans, aberrations in PFC connectivity have been associated with the dysfunction of the endogenous pain modulating system. This was observed in an fMRI study by Strigo and colleagues [23], where participants with a reported history of mTBI showed higher activation in the periaqueductal grey (PAG) matter, right DLPFC and cuneus during pain anticipation. While higher activation of the DLPFC might seem at odds with decreased activity observed under relatively-high cognitive control, Gosselin and colleagues [24] demonstrated that DLPFC shows variable activation levels in working memory vs. pain anticipation tasks. This suggests that connectivity changes in different regions due to pain rather than to cognitive responses cannot be generalized. Nevertheless, in TBI victims, the likelihood of partial overlap between these functional networks cannot be discounted.

1.2. Cognitive disturbances resulting from TBI

Concurrently with cognitive symptoms, chronic TBI presents other mental health comorbidities in military veterans, including affective dysregulation. Damage to PAG matter—which consists of neurons located around the central aqueduct within the tegmentum of the midbrain—is believed to be one of the central contributors to social withdrawal, depressive episodes, inactivity, anxiety and even panic attacks [25]. This can be traced to PAG matter receiving projections from the central nucleus of the amygdala and to dorsal PAG matter relaying stimuli information to the basolateral amygdalar complex, which directs both innate and learned fear responses [26,27]. A factor contributing to emotional dysregulation may involve reduced FA in the midbrain regions of TBI patients relative to uninjured volunteers, as shown by Newcombe and colleagues [28], and suggesting potential changes in PAG matter volume and/or connectivity.

A commonly-experienced symptom of mTBI involves the disturbance of sleeping patterns. Cross-sectional studies of military personnel—predominantly men—within the first few months after returning from combat report that an overwhelmingly-high number of such individuals are diagnosed with sleep disorders [29]. It has also been proposed that the incidence of insomnia increases significantly with the increment in the number of TBIs experienced [30]. Although these inferences are based on self-reported information obtained during clinical interviews, the study of sleep neurophysiology using radioisotope tracers has hinted that mTBI-exposed individuals can exhibit reduced cerebral glucose metabolism relative to the general population. In one such study, Stocker and colleagues [31] used [^{18}F]-fluorodeoxyglucose (FDG) positron emission tomography (PET) to compare wakefulness, rapid eye movement (REM) sleep and non-REM sleep between blast-exposed and otherwise-healthy military veterans. Their findings show significant decreases in the relative cerebral metabolic rate of glucose (CMRglu) during wakefulness and during REM sleep in blast-exposed veterans vs. control participants. Other factors

affecting the sleep-wake cycle include low levels of hypocretin-1 (orexin A) in cerebral spinal fluid (CSF) samples collected from acute TBI patients [32]. Hypocretin-1 is a wakefulness-promoting neuropeptide produced by hypocretin neurons in the posterolateral nucleus of the hypothalamus (PH). The downregulation of hypocretin-1 in many TBI cases is not permanent and the level of this neuropeptide typically recovers within 6 months after injury. In some patients, however, such recovery may be suboptimal and can additionally result in post-traumatic sleepiness—also known as hypersomnia—defined as sleeping significantly more than prior to TBI [33]. In conjunction with PH neurons, cholinergic and glutamatergic neurons in the basal forebrain (BF) are also responsible for regulating the waking cycle, as well as other neurocognitive functions like attention, learning and memory [34,35]. This role of the BF in wakefulness has also been demonstrated in rats, thereby highlighting the influence of the BF on the ACC [36]. Neuroimaging studies of TBI patients have indicated that injury to the BF, hippocampus, and to neocortical regions result in globally reduced grey matter (GM), which may cause fatigue and hypersomnia through damage to the reticular activation system [37,38]. As such symptoms persist, their cumulative effects can give rise to complex neuropsychological abnormalities.

1.3. Cellular dysfunction

Neurological and cognitive impairments may be elicited by diffuse axonal injury (DAI), which has been a key topic of discussion in the clinical and scientific communities due to insufficient understanding of its effects [39]. DAI is induced by the sheer physical force exerted upon neurons during TBI, resulting in the increased permeability of their axolemmas [40]. This change in permeability can be demonstrated using horseradish peroxidase as a tracer of axonal processes [41]. Increased axolemma permeability triggers abnormal influx of Ca^{2+} ions which activate cysteine pathways that degrade the intra-axonal cytoskeletal framework [42]. Contrary to this, however, Wolf and colleagues [43] did not find evidence for increased permeability—but rather dysregulation—in Ca^{2+} membrane transport pathways, where Na^+ channel activation results in axonal depolarization and in the subsequent opening of $\text{Na}^+/\text{Ca}^{2+}$ channels. While these studies offer complementary—although potentially contradictory—perspectives on the increase of Ca^{2+} uptake, they both appear to suggest a strong correlation between increased Ca^{2+} influx and the imminence of neuronal breakdown. Ca^{2+} influx can modify sub-axolemmal signaling networks via early induction of calpain-mediated spectrin proteolysis or may result in mitochondrial permeabilization through the

activation of mitochondrial membrane permeability transition (MPT) pores. This causes increased water intake, reactive oxygen species (ROSs) production and the trans-membrane migration of molecules which contribute to mitochondrial swelling [44,45]. Simultaneously, Ca^{2+} can activate calcineurin, resulting in the alteration of neurofilament phosphorylation followed by neurofilament compaction (NFC). NFC, in particular, has long been associated with TBI and can serve as a precursor to the breakdown of axonal transport, to subsequent cytoskeletal collapse and to the release of amyloid precursor proteins (APPs) in the parenchyma [46].

The potentially wide-ranging effects of DAI make its early detection imperative. Huang and colleagues [47] found that the joint use of magnetoencephalography (MEG) and diffusion tensor imaging (DTI) can be very useful for studying the functional correlates of DAI [48]. Acutely, shear-related injuries in specific brain regions can be followed by increased blood flow due to rising metabolic demands and may also represent intact vaso-reactivity [49]. Whereas increased cerebral blood flow (CBF) might represent a neuroprotective measure, persistent CBF increases can result in intracranial hypertension (IH). This can lead to intracranial haematomas, which occur in 5–10% of patients with moderate TBI and in 25–35% patients with severe TBI.

1.4. Intracranial pressure and cerebral blood flow

Unregulated intracranial pressure (ICP) can contribute to brain tissue displacement due to pressure gradients across the brain, leading to intraventricular hemorrhage (bleeding into the ventricular system), may lead to acute obstructive hydrocephalus, and subsequent cell death. Decompressive craniotomies, barbiturates and extreme hyperventilation are commonly-utilized procedures to remediate elevated ICP, although these interventions have poor efficacy (Table 1) [50,51]. Where these strategies have been used, studies have found that indomethacin, a non-steroidal anti-inflammatory agent, may reduce CBF substantially [52]. Indomethacin's other benefits include the prevention of edema formation and the inhibition of CSF formation, although not without side effects [53,54]. The latter impact 10–50% of patients, are dose-dependent and include dizziness, vertigo, fatigue and impaired renal function, although such symptoms are transient and predominantly affect patients with impaired renal function [55]. Hypertonic saline (HTS) is also used for reducing ICP and, in both humans and animal models, has been shown to reduce elevated ICP and cerebral edema, thereby improving cerebral hemodynamics [56,57]. HTS administration *in vitro* has been associated with attenuated astrogliosis post-injury, such that astrocytes may even

Table 1
Pharmacological agents used to treat TBI, their provider, and their potential side-effects.

Condition	Drug	Manufacturer	Side effects
Elevated ICP	Indomethacin	Merck & Co.	Dizziness, fatigue, vertigo, negative renal function (Harrigan, Tuteja et al. 1997)
Insomnia	Benzodiazepine	Roche	Dizziness, nausea, headache, non-verbal visual memory impairment (Barker, Greenwood et al. 2004)
	Prazosin	Pfizer	Dizziness, weakness, nausea (Koola, Varghese et al. 2014)
Hypersomnia PTE	Modafinil	Teva Pharmaceutical	Headache, nausea [166]
	Phenytoin	Pfizer	Poor cognitive recovery (Bhullar, Johnson et al. 2014)
Emotional dysregulation	Fluoxetine	Eli Lilly	Delusions, aggression and suicidal ideation [167]
	Methylphenidate	Novartis	Insomnia, decreased appetite, headache [168]

Table 1 Stages of brain injury in military personnel and pharmacological interventions thought to be effective against TBI-related comorbidities. Outlined are the benefits of current interventions, their side effects and how TBI polypharmacy, particularly at high dosages, may prevent the recovery of cognitive capacities.

exert neuroprotective effects to achieve substantial reduction in tissue loss [58]. Administering this agent between 30 and 60 min post-injury appears to elicit optimal neuroprotective effects [59,60].

Despite increased global CBF, brain regions affected by primary TBI frequently experience reduced blood flow leading to cellular organelle damage, including mitochondrial damage [61]. These modifications to the cellular machinery can alter the metabolic state of the neuron from aerobic to anaerobic, resulting in an uptick of reactive oxygen species (ROS) production [62]. ROSs—or free radicals—oxidize the cellular membrane through different pathways, including the lipoxygenase pathway, to create inflammatory precursors while also contributing to a reduction in the pH of the brain known as acidosis [63]. Changes in pH levels and subsequent acidosis can be measured via cerebral microdialysis, in which a probe is inserted into the brain at injury locations [64]. However, emerging contrast-enhanced methods such as CEST MRI, initially applied in mapping pH-related change in cancer patients [65], are providing means for non-invasive imaging of pH in TBI patients [65,66].

Early-stage clinical interventions like normobaric and hyperbaric hyperoxia can counter sudden loss in regional CBF while meeting increased global CBF demands. This impact is brought about by increases in adenosine triphosphate (ATP) synthesis and in the cerebral metabolic rate of oxygen, or CMRO₂ [67]. Increasing cerebral oxygen levels can, in turn, result in a global increase in aerobic metabolism and in a deceleration of ROS production, although there is a fixed window for this intervention to have a neuroprotective effect. In a cerebral microdialysis study by Magnoni and colleagues [68], the induction of hyperoxia post-injury resulted in a 40% reduction of lactate (Lac) levels in the cerebral extracellular fluid (CEF). Increases in Lac levels are generally accompanied by increases in the lactate/pyruvate ratio (LPR) in the CEF, as frequently observed under conditions of hypoxia or mitochondrial dysfunction [69,70]. This increase in Lac is also thought to be one of the early markers of brain injury [71] and can be measured using magnetic resonance spectroscopy (MRS) [72]. This allows Lac levels to be utilized as a metrics to gauge the efficacy of early-stage interventions post-injury.

1.5. Inflammation

Following brain injury, inflammation is one of the first physiological responses to limit the impact of neural damage. The immune system deploys leukocytes which attempt to contribute to the restoration of homeostasis by killing damaged cells, facilitating CBF increases and reducing infection severity [73]. These leukocytes exert their impact via a class of molecules known as leukotrienes. Leukotrienes upregulation after TBI is brought about by the binding of arachidonic acid to 5-lipoxygenase binding protein and then by the interaction of this complex with 5-lipoxygenase to produce leukotriene A₄ (LTA₄) [74]. LTA₄ further interacts with various synthases and hydrolases to produce other leukotrienes which contribute to cerebral edema [75]. An MRI modality called fluid-attenuated inversion recovery (FLAIR) allows imaging of such edema, which appears hyper-intense in FLAIR images [76]. Brain injury also causes cells to release danger-associated molecular patterns (DAMPs) such as ribonucleic acid (RNA), deoxyribonucleic acid (DNA) and heat shock proteins (HSPs) to sustain non-infectious inflammatory responses [77]. Some DAMPs bind to toll-like receptors, activating pathways for nuclear-factor κ B (NF κ B) and mitogen-activated protein kinase (MAPK) to release Interleukin-1 β (IL-1 β), Interleukin-6 and other chemokines [78]. The levels of these cytokines are higher in the early stages of injury—early as within a few minutes

post-trauma—and typically subside over time [79]. This phenomenon, termed a *cytokine storm*, is specifically observed in CSF and cannot typically be traced to blood samples. A cytokine storm in the brain's blood stream has both beneficial and detrimental effects, bringing about positive changes like astrogliosis stimulation and neurovascularization, but also negative ones like axonal dysfunction [80]. This dichotomous response to injury is also exhibited by other immune cells and neurotransmitters, as in the case of microglia.

In the healthy brain, microglia serve as highly-active cells scanning the central nervous system (CNS) for various signals which are indicative of homeostasis disruption [81]. From the early stages of brain development, these cells play a critical role in regulating cell death, contributing to neuronal plasticity and refining neuronal circuitry [82]. Such behavior assists in clearing out accumulated metabolites and cellular debris while maintaining normal phenotypic function [83]. During embryonic stages, cerebral microglial formation precedes blood brain barrier (BBB) endothelial cell formation. Certain studies suggests that these macrophages may also play a role in imparting tight junction properties to the cerebral vasculature [84]. This is critical, as any compromise in the BBB can result in the influx of harmful metabolites, especially after TBI. This phenomenon was shown in a study by Sumi and colleagues [85] where microglial activation in microvascular endothelial tissue of the rat brain — when mediated by lipo-poly saccharides—leads to BBB dysfunction by activating NADPH oxidase — the reduced form of nicotinamide adenine dinucleotide phosphate (NADP)—which results in the production of ROSs or superoxides (O₂⁻) to increase BBB permeability [85]. This allows the discharge of plasma proteins, like fibrinogen, into the brain. Fibrinogen deposition promotes further neuroinflammation via the activation of the tumor growth factor- β (TGF- β) pathway [86,87]. Cumulatively, fibrinogen deposition in the brain's vascular space and the permeabilization of the BBB makes the brain more susceptible to neurological diseases like AD.

Another manner in which microglia have been shown to respond to injury is via macrophage polarization, where the phenotype and functions of the latter are altered via two groups of mechanisms referred to M1-like and M2-like polarization, respectively [88]. Activation of M1-like (classical) microglia generally has a protective effect upon the injured brain, and their response to injury is downregulated once damage has been contained. In some cases, however, M1-like microglia may become unregulated, which brings about neurotoxicity and neurodegeneration via the uncontrolled release of pro-inflammatory factors such as IL-1 β , IL-12 and ROSs [89]. In military veterans, a higher concentration of these inflammatory markers is observed in patients with PTSD compared to PTSD-free subjects, and there is a correlation between neuroinflammation, on the one hand, and chronic behavioral and affective symptoms, on the other hand [90]. Maladaptive microglial activations may last for years after injury and their impact can be compounded through thinning of the corpus callosum [89,91,92].

M1-like macrophages upregulate chondroitin sulphate proteoglycans, which act as axonal regrowth inhibitors [93]. Whereas most research on M1-like, M2-like microglia and macrophages involves focal cortical injury models, the activity of macrophages observed in diffuse injuries exhibits characteristics which are like those of M1-like macrophages. These similarities involve the elevation of IL-1 β and TNF-alpha levels as early as 4 h post-injury and a return to baseline within 72 h [94]. By contrast, microglial activation in the presence of IL-4 also elicits neurogenesis in adult stem cells by recruiting M2-like microglia [95]. Similar neuronal proliferation is mediated by downstream effects of activated mitogen-activated protein kinases (MAPKs) and of other neurotrophic factors via microglia-released activators [96].

1.6. Post-traumatic neuropathophysiology

Military personnel's protective equipment may protect them from flesh wounds but does not eliminate the likelihood of TBI and soldiers' subsequent susceptibility to post-traumatic epilepsy (PTE). Injury severity modulates the extent of disruptions in neurotransmitter and neurometabolite pathways, leading to PTE which is partially due to the disruption of γ -aminobutyric acid (GABA) signaling pathways [97]. GABA serves as the main inhibitory neurotransmitter of the CNS and via the activation of GABA_A receptors. Aberrations in GABAergic signaling can result in epileptogenesis, which may chronically transform into PTE [98]. A head injury study in Vietnam War veterans showed that 53% of enrolled veterans who had suffered penetrating head injuries had at least one seizure, and that, in 50% of veterans, seizures occurred in the first year post-injury [99]. PTE seizures differ from those observed in non-traumatic epilepsy because they vary in symptoms and mortality [100]. PTE can be classified into immediate, early, and late, based on the time lag between the primary injury and the incidence of the first seizure. In each of the three categories, seizures may constitute a single episode within (A) the first 24 h, (B) the first week or (C) the period after the first week post-injury, respectively [101]. The likelihood that patients fall into any of these categories is dependent on the severity of the injury. The relative risk of epilepsy increases by factors of 2.22 and 7.40 after mild vs. severe injury, respectively [102]. Furthermore, the risk of seizures is found to be highest immediately after TBI and may remain high for more than ten years after injury.

To treat PTE, clinicians employ a range of pharmacological interventions. Seizure medications like magnesium, valproate, carbamazepine and phenytoin (PHT) are most commonly administered. Amongst these, phenytoin (PHT) is among the most commonly-used prophylactic agents and is typically administered within the first seven days after injury [103]. When compared to placebo, PHT has been shown to reduce early PTE seizure incidence from 14.2% to 3.6% – although this result should not be interpreted as implying that PHT can reduce PTE risk [104,105]. The risk in question is modulated by changes in brain neurochemistry over several weeks, by effective dosage and interaction between drugs administered acutely. Despite its effectiveness in reducing early-stage post-traumatic seizures, PHT elicits multiple side effects, which arise from its pharmacokinetic interactions with other drugs during the first few days after injury. Studies have shown that, in some forms of brain injury, PHT administration may have a negative impact upon cognitive recovery. One such study by Bhullar and colleagues [106] found that PHT prophylaxis results in lower functional outcome as measured using the Glasgow outcome scale (GOS) and the modified Rankin scale. Vespa and colleagues [107] showed that the incidence of seizures is closely linked to ICP increase, and proposed ICP as a metric for monitoring the effects of therapeutic interventions.

Modern neuroimaging allows the study of PTE both at the macro- and at the micro-scale. Epileptic seizures can be measured using electroencephalography (EEG), MEG, fMRI, as well as simultaneous EEG/fMRI [108]. Aberrant electrical rhythms due to interictal brain activity can be localized using EEG and MEG to identify epileptogenic foci [109]. At the molecular scale, GABA signaling cannot be directly measured due to its intercoupling with glutamate (Glu) pathways, but its downstream products can be measured to identify disruptions in its metabolism. This can be accomplished using a spectroscopy method termed MEGA-PRESS [110], which combines MEGA (a frequency-selective editing technique) and PRESS (point-resolved spectroscopy sequence). GABA_A can also be imaged using PET tracers like [¹¹C]-Flumazenil or [¹¹C]-Ro 15-4513, which is a partial inverse agonist of the benzodiazepine receptor [110,111]. Furthermore, MRS can also be utilized

to measure relative changes in GABA levels compared to N-acetyl aspartate (NAA) or to other neurometabolites [112].

Glutamate (Glu) is an important excitatory neurotransmitter which suffers from disruption of brain biochemistry following TBI [113]. Glu activates different classes of receptors such as AMPA, NMDA and metabotropic Glu receptors, leading to alterations in cognitive function, including learning and memory. Following TBI, there may be abnormal O₂/ATP metabolism causing Glu imbalance and the malfunction of Glu reuptake transporters. Neuroimaging methods like ¹H MRS, along with cerebral microdialysis, can be used to measure dwindling Glu levels, although microdialysis offers relatively-poor specificity compared to MRS [114]. Other neurotransmitters like acetylcholine (ACh) and nitric oxide (NO) serve as signal-relaying and vasodilating neurotransmitters under normal physiological circumstances, while also playing a role in sleep regulation to bring about REM sleep and REM-related changes in regional blood flow [115]. The vasodilatory effect of NO and, by association, changes in its levels can be measured using fMRI, whereas ACh levels can be imaged using 123I-3-[2(S)-2-azetidinyloxy]pyridine (¹²³I-5-IA) single-photon emission computed tomography (SPECT) [116,117].

Neurotransmitter signaling pathways may be markedly damaged in military veterans who have suffered TBI. For this reason, clinicians routinely prescribe benzodiazepines (BDZs), prazosin and/or cognitive behavioral therapy (CBT) in such patients to partially relieve symptoms of PTSD-related sleep disruption. BDZs act by non-selective activation of BDZ-subtype receptors in the GABA receptor complex. These drugs are very useful for reducing sleep latency but tend to have side-effects like dizziness, nausea, headache, as well as visual memory impairment [118]. In some patients, side effects are reversible post-treatment secession, whereas in others they may not [119]. These side effects and other cognitive deficits can often be successfully alleviated via administration of pregabalin, which has been suggested as a viable option in the treatment of patients who have been administered BDZs for more than a decade [120].

Prazosin, on the other hand, is a pharmacological agent which acts as an α -1 receptor antagonist. This drug has been found to be effective in military veterans with mTBI, where its administration can relieve trauma, reduce nightmare frequency and improve sleep quality [121]. The effects of prazosin extend to other disorders beyond insomnia, and may assist with alleviating daytime PTSD symptoms even at high daily doses like 45 mg, although potentially with side effects [122,123]. Thus, current TBI treatment may involve a wide array of narcotic analgesics, antidepressants, anti-convulsants and other psychotropic medications but not without side effects [124]. For this reason, tailoring pharmacological treatments to different patients may be difficult without underlying neuroimaging evidence, as well as the availability of key biomarkers which can accompany the alleviation of side effects.

1.7. Blood biomarkers

TBI disrupts the pharmacological homeostasis of the brain, resulting in dysregulation of biochemical compounds which can be detected in CSF and blood serum. Tracking these biomolecules could, thus, complement other prognostic tools to better gauge injury extent, but also to offer clinicians more options for identifying biochemical pathways whose disruption can have substantially-deleterious downstream effects. Common blood biomarkers which have been proposed to detect and gauge mTBI severity include serum ubiquitin C-terminal hydrolase L1 (UCH-L1), glial fibrillary acidic protein (GFAP) and monocyte protein-1 (MCP-1) [125–127]. UCH-L1 is a relatively promising biomarker since it is a protein whose presence and activity are specific to the brain, where it comprises as much as 1%–5% of soluble brain

proteins [128]. UCH-L1 shows high serum concentrations in TBI patients compared to normal control subjects, indicating its value as a potential biomarker of brain damage [129]. A dangerous increase in UCH-L1 levels has also been implicated as a risk factor for serious brain damage, including intracerebral hemorrhage [129]. The normal function of UCH-L1 is currently enigmatic, with researchers linking it to proteasomal, lysosomal or to ubiquitination functions in the cell [130]. No definitive link to a specific mechanistic pathway has been established, and often times UCH-L1 deficient mice do not exhibit a detectable lack of neural function [131]. Despite the uncertainty surrounding its mechanistic pathway, the role of UCH-L1 in maintaining axonal health and stability has been well documented [132]. For example, UCH-L1-deficient mice show marked impairment of synaptic transmission at the neuromuscular junction [133]. Due to its critical role in neuronal integrity, abnormal concentrations of UCH-L1 in blood serum could suggest several pathways through which TBI impacts the brain [134].

One such pathway involves the correlation of UCH-L1 with abnormal blood brain barrier (BBB) integrity as observed in patients with TBI or with non-traumatic headaches [135]. BBB function after TBI can be assessed using the CSF-serum albumin quotient (Q_A), where higher Q_A indicates decreased BBB integrity [136]. Studies indicate that Q_A and UCH-L1 are significantly elevated in TBI compared to headache patients, especially at the 12-hour mark after injury; Q_A and UCH-L1 levels are also strongly correlated. Furthermore, elevated serum levels of S100 calcium-binding protein B (S100B) are related to levels of Q_A , suggesting BBB damage [137] (Table 2).

An important serum biomarker thought to be an indicator of TBI is GFAP [138]. GFAP is a key component of glial filaments in differentiated astrocytes of the CNS [139]. It is responsible for the stable formation of astrocytic processes in response to neurons and hence imparts astrocytes their structural phenotype [140]. Given its role in astrocytic structural organization, changes in the serum levels of GFAP could be an indicator of astrocytic damage extent brought about by TBI. In samples collected from trauma patients over a period of three days after injury, serum GFAP levels showed trends like those of UCH-L1 [141]. Levels of serum GFAP, S100B, and neuron-specific enolase are elevated in TBI compared to non-TBI patients; the serum GFAP level one day after injury appears to have highest sensitivity and specificity for TBI [142].

Although biomarkers can assist in discriminating non-traumatic head injury from TBI by themselves, these compounds are typically more robust when used together, and one blood test measuring the serum levels of GFAP and UCH-L1 has in fact been approved by the

Food and Drug Administration (FDA) [143]. Due to the diffusion of injury-relevant proteins in the blood within the first few hours post-injury, these tests can be administered in circumstances where CT/MRI are not readily available and/or to reduce the number of diagnostic steps required to quickly ascertain the extent of brain damage. Such diagnostic measures can thus be useful in military field hospital situations, especially when there is no sterile environment or appropriate tools to extract CSF.

Proteins like tau are particularly useful for diagnostic purposes because not only can their levels be measured, but their spatial distribution can also be imaged [144–146]. In healthy individuals, tau proteins form a part of the microtubular architecture. In eukaryotic cells, microtubules carry out the process of transporting membrane organelles bi-directionally, thereby promoting fast axonal transport. Along with this, microtubules also contribute to the maintenance of cellular infrastructure. Microtubules are abundant in neurons, where inter-axonal, intra-axonal and dendritic microtubules link to provide cytoskeletal shape and stability. The tau protein is one of several microtubule-associated proteins (MAPs) which serve this function [147]. Microtubules degrade in degenerative diseases like Alzheimer's Disease [148].

In TBI, WM fibers are frequent sheared or otherwise damaged, which slowly results in a breakdown of neuronal structure, accompanied by the cleavage of tau proteins from microtubules and by their transportation into CSF. In samples extracted from head trauma patients' CSF about 3–8 h after post-injury resuscitation, cleaved-tau (C-tau) levels are found to be elevated by a factor in excess of 1000 compared to control subjects [149]. Furthermore, hypo-phosphorylated tau protein (P-tau), total tau levels and the P-tau-total-tau ratios in plasma samples of acute and chronic TBI patients are typically higher than in healthy control subjects [150]. There is also evidence of elevated peripheral blood tau levels in military personnel with a deployment history, where tau levels have been significantly associated with their TBI-related outcome prognosis [151]. In addition to being a promising indicator of TBI, tau protein levels also provide insight into how long a patient might take to recover for active duty. Results from studies of acute blast exposure suggest transient changes in cell membrane integrity in multiple organs leading to abnormal migration of proteins from the tissues to the plasma and vice versa, possibly contributing to the pathophysiology of TBI and polytrauma after blast exposure [152]. Moreover, elevated exosomal tau and p-tau correlate with post-traumatic and post-concussive symptoms, with exosomal tau also relating specifically to cognitive, affective, and somatic post-concussive symptoms [153]. It might be surmised that these metrics could be used in conjunction with conventional

Table 2
Blood biomarkers and neuroimaging modalities.

Sequela	Biomarker	Imaging modality	References
Wakefulness/insomnia	Glucose	[¹⁸ F]-FDG-PET	Stocker, Cieply et al. 2014
Neuronal integrity	NAA	MRS	Gujar, Maheshwari et al. 2005
Oxidative stress	pH	Cerebral microdialysis	Landolt, Langemann et al. 1994
Hypoxia	Lac	MRS cerebral microdialysis	Makoroff, Cecil et al. 2005 Magnoni, Ghisoni et al. 2003
Edema	LTA4	FLAIR	Irimia, Chambers et al. 2011
PTE/seizures	GABA	MEGA-PRESS [¹ C] flumazenil/[¹ C] Ro 15-4513-PET	Edden and Barker 2007; Edden and Barker 2007, Asahina, Shiga et al. 2008
Memory impairment	Glu	MRS	Benveniste, Drejer et al. 1984
Sleep dysregulation	NO	fMRI/DWI	Attwell and Iadecola 2002
	Ach	¹²³ I-5-IA SPECT	Esterlis, Hannestad et al. 2013
Microtubule disintegration	tau	[¹⁸ F] AV-1451-PET	Maruyama, Shimada et al. 2013; Dickstein, Pullman et al. 2016

Table 2 Blood biomarkers which can be imaged *in vivo*. Some measures require the use of imaging tracers to isolate their location and concentration. The molecules listed typically have a downstream neurological impact and imaging can be useful for the formulation of adequate therapeutic interventions.

neuroimaging methods, where specific radioisotopes used as tracers allow one to visualize the extent of tau accumulation. For example, [^{18}F]AV-1451, [^{18}F]THK5351 and [^{11}C]Pittsburgh compound-B are commonly-used PET ligands in clinical settings, with [^{18}F]AV-1451-PET being particularly useful for visualizing tau accumulation in CTE patients [154,155].

2. Conclusion

TBI is a severe condition having causes ranging from repetitive sub-concussive and/or object-related impact to the brain to blast-induced forces – these events are not uncommon for active duty military personnel during combat and/or tour duties. In particular, the current body of research suggests that military veterans notably experience emotional dysregulation, abnormal pain perception and insomnia, along with a variety of cognitive symptoms subsequent to TBI. A culmination of these symptoms can accelerate psychiatric disease evolution and, for instance, may result in a high likelihood of suicide attempts [156]. Suffering individuals often develop substance dependencies, including alcohol consumption, and studies have shown an association between TBI-induced loss of consciousness and a four-fold increase in negative, drinking-related daily-life outcomes [157]. The consequences of TBI can be debilitating over the sufferer's lifetime.

While the U.S. Department of Veterans Affairs does not currently recommend using neuroimaging, blood biomarkers, or EEG for the assessment and/or diagnosis of mTBI, a growing and quantifiable body of research results provides mixed evidence in favor of using such assessments for TBI assessment and characterization. Based on current knowledge, it appears that a combination of multimodal neuroimaging techniques and serum biomarkers could offer a more systematic picture of the existence and progression of TBI in military service members. Task-based fMRI methodologies and analyses may be important for the long-term monitoring of TBI, its prognosis, as well as efficacy of prescribed drugs. Additionally, the accessibility of various radioactive tracers for use with PET imaging may further enable precise tracking of specific biomarkers which can be measured and manipulated to improve functional outcomes.

Injury prognosis might also benefit from neuroimaging measurement of the glymphatic system and exploring how it is affected during these neuronal homeostatic disruptions [158]. Responsible for clearing the harmful metabolites, this system could play an important role in maintaining neuronal health during acute stages of the injury and could also serve as a potential drug target to minimize further damage [159]. Radiolabeled tracers using PET could provide opportunities to understand these systems via labeling receptors like Aquaporin-4 [160] that show selective permeability to water across the perivascular space [161].

Because pharmacological treatments and other therapies have been suggested for palliative care [162,163], more research should be dedicated to (A) identifying neurological changes which take place in the human brain post-injury and (B) determining how palliative solutions affect the brain's neurochemistry, if at all. Because symptom amelioration alone is likely to be insufficient for the full treatment of TBI in military service members, prescribed medications should be carefully researched against neuroimaging evidence on their effects on brain structure, function, and connectomics to inform the recovery process.

Finally, closer collaborative efforts between military-affiliated and academic research institutions should be undertaken using neuroimaging methods to understand how brain injuries progress, the role of blood biomarkers, and how best to attend to the needs of active and former military service members [164,165]. Doing so will necessitate greater sharing of data, consideration of how study

participation might affect service member VA benefits, accounting for variables unique to each military experience such as duration of a tour, length of active combat scenarios, etc. However, greater cooperation between military service agency and university researchers is the likely best path forward in the identification of blood and neuroimaging biomarkers having the greatest predictive power concerning clinical outcome and recovery from TBI in active and former military service members.

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Author contributions

A.B. performed the detailed review of the literature and undertook the writing of the manuscript; J.D.V.H. conceived of the rationale for the article and worked to provide content, to edit, and to refine the manuscript; A.I. provided critical comments, recommendations, and editorial contributions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jocn.2019.07.001>.

References

- [1] Dewan MC, Rattani A, Gupta S, Baticulon RE, Hung YC, Punchak M, et al. Estimating the global incidence of traumatic brain injury. *J Neurosurg*. 2018;1–18.
- [2] Langlois JA, Rutland-Brown W, Wald MM. The epidemiology and impact of traumatic brain injury: a brief overview. *J Head Trauma Rehabil*. 2006;21(5):375–8.
- [3] Kotapka MJ, Graham DI, Adams JH, Gennarelli TA. Hippocampal pathology in fatal non-missile human head injury. *Acta Neuropathol*. 1992;83(5):530–4.
- [4] Mortimer JA, French LR, Hutton JT, Schuman LM. Head injury as a risk factor for Alzheimer's disease. *Neurology*. 1985;35(2):264–7.
- [5] Wojcik BE, Stein CR, Bagg K, Humphrey RJ, Orosco J. Traumatic brain injury hospitalizations of U.S. army soldiers deployed to Afghanistan and Iraq. *Am J Prev Med* 2010;38(1 Suppl):S108–16.
- [6] CDC. Traumatic Brain Injury in the United States: Emergency Department Visits, Hospitalizations and Deaths 2002–2006 (Blue Book). 2010.
- [7] Borczuk P. Mild head trauma. *Emerg Med Clin North Am*. 1997;15(3):563–79.
- [8] Paniak C, Toller-Lobe G, Durand A, Nagy J. A randomized trial of two treatments for mild traumatic brain injury. *Brain Inj* 1998;12(12):1011–23.
- [9] Weight DG. Minor head trauma. *Psychiatr Clin North Am*. 1998;21(3):609–24.
- [10] Hall RC, Hall RC, Chapman MJ. Definition, diagnosis, and forensic implications of postconcussional syndrome. *Psychosomatics* 2005;46(3):195–202.
- [11] Helmick KM, Spells CA, Malik SZ, Davies CA, Marion DW, Hinds SR. Traumatic brain injury in the US military: epidemiology and key clinical and research programs. *Brain Imaging Behav* 2015;9(3):358–66.
- [12] McKee AC, Robinson ME. Military-related traumatic brain injury and neurodegeneration. *Alzheimers Dement* 2014;10(3 Suppl):S242–53.
- [13] Taber KH, Hurley RA, Haswell CC, Rowland JA, Hurt SD, Lamar CD, et al. White matter compromise in veterans exposed to primary blast forces. *J Head Trauma Rehabil*. 2015;30(1):E15–25.
- [14] Nathan DE, Bellgowan JAF, French LM, Wolf J, Oakes TR, Mielke J, et al. Assessing the impact of post-traumatic stress symptoms on the resting-state default mode network in a military chronic mild traumatic brain injury sample. *Brain Connect*. 2017;7(4):236–49.

- [15] Beckmann CF, DeLuca M, Devlin JT, Smith SM. Investigations into resting-state connectivity using independent component analysis. *Philos Trans R Soc Lond B Biol Sci* 2005;360(1457):1001–13.
- [16] Fujii DE, Ahmed I. Risk factors in psychosis secondary to traumatic brain injury. *J Neuropsychiatry Clin Neurosci* 2001;13(1):61–9.
- [17] Zhou Y, Milham MP, Lui YW, Miles L, Reaume J, Sodickson DK, et al. Default-mode network disruption in mild traumatic brain injury. *Radiology* 2012;265(3):882–92.
- [18] Zhang Z, Lu G, Zhong Y, Tan Q, Liao W, Wang Z, et al. Altered spontaneous neuronal activity of the default-mode network in mesial temporal lobe epilepsy. *Brain Res* 2010;1323:152–60.
- [19] Andrews-Hanna JR, Reidler JS, Sepulcre J, Poulin R, Buckner RL. Functional-anatomic fractionation of the brain's default network. *Neuron* 2010;65(4):550–62.
- [20] MacDonald 3rd AW, Cohen JD, Stenger VA, Carter CS. Dissociating the role of the dorsolateral prefrontal and anterior cingulate cortex in cognitive control. *Science* 2000;288(5472):1835–8.
- [21] Witt ST, Lovejoy DW, Pearson GD, Stevens MC. Decreased prefrontal cortex activity in mild traumatic brain injury during performance of an auditory oddball task. *Brain Imaging Behav* 2010;4(3):232–47.
- [22] Lipton ML, Gulko E, Zimmerman ME, Friedman BW, Kim M, Gellella E, et al. Diffusion-tensor imaging implicates prefrontal axonal injury in executive function impairment following very mild traumatic brain injury. *Radiology* 2009;252(3):816–24.
- [23] Strigo IA, Spadoni AD, Lohr J, Simmons AN. Too hard to control: compromised pain anticipation and modulation in mild traumatic brain injury. *Transl Psychiatry* 2014;4:e340.
- [24] Gosselin N, Chen JK, Bottari C, Petrides M, Jubault T, Tinawi S, et al. The influence of pain on cerebral functioning after mild traumatic brain injury. *J Neurotrauma* 2012;29(17):2625–34.
- [25] Price JL. Prefrontal cortical networks related to visceral function and mood. *Ann N Y Acad Sci* 1999;877:383–96.
- [26] Kim EJ, Horowitz O, Pellman BA, Tan LM, Li Q, Richter-Levin G, et al. Dorsal periaqueductal gray-amygdala pathway conveys both innate and learned fear responses in rats. *Proc Natl Acad Sci USA* 2013;110(36):14795–800.
- [27] Rizvi TA, Ennis M, Behbehani MM, Shipley MT. Connections between the central nucleus of the amygdala and the midbrain periaqueductal gray: topography and reciprocity. *J Comp Neurol* 1991;303(1):121–31.
- [28] Newcombe VF, Williams GB, Scoffings D, Cross J, Carpenter TA, Pickard JD, et al. Aetiological differences in neuroanatomy of the vegetative state: insights from diffusion tensor imaging and functional implications. *J Neurol Neurosurg Psychiatry* 2010;81(5):552–61.
- [29] Mysliwiec V, Gill J, Lee H, Baxter T, Pierce R, Barr TL, et al. Sleep disorders in US military personnel: a high rate of comorbid insomnia and obstructive sleep apnea. *Chest* 2013;144(2):549–57.
- [30] Bryan CJ. Repetitive traumatic brain injury (or concussion) increases severity of sleep disturbance among deployed military personnel. *Sleep* 2013;36(6):941–6.
- [31] Stocker RP, Cieply MA, Paul B, Khan H, Henry L, Kontos AP, et al. Combat-related blast exposure and traumatic brain injury influence brain glucose metabolism during REM sleep in military veterans. *Neuroimage* 2014;99:207–14.
- [32] Baumann CR, Stocker R, Imhof HG, Trentz O, Hersberger M, Mignot E, et al. Hypocretin-1 (orexin A) deficiency in acute traumatic brain injury. *Neurology* 2005;65(1):147.
- [33] Baumann CR. Traumatic brain injury and disturbed sleep and wakefulness. *Neuromolecular Med* 2012;14(3):205–12.
- [34] Anaclet C, Pedersen NP, Ferrari LL, Vennar A, Bass CE, Arrigoni E, et al. Basal forebrain control of wakefulness and cortical rhythms. *Nat Commun* 2015;6:8744.
- [35] Cape EG, Jones BE. Effects of glutamate agonist versus procaine microinjections into the basal forebrain cholinergic cell area upon gamma and theta EEG activity and sleep-wake state. *Eur J Neurosci* 2000;12(6):2166–84.
- [36] Nair J, Klaassen AL, Arato J, Vyssotski AL, Harvey M, Rainer G. Basal forebrain contributes to default mode network regulation. *Proc Natl Acad Sci USA* 2018;115(6):1352–7.
- [37] Jang SH, Kwon HG. Injury of the ascending reticular activating system in patients with fatigue and hypersomnia following mild traumatic brain injury: two case reports. *Medicine (Baltimore)* 2016;95(6):e2628.
- [38] Salmond CH, Chatfield DA, Menon DK, Pickard JD, Sahakian BJ. Cognitive sequelae of head injury: involvement of basal forebrain and associated structures. *Brain* 2005;128(Pt 1):189–200.
- [39] Irimia A, Wang B, Aylward SR, Prastawa MW, Pace DF, Gerig G, et al. Neuroimaging of structural pathology and connectomics in traumatic brain injury: toward personalized outcome prediction. *NeuroImage Clin* 2012;1(1):1–17.
- [40] Povlishock JT, Pettus EH. Traumatically induced axonal damage: evidence for enduring changes in axolemmal permeability with associated cytoskeletal change. *Acta Neurochir Suppl* 1996;66:81–6.
- [41] Pettus EH, Christman CW, Giebel ML, Povlishock JT. Traumatically induced altered membrane permeability: its relationship to traumatically induced reactive axonal change. *J Neurotrauma* 1994;11(5):507–22.
- [42] Buki A, Povlishock JT. All roads lead to disconnection?—Traumatic axonal injury revisited. *Acta Neurochir (Wien)* 2006;148(2):181–93. discussion 93–4.
- [43] Wolf JA, Stys PK, Lusardi T, Meaney D, Smith DH. Traumatic axonal injury induces calcium influx modulated by tetrodotoxin-sensitive sodium channels. *J Neurosci* 2001;21(6):1923–30.
- [44] Lemasters JJ, Nieminen AL, Qian T, Trost LC, Elmore SP, Nishimura Y, et al. The mitochondrial permeability transition in cell death: a common mechanism in necrosis, apoptosis and autophagy. *Biochim Biophys Acta*. 1998;1366(1–2):177–96.
- [45] Zoratti M, Szabo I. The mitochondrial permeability transition. *Biochim Biophys Acta* 1995;1241(2):139–76.
- [46] Okonkwo DO, Pettus EH, Moroi J, Povlishock JT. Alteration of the neurofilament sidearm and its relation to neurofilament compaction occurring with traumatic axonal injury. *Brain Res* 1998;784(1–2):1–6.
- [47] Huang MX, Theilmann RJ, Robb A, Angeles A, Nichols S, Drake A, et al. Integrated imaging approach with MEG and DTI to detect mild traumatic brain injury in military and civilian patients. *J Neurotrauma* 2009;26(8):1273–26.
- [48] Inglese M, Makani S, Johnson G, Cohen BA, Silver JA, Gonen O, et al. Diffuse axonal injury in mild traumatic brain injury: a diffusion tensor imaging study. *J Neurosurg*. 2005;103(2):298–303.
- [49] Kelly DF, Martin NA, Kordestani R, Counelis G, Hovda DA, Bergsneider M, et al. Cerebral blood flow as a predictor of outcome following traumatic brain injury. *J Neurosurg* 1997;86(4):633–41.
- [50] Maas AI, Stocchetti N, Bullock R. Moderate and severe traumatic brain injury in adults. *Lancet Neurol* 2008;7(8):728–41.
- [51] Meyer MJ, Megyesi J, Meythaler J, Murie-Fernandez M, Aubut JA, Foley N, et al. Acute management of acquired brain injury part II: an evidence-based review of pharmacological interventions. *Brain Inj* 2010;24(5):706–21.
- [52] Jensen K, Ohrstrom J, Cold GE, Astrup J. The effects of indomethacin on intracranial pressure, cerebral blood flow and cerebral metabolism in patients with severe head injury and intracranial hypertension. *Acta Neurochir (Wien)* 1991;108(3–4):116–21.
- [53] Schalk KA, Faraci FM, Heistad DD. Effect of endothelin on production of cerebrospinal fluid in rabbits. *Stroke* 1992;23(4):560–3.
- [54] Dempsey RJ, Roy MW, Meyer KL, Donaldson DL. Indomethacin-mediated improvement following middle cerebral artery occlusion in cats. Effects of anesthesia. *J Neurosurg* 1985;62(6):874–81.
- [55] Harrigan MR, Tuteja S, Neudeck BL. Indomethacin in the management of elevated intracranial pressure: a review. *J Neurotrauma* 1997;14(9):637–50.
- [56] Horn P, Munch E, Vajkoczy P, Herrmann P, Quintel M, Schilling L, et al. Hypertonic saline solution for control of elevated intracranial pressure in patients with exhausted response to mannitol and barbiturates. *Neurol Res* 1999;21(8):758–64.
- [57] Shackford SR, Bourguignon PR, Wald SL, Rogers FB, Osler TM, Clark DE. Hypertonic saline resuscitation of patients with head injury: a prospective, randomized clinical trial. *J Trauma* 1998;44(1):50–8.
- [58] Elliott MB, Jallo JJ, Barbe MF, Tuma RF. Hypertonic saline attenuates tissue loss and astrocyte hypertrophy in a model of traumatic brain injury. *Brain Res* 2009;1305:183–91.
- [59] Sell SL, Avila MA, Yu G, Vergara L, Prough DS, Grady JJ, et al. Hypertonic resuscitation improves neuronal and behavioral outcomes after traumatic brain injury plus hemorrhage. *Anesthesiology* 2008;108(5):873–81.
- [60] Soustiel JF, Vlodavsky E, Zaaroor M. Relative effects of mannitol and hypertonic saline on calpain activity, apoptosis and polymorphonuclear infiltration in traumatic focal brain injury. *Brain Res*. 2006;1101(1):136–44.
- [61] Barkhoudarian G, Hovda DA, Giza CC. The molecular pathophysiology of concussive brain injury. *Clin Sports Med* 2011;30(1):33–48. vii–iii.
- [62] Slemmer JE, Shacka JJ, Sweeney MI, Weber JT. Antioxidants and free radical scavengers for the treatment of stroke, traumatic brain injury and aging. *Curr Med Chem*. 2008;15(4):404–14.
- [63] Hall ED, Vaishnav RA, Mustafa AG. Antioxidant therapies for traumatic brain injury. *Neurotherapeutics* 2010;7(1):51–61.
- [64] Landolt H, Langemann H, Mendelowitsch A, Gratzl O. Neurochemical monitoring and on-line pH measurements using brain microdialysis in patients in intensive care. *Acta Neurochir Suppl (Wien)* 1994;60:475–8.
- [65] Ellington BM, Nguyen HN, Lai A, Nechifor RE, Zaw O, Pope WB, et al. Contrast-enhancing tumor growth dynamics of preoperative, treatment-naïve human glioblastoma. *Cancer* 2016.
- [66] Ellington BM, Yao J, Raymond C, Chakhoyan A, Khatibi K, Salamon N, et al. pH-weighted molecular MRI in human traumatic brain injury (TBI) using amine proton chemical exchange saturation transfer echoplanar imaging (CEST EPI). *NeuroImage Clin* 2019;22:101736.
- [67] von Elm E, Schoettler P, Henzi I, Osterwalder J, Walder B. Pre-hospital tracheal intubation in patients with traumatic brain injury: systematic review of current evidence. *Br J Anaesth* 2009;103(3):371–86.
- [68] Magnoni S, Ghisoni L, Locatelli M, Caimi M, Colombo A, Valeriani V, et al. Lack of improvement in cerebral metabolism after hyperoxia in severe head injury: a microdialysis study. *J Neurosurg* 2003;98(5):952–8.
- [69] Carpenter S. The Roaring 80s: The Bicycle Economy Out of Control. Japan Inc on the Brink: Institutional Corruption and Agency Failure. London: Palgrave Macmillan UK; 2015. p. 112–22.
- [70] Timofeev I, Carpenter KL, Nortje J, Al-Rawi PG, O'Connell MT, Czosnyka M, et al. Cerebral extracellular chemistry and outcome following traumatic brain injury: a microdialysis study of 223 patients. *Brain* 2011;134(Pt 2):484–94.
- [71] Vespa P. Traumatic brain injury is a longitudinal disease process. *Curr Opin Neurol* 2017;30(6):563–4.

- [72] Makoroff KL, Cecil KM, Care M, Ball Jr WS. Elevated lactate as an early marker of brain injury in inflicted traumatic brain injury. *Pediatr Radiol* 2005;35(7):668–76.
- [73] Woodcock T, Morganti-Kossmann MC. The role of markers of inflammation in traumatic brain injury. *Front Neurol* 2013;4:18.
- [74] Boyce JA. Mast cells and eicosanoid mediators: a system of reciprocal paracrine and autocrine regulation. *Immunol Rev* 2007;217(1):168–85.
- [75] Rao AM, Hatcher JF, Kindy MS, Dempsey RJ. Arachidonic acid and leukotriene c4: Role in transient cerebral ischemia of gerbils. *Neurochem Res* 1999;24(10):1225–32.
- [76] Irimia A, Chambers MC, Alger JR, Filippou M, Prastawa MW, Wang B, et al. Comparison of acute and chronic traumatic brain injury using semi-automatic multimodal segmentation of MR volumes. *J Neurotrauma* 2011;28(11):2287–306.
- [77] Manson J, Thiernemann C, Brohi K. Trauma alarmins as activators of damage-induced inflammation. *Br J Surg* 2012;99(Suppl 1):12–20.
- [78] Buchanan MM, Hutchinson M, Watkins LR, Yin H. Toll-like receptor 4 in CNS pathologies. *J Neurochem* 2010;114(1):13–27.
- [79] Frugier T, Morganti-Kossmann MC, O'Reilly D, McLean CA. In situ detection of inflammatory mediators in post mortem human brain tissue after traumatic injury. *J Neurotrauma* 2010;27(3):497–507.
- [80] Kumar A, Stoica BA, Loane DJ, Yang M, Abulwerdi G, Khan N, et al. Microglial-derived microparticles mediate neuroinflammation after traumatic brain injury. *J Neuroinflammation* 2017;14(1):47.
- [81] Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science* 2005;308(5726):1314–8.
- [82] Salter MW, Beggs S. Sublime microglia: expanding roles for the guardians of the CNS. *Cell* 2014;158(1):15–24.
- [83] Loane DJ, Kumar A. Microglia in the TBI brain: the good, the bad, and the dysregulated. *Exp Neurol* 2016;275(Pt 3(0 3)):316–27.
- [84] da Fonseca AC, Matias D, Garcia C, Amaral R, Geraldo LH, Freitas C, et al. The impact of microglial activation on blood-brain barrier in brain diseases. *Front Cell Neurosci* 2014;8:362.
- [85] Sumi N, Nishioku T, Takata F, Matsumoto J, Watanabe T, Shuto H, et al. Lipopolysaccharide-activated microglia induce dysfunction of the blood-brain barrier in rat microvascular endothelial cells co-cultured with microglia. *Cell Mol Neurobiol* 2010;30(2):247–53.
- [86] Muradashvili N, Lominadze D. Role of fibrinogen in cerebrovascular dysfunction after traumatic brain injury. *Brain Inj* 2013;27(13–14):1508–15.
- [87] Schachtrup C, Ryu JK, Helmrick MJ, Vagena E, Galanakis DK, Degen JL, et al. Fibrinogen triggers astrocyte scar formation by promoting the availability of active TGF-beta after vascular damage. *J Neurosci* 2010;30(17):5843–54.
- [88] Sica A, Mantovani A. Macrophage plasticity and polarization: in vivo veritas. *J Clin Invest* 2012;122(3):787–95.
- [89] Johnson VE, Stewart JE, Begbie FD, Trojanowski JQ, Smith DH, Stewart W. Inflammation and white matter degeneration persist for years after a single traumatic brain injury. *Brain*. 2013;136(1):28–42.
- [90] Devoto C, Arcurio L, Fetta J, Ley M, Rodney T, Kanefsky R, et al. Inflammation relates to chronic behavioral and neurological symptoms in military personnel with traumatic brain injuries. *Cell Transplant* 2017;26(7):1169–77.
- [91] Loane DJ, Kumar A, Stoica BA, Cabatbat R, Faden AL. Progressive neurodegeneration after experimental brain trauma: association with chronic microglial activation. *J Neuropathol Exp Neurol* 2014;73(1):14–29.
- [92] Ramlackhansingh AF, Brooks DJ, Greenwood RJ, Bose SK, Turkheimer FE, Kinnunen KM, et al. Inflammation after trauma: Microglial activation and traumatic brain injury. *Ann Neurol* 2011;70(3):374–83.
- [93] Galtrey CM, Fawcett JW. The role of chondroitin sulfate proteoglycans in regeneration and plasticity in the central nervous system. *Brain Res Rev* 2007;54(1):1–18.
- [94] Fenn AM, Gensel JC, Huang Y, Popovich PG, Lifshitz J, Godbout JP. Immune activation promotes depression 1 month after diffuse brain injury: a role for primed microglia. *Biol Psychiatry* 2014;76(7):575–84.
- [95] Butovsky O, Ziv Y, Schwartz A, Landa G, Talpalar AE, Pluchino S, et al. Microglia activated by IL-4 or IFN-gamma differentially induce neurogenesis and oligodendrogenesis from adult stem/progenitor cells. *Mol Cell Neurosci* 2006;31(1):149–60.
- [96] Morgan SC, Taylor DL, Pocock JM. Microglia release activators of neuronal proliferation mediated by activation of mitogen-activated protein kinase, phosphatidylinositol-3-kinase/Akt and delta-Notch signalling cascades. *J Neurochem* 2004;90(1):89–101.
- [97] Guerrier RM, Giza CC, Rotenberg A. Glutamate and GABA imbalance following traumatic brain injury. *Curr Neurol Neurosci Rep* 2015;15(5):27.
- [98] Van Horn JD, Bhattra A, Irimia A. Multimodal imaging of neurometabolic pathology due to traumatic brain injury. *Trends Neurosci* 2017;40(1):39–59.
- [99] Salazar AM, Jabbari B, Vance SC, Grafman J, Amin D, Dillon JD. Epilepsy after penetrating head injury. I. Clinical correlates: a report of the Vietnam Head Injury Study. *Neurology* 1985;35(10):1406–14.
- [100] Hesdorffer DC, Benn EK, Cascino GD, Hauser WA. Is a first acute symptomatic seizure epilepsy? Mortality and risk for recurrent seizure. *Epilepsia*. 2009;50(5):1102–8.
- [101] Lowenstein DH. Epilepsy after head injury: an overview. *Epilepsia* 2009;50(Suppl 2(s2)):4–9.
- [102] Christensen J, Pedersen MG, Pedersen CB, Sidenius P, Olsen J, Vestergaard M. Long-term risk of epilepsy after traumatic brain injury in children and young adults: a population-based cohort study. *Lancet* 2009;373(9669):1105–10.
- [103] Chang BS, Lowenstein DH. Quality Standards Subcommittee of the American Academy of N. Practice parameter: antiepileptic drug prophylaxis in severe traumatic brain injury: report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology* 2003;60(1):10–6.
- [104] Szaflarski JP, Nazzari Y, Dreier LE. Post-traumatic epilepsy: current and emerging treatment options. *Neuropsychiatr Dis Treat* 2014;10:1469–77.
- [105] Temkin NR, Dikmen SS, Wilensky AJ, Keihm J, Chabal S, Winn HR. A randomized, double-blind study of phenytoin for the prevention of post-traumatic seizures. *N Engl J Med* 1990;323(8):497–502.
- [106] Bhullar IS, Johnson D, Paul JP, Kerwin AJ, Tepas 3rd JJ, Frykberg ER. More harm than good: antiseizure prophylaxis after traumatic brain injury does not decrease seizure rates but may inhibit functional recovery. *J Trauma Acute Care Surg* 2014;76(1):54–60. discussion -1.
- [107] Vespa PM, Miller C, McArthur D, Eliseo M, Etchepare M, Hirt D, et al. Nonconvulsive electrographic seizures after traumatic brain injury result in a delayed, prolonged increase in intracranial pressure and metabolic crisis. *Crit Care Med* 2007;35(12):2830–6.
- [108] Storti SF, Formaggio E, Franchini E, Bongiovanni LG, Cerini R, Fiaschi A, et al. A multimodal imaging approach to the evaluation of post-traumatic epilepsy. *Magn Reson Mater Phys, Biol Med* 2012;25(5):345–60.
- [109] Irimia A, Van Horn JD. Epileptogenic focus localization in treatment-resistant post-traumatic epilepsy. *J Clin Neurosci* 2015;22(4):627–31.
- [110] Edden RA, Barker PB. Spatial effects in the detection of gamma-aminobutyric acid: improved sensitivity at high fields using inner volume saturation. *Magn Reson Med* 2007;58(6):1276–82.
- [111] Asahina N, Shiga T, Egawa K, Shiraishi H, Kohsaka S, Saitoh S. [¹¹C]Flumazenil positron emission tomography analyses of brain gamma-aminobutyric acid type A receptors in angelman syndrome. *J Pediatr* 2008;152(4):546 E93.
- [112] Gujar SK, Maheshwari S, Bjorkman-Burtscher I, Sundgren PC. Magnetic resonance spectroscopy. *J Neuroophthalmol* 2005;25(3):217–26.
- [113] Veeramuthu V, Seow P, Narayanan V, Wong JHD, Tan LK, Hernowo AT, et al. Neurometabolites Alteration in the Acute Phase of Mild Traumatic Brain Injury (mTBI): an In Vivo Proton Magnetic Resonance Spectroscopy (1H-MRS) Study. *Acad Radiol* 2018.
- [114] Benveniste H, Dreier J, Schousboe A, Diemer NH. Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. *J Neurochem* 1984;43(5):1369–74.
- [115] Braun AR, Balkin TJ, Wesenten NJ, Carson RE, Varga M, Baldwin P, et al. Regional cerebral blood flow throughout the sleep-wake cycle. An H2(15)O PET study. *Brain* 1997;120(Pt 7):1173–97.
- [116] Attwell D, Iadecola C. The neural basis of functional brain imaging signals. *Trends Neurosci* 2002;25(12):621–5.
- [117] Esterlis I, Hannestad JO, Bois F, Sewell RA, Tyndale RF, Seibyl JP, et al. Imaging changes in synaptic acetylcholine availability in living human subjects. *J Nucl Med* 2013;54(1):78–82.
- [118] Kontos AP, Collins MW, Holland CL, Reeves VL, Edelman K, Benso S, et al. Preliminary evidence for improvement in symptoms, cognitive, vestibular, and oculomotor outcomes following targeted intervention with chronic mTBI patients. *Mil Med* 2018;183(suppl_1):333–8.
- [119] Barker M. Persistence of cognitive effects after withdrawal from long-term benzodiazepine use: a meta-analysis. *Arch Clin Neuropsychol* 2004;19(3):437–54.
- [120] Oulis P, Kalogerakou S, Anyfandi E, Konstantakopoulos G, Papakosta VM, Madsdrakis V, et al. Cognitive effects of pregabalin in the treatment of long-term benzodiazepine-use and dependence. *Hum Psychopharmacol* 2014;29(3):224–9.
- [121] Raskind MA, Peterson K, Williams T, Hoff DJ, Hart K, Holmes H, et al. A Trial of Prazosin for Combat Trauma PTSD With Nightmares in Active-Duty Soldiers Returned From Iraq and Afghanistan. *Amer J Psychiat* 2013;170(9):1003–10.
- [122] Germain A, Richardson R, Moul DE, Mammen O, Haas G, Forman SD, et al. Placebo-controlled comparison of prazosin and cognitive-behavioral treatments for sleep disturbances in US Military Veterans. *J Psychosom Res* 2012;72(2):89–96.
- [123] Koola MM, Varghese SP, Fawcett JA. High-dose prazosin for the treatment of post-traumatic stress disorder. *Ther Adv Psychopharmacol* 2014;4(1):43–7.
- [124] Formisano R, Barba C, Buzzi MG, Newcomb-Fernandez J, Menniti-Ippolito F, Zafonte R, et al. The impact of prophylactic treatment on post-traumatic epilepsy after severe traumatic brain injury. *Brain Inj* 2007;21(5):499–504.
- [125] Ho L, Zhao W, Dams-O'Connor K, Tang CY, Gordon W, Pensked ER, et al. Elevated plasma MCP-1 concentration following traumatic brain injury as a potential “predisposition” factor associated with an increased risk for subsequent development of Alzheimer's disease. *J Alzheimers Dis* 2012;31(2):301–13.
- [126] Lei J, Gao G, Feng J, Jin Y, Wang C, Mao Q, et al. Glial fibrillary acidic protein as a biomarker in severe traumatic brain injury patients: a prospective cohort study. *Crit Care* 2015;19:362.
- [127] Li J, Yu C, Sun Y, Li Y. Serum ubiquitin C-terminal hydrolase L1 as a biomarker for traumatic brain injury: a systematic review and meta-analysis. *Am J Emerg Med* 2015;33(9):1191–6.
- [128] Wilkinson K, Lee K, Deshpande S, Duerksen-Hughes P, Boss J, Pohl J. The neuron-specific protein PGP 9.5 is a ubiquitin carboxyl-terminal hydrolase. *Science* 1989;246(4930):670–3.
- [129] Lewis SB, Wolper R, Chi YY, Miralía L, Wang Y, Yang C, et al. Identification and preliminary characterization of ubiquitin C terminal hydrolase 1 (UCHL1) as a

- biomarker of neuronal loss in aneurysmal subarachnoid hemorrhage. *J Neurosci Res* 2010;88(7):1475–84.
- [130] Bazarian JJ, Biberthaler P, Welch RD, Lewis LM, Barzo P, Bogner-Flatz V, et al. Serum GFAP and UCH-L1 for prediction of absence of intracranial injuries on head CT (ALERT-TBI): a multicentre observational study. *Lancet Neurol* 2018;17(9):782–9.
- [131] Bishop P, Rocca D, Henley JM. Ubiquitin C-terminal hydrolase L1 (UCH-L1): structure, distribution and roles in brain function and dysfunction. *Biochem J* 2016;473(16):2453–62.
- [132] Rezaei P, Grant G, Zeineh M, Richardson KJ, Coburn ML, Bet AM, et al. Stability of Blood Biomarkers of Traumatic Brain Injury The Levels of Glial Fibrillary Acidic Protein and Ubiquitin C-Terminal Hydrolase-L1 During the First Week After a Traumatic Brain Injury: Correlations With Clinical and Imaging Findings. *J Neurotrauma* 2019;79(3):456–64.
- [133] Chen F, Sugiura Y, Myers KG, Liu Y, Lin W. Ubiquitin carboxyl-terminal hydrolase L1 is required for maintaining the structure and function of the neuromuscular junction. *Proc Natl Acad Sci USA* 2010;107(4):1636–41.
- [134] Korley FK, Diaz-Arrastia R, Wu AH, Yue JK, Manley GT, Sair HI, et al. Circulating brain-derived neurotrophic factor has diagnostic and prognostic value in traumatic brain injury. *J Neurotrauma* 2016;33(2):215–25.
- [135] Blyth BJ, Farhavar A, Gee C, Hawthorn B, He H, Nayak A, et al. Validation of serum markers for blood-brain barrier disruption in traumatic brain injury. *J Neurotrauma* 2009;26(9):1497–507.
- [136] Reiber H. Dynamics of brain-derived proteins in cerebrospinal fluid. *Clin Chim Acta*. 2001;310(2):173–86.
- [137] Dadas A, Washington J, Diaz-Arrastia R, Janigro D. Biomarkers in traumatic brain injury (TBI): a review exploring serum biomarkers for mild traumatic brain injury. *Neuropsychiatr Dis Treat* 2018;14:2989–3000.
- [138] Bogoslovsky T, Wilson D, Chen Y, Hanlon D, Gill J, Jeromin A, et al. Increases of plasma levels of glial fibrillary acidic protein, tau, and amyloid beta up to 90 days after traumatic brain injury. *J Neurotrauma* 2017;34(1):66–73.
- [139] Eng LF. Glial fibrillary acidic protein (GFAP): the major protein of glial intermediate filaments in differentiated astrocytes. *J Neuroimmunol* 1985;8(4–6):203–14.
- [140] Weinstein DE, Shelanski ML, Liem RK. Suppression by antisense mRNA demonstrates a requirement for the glial fibrillary acidic protein in the formation of stable astrocytic processes in response to neurons. *J Cell Biol* 1991;112(6):1205–13.
- [141] Honda M, Tsuruta R, Kaneko T, Kasaoka S, Yagi T, Todani M, et al. Serum glial fibrillary acidic protein is a highly specific biomarker for traumatic brain injury in humans compared with S-100B and neuron-specific enolase. *J Trauma* 2010;69(1):104–9.
- [142] Frankel M, Fan L, Yeatts SD, Jeromin A, Vos PE, Wagner AK, et al. The Association of Very Early Serum levels of S100B, GFAP, UCH-L1, and SBDP with Outcome in ProTECT III. *J Neurotrauma* 2019.
- [143] Samson K. In the Clinic-Traumatic Brain Injury: FDA Approves First Blood Test for Brain Bleeds After Mild TBI/Concussion. *Neurology Today* 2018;18(6):12–8.
- [144] Vogel JW, Mattsson N, Iturria-Medina Y, Strandberg OT, Scholl M, Dansereau C, et al. Data-driven approaches for tau-PET imaging biomarkers in Alzheimer's disease. *Hum Brain Mapp* 2019;40(2):638–51.
- [145] Kreisl WC, Henter ID, Innis RB. Imaging translocator protein as a biomarker of neuroinflammation in dementia. *Adv Pharmacol* 2018;82:163–85.
- [146] Schwarz AJ, Shcherbinin S, Sliker LJ, Risacher SL, Charil A, Irizarry MC, et al. Topographic staging of tau positron emission tomography images. *Alzheimers Dement (Amst)* 2018;10:221–31.
- [147] Zinsmaier KE, Babic M, Russo GJ. Mitochondrial Transport Dynamics in Axons and Dendrites. In: Koenig E, editor. *Cell Biology of the Axon*. Springer, Berlin Heidelberg; 2009. p. 361–81.
- [148] Goedert M. Tau protein and the neurofibrillary pathology of Alzheimer's disease. *Ann N Y Acad Sci* 1996;777:121–31.
- [149] Zemlan FP, Rosenberg WS, Luebke PA, Campbell TA, Dean GE, Weiner NE, et al. Quantification of axonal damage in traumatic brain injury: affinity purification and characterization of cerebrospinal fluid tau proteins. *J Neurochem* 1999;72(2):741–50.
- [150] Rubenstein R, Chang B, Yue JK, Chiu A, Winkler EA, Puccio AM, et al. Comparing plasma phospho tau, total tau, and phospho tau-total tau ratio as acute and chronic traumatic brain injury biomarkers. *JAMA Neurol* 2017;74(9):1063–72.
- [151] Olivera A, Lejbman N, Jeromin A, French LM, Kim HS, Cashion A, et al. Peripheral total tau in military personnel who sustain traumatic brain injuries during deployment. *JAMA Neurol* 2015;72(10):1109–16.
- [152] Arun P, Abu-Taleb R, Oguntayo S, Tanaka M, Wang Y, Valiyaveetil M, et al. Distinct patterns of expression of traumatic brain injury biomarkers after blast exposure: role of compromised cell membrane integrity. *Neurosci Lett* 2013;552:87–91.
- [153] Kenney K, Qu BX, Lai C, Devoto C, Motamedi V, Walker WC, et al. Higher exosomal phosphorylated tau and total tau among veterans with combat-related repetitive chronic mild traumatic brain injury Acute decrease in alkaline phosphatase after brain injury: a potential mechanism for tauopathy. *Brain Inj* 2018;32(10):1276–84.
- [154] Dickstein DL, Pullman MY, Fernandez C, Short JA, Kostakoglu L, Knesaurek K, et al. Cerebral [(18)F]T807/AV1451 retention pattern in clinically probable CTE resembles pathognomonic distribution of CTE tauopathy. *Transl Psychiatry* 2016;6(9):e900.
- [155] Maruyama M, Shimada H, Suhara T, Shinotoh H, Ji B, Maeda J, et al. Imaging of tau pathology in a tauopathy mouse model and in Alzheimer patients compared to normal controls. *Neuron* 2013;79(6):1094–108.
- [156] Fonda JR, Fredman L, Brogly SB, McGlinchey RE, Milberg WP, Gradus JL. Traumatic brain injury and attempted suicide among veterans of the wars in Iraq and Afghanistan. *Am J Epidemiol* 2017;186(2):220–6.
- [157] Adams RS, Larson MJ, Corrigan JD, Ritter GA, Williams TV. Traumatic brain injury among U.S. active duty military personnel and negative drinking-related consequences. *Subst Use Misuse* 2013;48(10):821–36.
- [158] Sullan MJ, Asken BM, Jaffee MS, DeKosky ST, Bauer RM. Glymphatic system disruption as a mediator of brain trauma and chronic traumatic encephalopathy. *Neurosci Biobehav Rev* 2018;84:316–24.
- [159] Iliff JJ, Lee H, Yu M, Feng T, Logan J, Nedergaard M, et al. Brain-wide pathway for waste clearance captured by contrast-enhanced MRI. *J Clin Invest* 2013;123(3):1299–309.
- [160] Suzuki Y, Nakamura Y, Yamada K, Huber VJ, Tsujita M, Nakada T. Aquaporin-4 positron emission tomography imaging of the human brain: first report. *J Neuroimaging* 2013;23(2):219–23.
- [161] Badaut J, Brunet JF, Regli L. Aquaporins in the brain: from aqueduct to “multi-duct”. *Metab Brain Dis* 2007;22(3–4):251–63.
- [162] Leonard JM, Polites SF, Martin ND, Glasgow AE, Habermann EB, Kaplan LJ. Comfort care in trauma patients without severe head injury: In-hospital complications as a trigger for goals of care discussions. *Injury* 2019.
- [163] Kahveci K, Dincer M, Doger C, Yariç AK. Traumatic brain injury and palliative care: a retrospective analysis of 49 patients receiving palliative care during 2013–2016 in Turkey. *Neural Regen Res* 2017;12(1):77–83.
- [164] Smith BM, Martinez RN, Evans CT, Saban KL, Balbale S, Proeschner EJ, et al. Barriers and strategies for coordinating care among veterans with traumatic brain injury: a mixed methods study of VA polytrauma care team members. *Brain Inj* 2018;32(6):755–62.
- [165] Helmick K, Baugh L, Lattimore T, Goldman S. Traumatic brain injury: next steps, research needed, and priority focus areas. *Mil Med* 2012;177(8 Suppl):86–92.
- [166] Borghol A, Aucoin M, Onor I, Jamero D, Hawawini F. Modafinil for the improvement of patient outcomes following traumatic brain injury. *Innov Clin Neurosci* 2018;15(3–4):17–23.
- [167] Fisher S, Bryant SG, Kent TA. Postmarketing surveillance by patient self-monitoring: trazodone versus fluoxetine. *J Clin Psychopharmacol* 1993;13(4):235–42.
- [168] Huang CH, Huang CC, Sun CK, Lin GH, Hou WH. Methylphenidate on cognitive improvement in patients with traumatic brain injury: a meta-analysis. *Curr Neuropharmacol* 2016;14(3):272–81.

POST-TRAUMATIC CEREBRAL MICROHEMORRHAGES AND THEIR EFFECTS UPON WHITE MATTER CONNECTIVITY IN THE AGING HUMAN BRAIN

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Abstract—Cerebral microbleeds (CMBs), a common manifestation of mild traumatic brain injury (mTBI), have been sporadically implicated in the neurocognitive deficits of mTBI victims but their clinical significance has not been established adequately. Here we investigate the longitudinal effects of post-mTBI CMBs upon the fractional anisotropy (FA) of white matter (WM) in 21 older mTBI patients across the first ~6 months post-injury. CMBs were segmented automatically from susceptibility-weighted imaging (SWI) by leveraging the intensity gradient properties of SWI to identify CMB-related hypointensities using gradient-based edge detection. A detailed diffusion magnetic resonance imaging (dMRI) atlas of WM was used to segment and cluster tractography streamlines whose prototypes were then identified. The correlation coefficient was calculated between (A) FA values at vertices along streamline prototypes and (B) topological (along-streamline) distances between these vertices and the nearest CMB. Across subjects, the CMB identification approach achieved a sensitivity of $97.1\% \pm 4.7\%$ and a precision of $72.4\% \pm 11.0\%$ across subjects. The correlation coefficient was found to be negative and, additionally, statistically significant for $12.3\% \pm 3.5\%$ of WM clusters ($p < 0.05$, corrected), whose FA was found to decrease, on average, by $11.8\% \pm 5.3\%$ across the first 6 months post-injury. These results suggest that CMBs can be associated with deleterious effects upon peri-lesional WM and highlight the vulnerability of older mTBI patients to neurovascular injury.

I. INTRODUCTION

Cerebral microbleeds (CMBs) are a consistent manifestation of traumatic brain injuries (TBIs) regardless of their severity. Although CMB impact upon clinical outcome is unclear, it has been proposed that TBI-related CMBs may result in widespread, long-term white matter (WM) alterations and in neurocognitive deficits [1]. Because CMBs are associated with neurotoxicity after the breakdown of the

blood brain barrier (BBB), their sequelae may be more severe in geriatric TBI because the brains of older adults (A) exhibit greater BBB permeability and (B) have more deficient neuroinflammatory responses after TBI [1]. Given the uncertainty over the potential significance of TBI-related CMBs to clinical decision making, their identification from magnetic resonance imaging (MRI) and the assessment of their effects upon WM are worthwhile tasks. The detection of intra-parenchymal CMBs can be challenging because standard, anatomic, T_1 - or T_2 -weighted MRI does not obviate them clearly. In addition, MRI sequence parameters affect the ability to delineate CMBs, whereas magnetic susceptibility artefacts (e.g. flow voids in blood vessels, calcium deposits, deoxyhemoglobin in venules) can complicate their identification [2]. Recent studies utilize susceptibility weighted-imaging (SWI), which provides a higher contrast index for individual CMBs than conventional T_2 -weighted MRI, and which has been shown to increase the sensitivity and reliability of CMB detection [3].

An important reason for which CMB effects upon WM have been difficult to study is the relative paucity of reliable techniques for the longitudinal comparison of WM connections via diffusion magnetic resonance imaging (dMRI). Tract-based spatial statistics (TBSS) is arguably the best-known such approach for both within- and between-subject comparison, and this method is available as part of the FMRIB Software Library (FSL) software library. Although adequate for voxel-wise analysis of WM fractional anisotropy (FA), TBSS is suboptimal for quantifying FA changes along specific WM bundles. By contrast, tract-based morphometry (TBM) uses a detailed dMRI WM atlas to facilitate the consistent and reliable identification of WM bundles across subjects, together with their grouping into WM streamline clusters [4]. When combined with streamline prototyping [5], this strategy allows one to identify WM connections confidently across subjects and to assess differences in FA across time.

The first aim of this study is to report on the efficacy of a gradient-based CMB identification algorithm which leverages gradient-based edge detection concepts to identify CMBs in mild TBI (mTBI) victims. The second aim is to utilize the said CMB segmentation algorithm in concert with automatic WM clustering to quantify longitudinal differences in WM FA in mTBI victims. The third aim is to report the statistical association between the presence of CMBs and the FA changes observed along WM streamlines which pass through their spatial neighborhoods (penumbras). The results of this study suggest that CMBs can be identified accurately using the proposed intensity gradient-based algorithm, and that cerebral microhemorrhages can be associated with

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statistically-significant and deleterious effects upon penumbral WM. These findings highlight the vulnerability of mTBI victims not only to neurovascular injury but also to changes in WM over the first 6-8 months post-injury.

II. METHODS

A. Participants

The study was conducted with the approval of the Institutional Review Board at the University of Southern California and all participants provided written informed consent. Our cohort includes 21 individuals who suffered an mTBI about 5½ months (mo) prior to SWI acquisition ($\mu = 5.6$ mo, $\sigma = 0.2$ mo) and who had an acute Glasgow Coma Scale (GCS) score of at least 13 at the time of the initial medical assessment. Participants had to be adults over 21 years (y) of age with no clinical histories of neurological or psychiatric disease prior to their injuries. Volunteers consisted of adults over the age of 45 ($N = 21$; 8 females; age: $\mu = 66.2$ y, $\sigma = 7.8$ y, range: 50-79 y).

B. Imaging

Imaging was performed at 3 T (Prisma MAGNETOM Trio TIM, Siemens Corp., Erlangen, Germany). T_1 - and T_2 -MRI, gradient recalled echo (GRE)/SWI and dMRI volumes were acquired. T_1 -weighted images were acquired using a 3D magnetization-prepared rapid acquisition gradient echo (MP-RAGE) sequence [repetition time (T_R) = 1,950 ms; echo time (T_E) = 2.98 ms; inversion time (T_I) = 900 ms; voxel size = $1.0 \times 1.0 \times 1.0$ mm]. T_2 -weighted images were acquired using a 3D sequence (T_R = 2,500 ms; T_E = 360 ms; voxel size = $1.0 \times 1.0 \times 1.0$ mm). Flow-compensated GRE/SWI volumes were acquired axially (T_R = 30 ms; T_E = 20 ms; voxel size = $1.33 \times 1.33 \times 1.6$ mm). dMRI volumes were acquired axially in 64 gradient directions (T_R = 8,300 ms; T_E = 72 ms; voxel size = $2.7 \times 2.7 \times 2$ mm). One volume with $b = 0$ s/mm² and another with $b = 1,000$ s/mm² were also acquired. All neuroimaging data were de-identified and de-linked after their acquisition.

C. Preprocessing

Volumes were corrected for motion and eddy current artefacts using the FSL image processing toolbox (<https://fsl.fmrib.ox.ac.uk/fsl>). The FSL brain extraction tool (BET) was used to exclude voxels outside the brain from analysis. Each subject's SWI volume was skull-stripped and co-registered rigidly to her/his T_1 volume. Diffusion tensor estimation, dMRI streamline tractography and FA calculations were implemented using TrackVis (trackvis.org) software. WM streamlines whose lengths were smaller than 40 mm were discarded from the analysis.

D. CMB identification

First, the gradient of the image intensities I in the skull-stripped SWI volume was calculated at each brain voxel and the histogram of gradient magnitudes was computed over all brain voxels. The gradient magnitude volume was subjected to binary erosion using a morphological structuring element of spherical shape (11 mm radius). This allowed the exclusion of voxels on the outer edges of the brain volume. The resulting volume was thresholded to discard voxels whose gradient magnitudes belonged to the two tails of their distribution. All connected components consisting of contiguous non-zero voxels within this thresholded volume

were identified and their volumes were computed. The components in question were found to be associated with groups of voxels straddling (A) boundaries between cerebrospinal fluid (CSF) and WM, (B) sulcal banks of the neocortex and boundaries between brain and blood vessels, and (C) boundaries between brain and CMBs. Voxels in components from group A could be set to zero by applying an island removal algorithm which targeted the connected component with the largest volume among all identified components. Components in group B could be distinguished from those in group C and isolated for removal by noting that GM and WM are isointense (within $\mu \pm 2\sigma$ of the image intensity distribution) in SWI whereas the vasculature and CMBs are hypointense ($I < \mu - 2\sigma$). After setting the voxels in group B to zero, the gradient magnitude map contained only connected components associated with CMBs (group C). The approach described above allowed connected components associated with CMBs to be identified with sensitivity and precision. Two raters trained in neuroimaging and blinded to the results of the automatic labeling process identified blood vessels and CMBs visually and independently from each other. All identified features of interest were either confirmed or infirmed by a third blinded rater. This allowed the sensitivity and precision of the classification algorithm to be calculated.

E. WM parcellation and prototyping

WM streamline parcellation was implemented using an anatomically-curated streamline clustering atlas described elsewhere [6]. Briefly, an annotation method was used to leverage expert neuroanatomical knowledge and population-based brain anatomical information and to then annotate and categorize streamline clusters. WM structures were annotated in an atlas containing deep WM bundles, including major long-range association and projection bundles, commissural bundles, bundles related to cerebellar connections and to the brainstem, as well as short and medium-range superficial bundle clusters organized into categories based on which brain lobes they connect. Potential false positive connections were annotated to facilitate their exclusion. The atlas allows whole-brain WM parcellation into 800 streamline clusters.

After parcellation, the most representative WM streamline in each cluster was identified using a streamline prototyping approach, as described elsewhere [4, 5, 7]. Briefly, this method can be applied to each WM cluster to facilitate the identification of a unique WM streamline whose trajectory and other properties are representative of the group of streamlines in the cluster. Streamline prototyping is necessary in a study like ours partly because dMRI tractography may generate spurious streamlines which are not associated with physical WM connections; the prototype of an entire cluster of streamlines is a more fiducial descriptor of the underlying WM architecture within the cluster and of its overall properties, including its trajectory.

F. Statistical analysis

The biological hypothesis of the WM analysis is that, within the first ~5½ months after injury, a CMB leads to decreases in FA whose magnitudes are largest in the immediate spatial neighborhood (penumbra) of the CMB. In other words, the hypothesis is that TBI leads to substantial

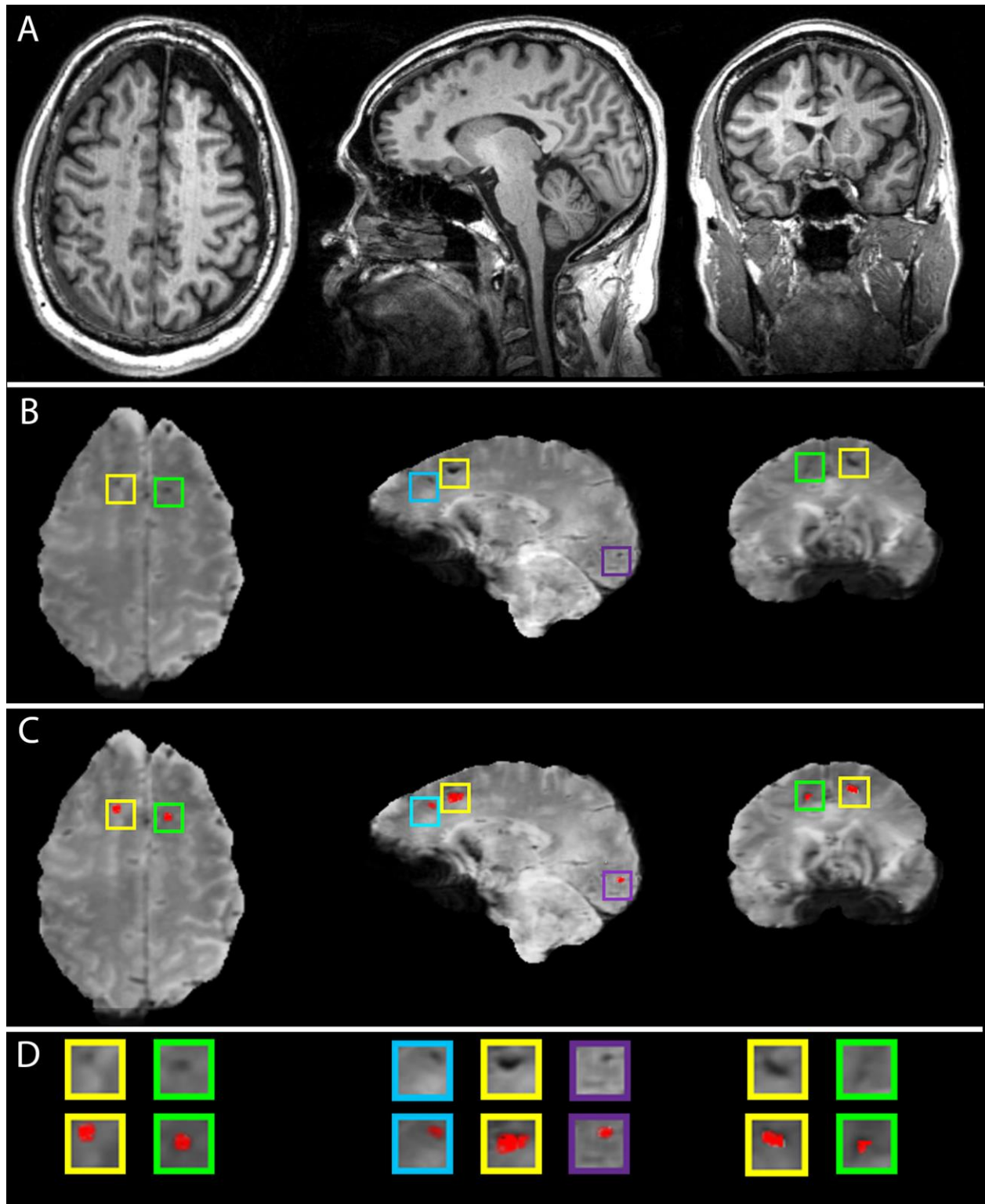


Fig. 1. Examples of automatic CMB segmentations in a geriatric mTBI patient. (A) T_1 -weighted images. (B) SWI images with CMBs highlighted in color-coded boxes. (C) SWI images with superimposed CMB segmentations. (D) Enlarged views of CMBs in (B) and (C).

decreases in FA around the CMB, and that the magnitude of these decreases tapers off within the WM as a function of the distance to the nearest CMB. This hypothesis is inspired by the observation that CMBs are associated with breakdown of the BBB, with the release of neurotoxic compounds into the brain parenchyma and with the gradual infiltration of these compounds along WM tracts [1]. Formally, for each WM

streamline prototype, the null hypothesis was formulated as the statement that there is no statistically significant correlation between (A) the longitudinal difference in FA measured at vertices along the prototype streamline and (B) the topological (along-tract) distance between the closest CMB and each vertex along the streamline prototype. The topological distance was selected over the Euclidean distance

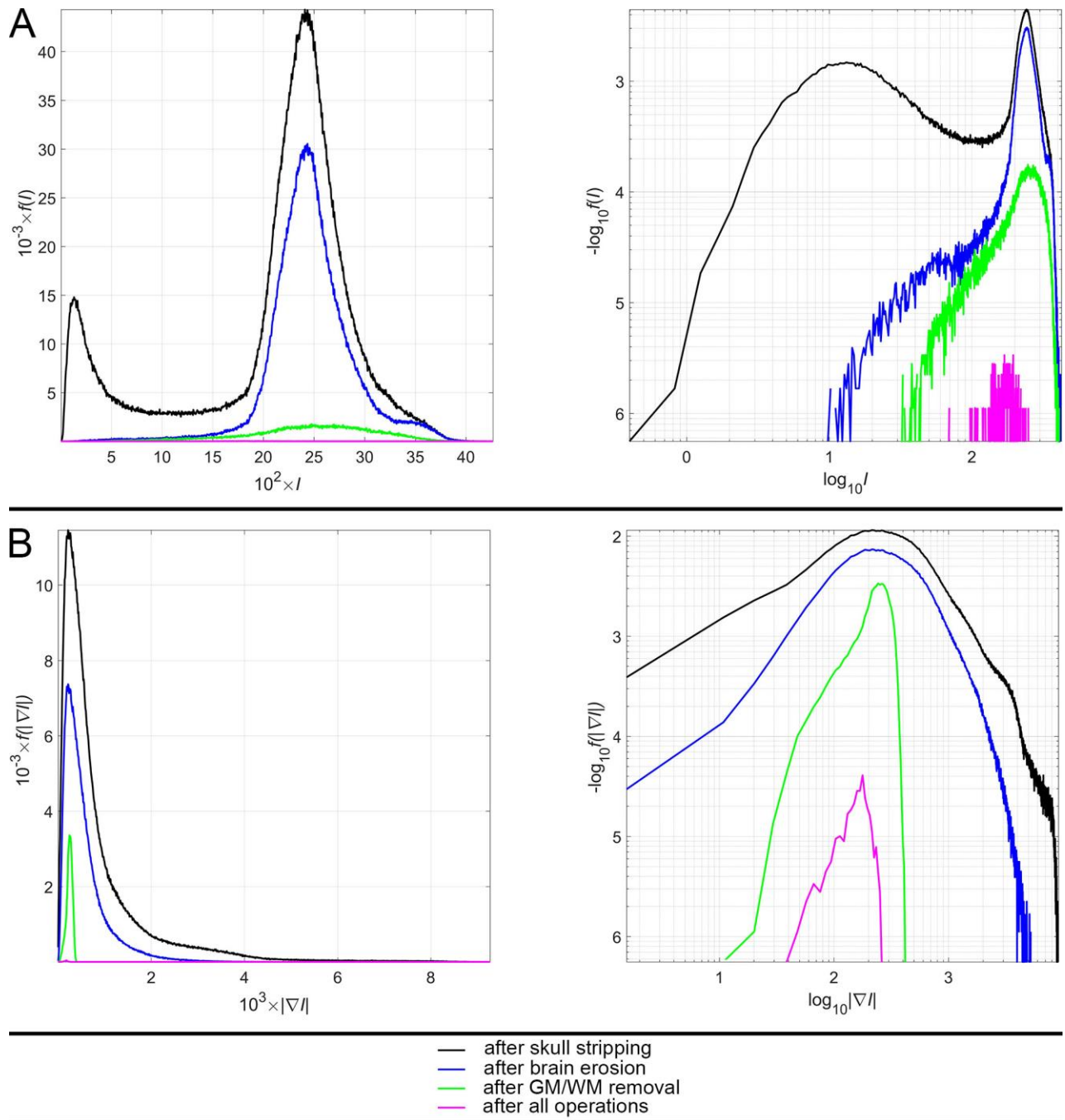


Fig. 2. The PDFs $f(\cdot)$ for (A) SWI intensities I and (B) SWI intensity gradient magnitudes $|\nabla I|$ in a sample participant. For convenience, plots are scaled either linearly (first column) or logarithmically (second column) along both axes. Traces depict the corresponding PDF computed over all brain voxels after skull stripping (black), over the subset of voxels remaining after brain erosion (blue), over voxels remnant after the subsequent removal of GM/WM (green) and over CMB voxels alone, after all operations (magenta). In the first column, for visual convenience, values are scaled linearly; for example, $10^2 \times I$ is plotted on the abscissa in A (instead of I) and $10^3 \times |\nabla I|$ is plotted on the abscissa in B (rather than $|\nabla I|$). In the second column, the logarithm of the quantity being depicted is shown; thus, in A, the abscissa displays $\log_{10}(I)$ rather than I ; similarly, in B, the abscissa displays $\log_{10}(|\nabla I|)$ rather than $|\nabla I|$ itself. Thus, in each of A and B, the left and right columns display the same information, albeit using different scaling, i.e. linear scaling (first column) or logarithmic scaling (second column).

because the diffusion of neurotoxins away from the CMB site is thought to be constrained by WM axon trajectories [7]. This suggests that the topological (along-tract) distance is a measure which reflects the parameters of the biophysics problem more realistically. The statistical significance of the correlation coefficient between (A) longitudinal differences in FA at prototype streamline vertices and (B) topological vertex-to-CMB distances was evaluated using Student's

dependent t test for paired samples at a significance level α equal to 0.05, subject to the Benjamini-Hochberg correction for multiple comparisons.

III. RESULTS

Examples of CMB segmentations are shown in Figure 1. Across all subjects, the average CMB count was 16.6 ± 11.5

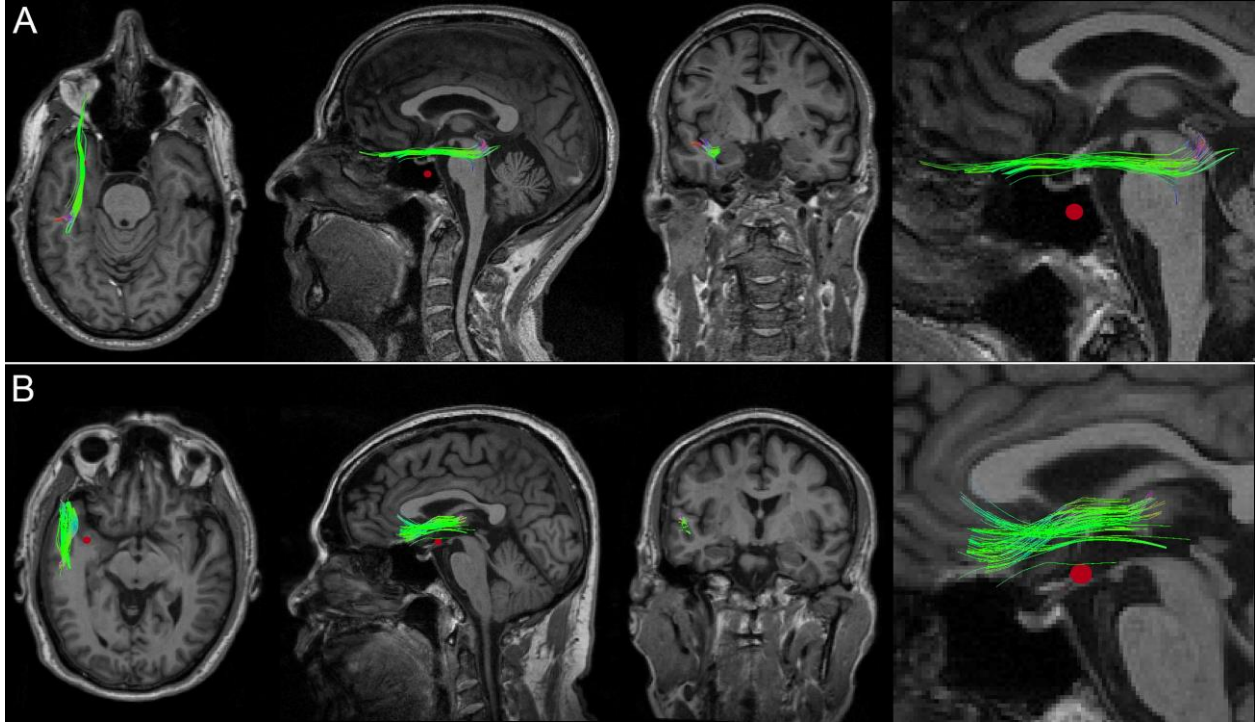


Fig. 2. WM streamline clusters in two mTBI victims who exhibit a CMB in the WM of the left anterior temporal lobe. In both (A) and (B), FA changes at vertices along the fasciculi were significantly correlated with the distances from these points to the nearest CMB (red). Whereas only some of the WM streamlines in each cluster pass through the CMB penumbra, the observed FA changes were found to extend beyond the immediate spatial neighborhood of the vascular lesion. From left to right: axial view, sagittal view, coronal view, and inset.

(range: 5 to 44). Volunteers aged 65 or under had an average of 17.0 ± 11.8 CMBs (range: 5 to 38), whereas participants over 65 had 16.4 ± 11.8 CMBs (range: 5 to 44). No sex-related significant difference in CMB count was found: Welch's $t = 0.116$, d.f. = 14.94, $p > 0.909$, 95% CI = (-10.690, 11.921). The average sensitivity of the CMB classification was $97.1\% \pm 4.7\%$ and its mean precision was $72.4\% \pm 11.0\%$.

Figure 2 displays the probability density functions (PDFs) f of SWI intensities I (Figure 2A) and of SWI gradient magnitudes (Figure 2B) for voxels within the brain of a sample participant. In other words, Figure 2A depicts $f(I)$ whereas Figure 2B depicts $f(|\nabla I|)$ for voxels within the sample brain. In both A and B, the PDF computed over all voxels within the brain are plotted in black; that computed over voxels remaining after the brain erosion operation is drawn in blue; the PDF computed over remnant voxels after GM/WM removal is shown in green; the PDF associated with CMB voxels is plotted in magenta (see figure caption).

Figure 3 displays CMBs and dMRI-derived streamlines for a perilesional WM cluster in two representative mTBI victims. Across all participants, the null hypothesis was rejected ($p < 0.05$, corrected), on average, for $12.3 \pm 3.5\%$ of WM clusters (range: 6.34% to 17.43%). In other words, for $12.3 \pm 3.5\%$ of the WM clusters identified in the average participant, the magnitude of longitudinal FA changes observed along the streamline prototype of the cluster was negatively and significantly correlated with the distance to the closest CMB. Across subjects, the FA changes in question averaged $11.8\% \pm 5.3\%$ of the FA measured at the first time point. No immediately obvious pattern in the spatial distribution of CMBs associated with significant WM FA changes was identified. WM structures whose FA decreases

were found to be correlated significantly with the distance to the closest CMB included the inferior fronto-occipital, inferior longitudinal and superior longitudinal fasciculi.

IV. DISCUSSION

Figure 2 allows the reader to observe the effects of successive operations applied by the CMB identification algorithm to a sample SWI volume. The PDF of SWI intensities for voxels within the brain (black trace in the left plot of Figure 2A) reflects the presence of hypointense voxels around the brain (peak at $I \approx 200$) in addition to that of isointense voxels associated with GM/WM (peak at $I \approx 2500$). The former group of voxels is removed via 3D erosion, as reflected by the blue trace in the left plot of Figure 2A. The subsequent removal of GM/WM voxels, which account for most voxels in the brain, is reflected by the fact that the peak of the green trace in the left plot of Figure 2A is considerably smaller than that of the blue trace in the same plot. Subsequent operations leading to the identification of the CMBs further reduce the number of voxels requiring labeling, such that only a logarithmic plot (right column of Figure 2A) allows one to inspect their distribution relative to other PDFs. The logarithmic plot in Figure 2A also allows one to take note of the fact that CMB-related voxels, although hypointense relative to GM/WM voxels, are nevertheless brighter than those which reside on—or close to—the outer edge of the brain. Finally, Figure 2B suggests that the magnitudes of the image intensity gradient at CMB voxel locations are similar to those at most other locations in the brain (right column of Figure 2B), despite the fact that CMB edges are hypointense relative to the GM/WM (right column of Figure 2B). This is consistent with the observation

that the intensities of contiguous, CMB-related voxels are homogeneous relative to one another. Interestingly, however, the logarithmic plot in Figure 2B also suggests that CMB voxels have a much narrower SWI intensity range than other structures of similar SWI intensity in the brain. To segment CMBs, SVMs and similar methods entail the use of large training data sets as well as highly-precise feature scaling and class weighting. Semi-automatic methods using random forests have used hand-crafted features dependent on CMB shape and size, but this can lead to erroneous results because CMBs do not always have well-defined shapes [8]. Convolutional neural networks (CNNs) have required large training data sets and substantial processing times due to the complex artificial neural layer architecture needed to solve this problem [9]. Because MRI scans are three-dimensional, conventional 2D CNNs are of limited applicability here, given that their kernels neglect information across slices whereas 3D CNNs are computationally intensive [9, 10].

Two prior ML studies report average sensitivities of 82% (SVM, [11]) and 93% (CNN, [9]) over multiple cross-fold validations. For the CNN method, the average precision of the classification was 44%. Whereas ML implementations are more computationally intensive and require cross-fold validations, our algorithm requires only one execution and achieves higher sensitivity and precision than the two ML approaches. Across subjects, our CMB identification method achieves an average sensitivity of $97.1 \pm 4.7\%$ and a mean precision of $72.4 \pm 11.0\%$, which are higher than in the studies of ML applications to similar SWI data sets. These results suggest that concepts inspired by gradient-based edge detection methods can be applied to SWI data like ours to perform CMB identification with sensitivity and precision which are comparable to those of some ML methods. Nevertheless, our approach has several limitations. Firstly, we have not used either SVMs or CNNs to segment CMBs from our SWI data set. CMBs may have been more difficult to segment from SWI in previous studies due to reasons like image quality. Comparison of our method to SVMs, CNNs and to other ML approaches should therefore account for such factors unknown to us. Secondly, CMB presentation can vary substantially across mTBI cases; thus, it would be useful to validate the method proposed here in a larger sample. Thirdly, our method was tested on SWI volumes acquired using one set of acquisition parameters. Because the ability of SWI to identify CMBs is sensitive to these, the utility of our approach when applied to SWI volumes acquired with other acquisition parameters is unknown. Fourthly, methods like ours should be tested and validated on SWI volumes acquired not only from mTBI subjects, but also from individuals with other conditions involving CMBs, such as cerebral amyloid angiopathy and similar neurovasculopathies. Finally, sex and age may affect CMB presentation, and therefore confound the efficacy of our algorithm; future research should therefore investigate the effects of these biological variables upon the sensitivity and precision of methods like ours. Despite these limitations, this study illustrates how an interesting and clinically-relevant image analysis problem can be addressed efficiently and parsimoniously in cases like ours using concepts inspired from gradient-based edge detection, rather than more sophisticated methods like SVMs and CNNs.

The analysis of CMB effects upon WM suggests that, in middle-aged and geriatric mTBI participants, CMBs can have

deleterious effects upon brain connectivity. The WM structures most frequently associated with such effects included the inferior fronto-occipital, inferior longitudinal and superior longitudinal fasciculi. These relatively long WM structures extend along the anteroposterior axis of the brain, and their association with CMB-related FA decreases may reflect the coup-contrecoup effects of fronto-occipital TBI, which is a very frequent mechanism of brain injury [1]. The statistical inferences made in this study appear to support the hypothesis that CMBs are associated with effects upon FA which are most deleterious in the CMB penumbra. Furthermore, the magnitude of these outcomes appears to decrease as the distance from the nearest CMB increases. This is consistent with the scenario where the occurrence of CMBs results in neurotoxic compounds being released into the brain parenchyma and in the gradual infiltration of such compounds into the WM, along the trajectories of WM tracts. One limitation of our analysis is that the statistical model involved only the distance to the nearest CMB, rather than distances to several CMBs. A corollary of this statement is that statistical interactions between distinct CMBs were not modelled and that their cumulative effects upon WM was not investigated. The effect of a CMB upon neighboring FA is likely much larger than the effects of more distant ones if CMBs are relatively far from one another. This assumption, however, may not hold if CMBs are close to each other, and future research should investigate this scenario.

REFERENCES

- [1] A. Irimia, J. D. Van Horn, and P. M. Vespa, "Cerebral microhemorrhages due to traumatic brain injury and their effects on the aging human brain," *Neurobiology of Aging*, vol. 66, pp. 158-164, 2018.
- [2] S. M. Greenberg *et al.*, "Cerebral microbleeds: a guide to detection and interpretation," *Lancet Neurology*, vol. 8, pp. 165-174, 2009.
- [3] R. N. Nandigam *et al.*, "MR imaging detection of cerebral microbleeds: effect of susceptibility-weighted imaging, section thickness, and field strength," *AJNR Am J Neuroradiol*, vol. 30, pp. 338-43, 2009.
- [4] L. J. O'Donnell and C. F. Westin, "Automatic tractography segmentation using a high-dimensional white matter atlas," *IEEE Transactions on Medical Imaging*, vol. 26, pp. 1562-1575, 2007.
- [5] L. J. O'Donnell, C. F. Westin, and A. J. Golby, "Tract-based morphometry for white matter group analysis," *Neuroimage*, vol. 45, pp. 832-44, 2009.
- [6] F. Zhang *et al.*, "An anatomically curated fiber clustering white matter atlas for consistent white matter tract parcellation across the lifespan," *Neuroimage*, vol. 179, pp. 429-447, 2018.
- [7] K. A. Rostowsky, A. S. Maher, and A. Irimia, "Macroscale white matter alterations due to traumatic cerebral microhemorrhages are revealed by diffusion tensor imaging," *Frontiers in Neurology*, vol. 9, 2018.
- [8] A. Fazlollahi *et al.*, "Efficient machine learning framework for computer-aided detection of cerebral microbleeds using the radon transform," *Biomedical Imaging (ISBI), 2014 IEEE 11th International Symposium on*, pp. 113-116, 2014.
- [9] Q. Dou *et al.*, "Automatic detection of cerebral microbleeds from MR images via 3D convolutional neural networks," vol. 35, pp. 1182-1195, 2016.
- [10] H. Chen, L. Yu, Q. Dou, L. Shi, V. C. Mok, and P. A. Heng, "Automatic detection of cerebral microbleeds via deep learning based 3d feature representation," *Biomedical Imaging (ISBI), 2015 IEEE 12th International Symposium on*, pp. 764-767, 2015.
- [11] S. R. Barnes, E. M. Haacke, M. Ayaz, A. S. Boikov, W. Kirsch, and D. Kido, "Semiautomated detection of cerebral microbleeds in magnetic resonance images," *Magn Reson Imaging*, vol. 29, pp. 844-52, 2011.

MAPPING CEREBRAL CONNECTIVITY CHANGES AFTER MILD TRAUMATIC BRAIN INJURY IN OLDER ADULTS USING DIFFUSION TENSOR IMAGING AND RIEMANNIAN MATCHING OF ELASTIC CURVES

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ABSTRACT

Although diffusion tensor imaging (DTI) can identify white matter (WM) changes due to mild traumatic brain injury (mTBI), the task of within-subject longitudinal matching of DTI streamlines remains challenging in this condition. Here we combine (A) automatic, atlas-informed labeling of WM streamline clusters with (B) streamline prototyping and (C) Riemannian matching of elastic curves to quantify within-subject changes in WM structure properties, focusing on the arcuate fasciculus. The approach is demonstrated in a group of geriatric mTBI patients imaged acutely and ~6 months post-injury. Results highlight the utility of differential geometry approaches when quantifying brain connectivity alterations due to mTBI.

Index Terms— Traumatic brain injury, diffusion tensor imaging, Riemannian matching, elastic curve

1. INTRODUCTION

Mild traumatic brain injury (mTBI) affects over 1.7 million Americans every year, and its all-time prevalence in US adults has been estimated to exceed 10% [1]. Whereas the magnetic resonance imaging (MRI) of moderate-to-severe TBI is frequently positive for intraparenchymal hemorrhage and mass edema, the MRI readings of mTBI patients are often free of such findings despite victims' persistent neurocognitive deficits as late as one year after injury. mTBI frequently results in traumatic axonal injury (TAI) despite the absence of gross pathology on T_1 - and T_2 -weighted MRI, such that there has been increasing interest in mapping the effects of mTBI upon the white matter (WM) connectivity of the brain over time [1]. MRI studies of mTBI have frequently leveraged diffusion MRI (dMRI) to quantify the fractional anisotropy (FA) of water in the brain and to indicate that relatively-low FA may be indicative of TAI [2]. Although some research has focused on identifying (sub-) acute mTBI effects upon WM, few investigations have studied such phenomena longitudinally. One key reason for this is the relative scarcity of reliable techniques for the longitu-

dinal evaluation of WM connections via dMRI. Tract-based spatial statistics (TBSS) is arguably among the best-known such approaches for both within- and between-subject comparison, and this technique is available as part of the FMRIB Software Library (FSL) software library. Although appropriate for voxel-wise analysis of WM FA, TBSS is inadequate for quantifying FA alterations along WM bundles. By contrast, tract-based morphometry (TBM) uses a detailed dMRI WM atlas to enable the consistent and reliable identification of WM fasciculi across subjects, in addition to their grouping into WM streamline clusters [4]. When combined with streamline prototyping [5], this strategy allows one to identify WM connections fiducially across subjects and to assess changes in FA across time. When comparing WM streamline properties longitudinally using dMRI, one important operation involves the matching of WM streamlines across time points. In this contribution, we propose that this task can be addressed using a novel combination of three sequential procedures, i.e. (A) automatic, atlas-informed labeling of WM streamline clusters, (B) streamline prototyping, and (C) modeling streamline prototypes as elastic curves which undergo Riemannian matching to calculate point-to-point correspondence. In this last step, points along (A) a streamline whose trajectory is inferred from dMRI data acquired at an initial (acute) timepoint are matched to corresponding points along (B) the streamline's trajectory as imaged at a second (chronic) timepoint. The approach is demonstrated in a group of geriatric mTBI victims imaged both acutely (within two weeks after injury) and chronically (about 6 months after injury). Our results highlight the utility of scale-invariant elastic curve matching for the quantification of WM alterations associated with mTBI in a geriatric patient population.

2. METHODS

2.1. Participants. The study received approval from the Institutional Review Board of the University of Southern California; all patients provided written informed consent. Participants had suffered an mTBI ~6 months (mo) prior to data acquisition ($\mu = 5.6$ mo, $\sigma = 0.2$ mo) and had had an

acute Glasgow Coma Scale (GCS) score over 12. They were older adults ($N = 21$; 8 females; age: $\mu = 66.2$ y, $\sigma = 7.8$ y, range: 50–79 y) with no history of psychiatric or neurological prior to mTBI.

2.2. MRI acquisition. Anatomic T_1 -, T_2 -MRI and dMRI volumes were acquired at 3 T (Prisma MAGNETOM Trio TIM, Siemens Corp., Erlangen, Germany). T_1 -weighted images were obtained using a 3D magnetization-prepared rapid acquisition gradient echo (MP-RAGE) sequence [repetition time (T_R) = 1.95 s; echo time (T_E) = 2.98 ms; inversion time (T_I) = 900 ms; voxel size = $1.0 \times 1.0 \times 1.0$ mm]. T_2 -weighted images were acquired using a 3D sequence (T_R = 2.5 s; T_E = 360 ms; voxel size = $1.0 \times 1.0 \times 1.0$ mm). dMRI volumes were acquired axially in 64 gradient directions (T_R = 8.3 s; T_E = 72 ms; voxel size = $2.7 \times 2.7 \times 2$ mm). A volume with $b = 0$ s/mm² and another with $b = 1,000$ s/mm² were also acquired. Data were de-identified and de-linked after acquisition.

2.3. Preprocessing. Motion and eddy current artefact corrections were implemented using the FSL image processing toolbox (<https://fsl.fmrib.ox.ac.uk/fsl>). Voxels outside the brain were excluded from analysis using the FSL brain extraction tool (BET). Diffusion tensor estimation, dMRI two-tensor streamline tractography and FA calculations were implemented in 3D Slicer ([slicer.org](https://www.slicer.org)). WM streamlines shorter than 4 cm were discarded.

2.4. WM parcellation. An anatomically curated streamline clustering atlas [3] was used to implement WM streamline parcellation. Briefly, the atlas allows whole-brain WM parcellation into 800 streamline clusters. An annotation method which leveraged population-based brain anatomical information and expert neuroanatomical knowledge was used to annotate and categorize streamline clusters. WM structure annotation was implemented in an atlas containing deep WM bundles—including major long-range association and projection bundles—commissural bundles, bundles related to cerebellar connections and to the brainstem, and both short and medium-range superficial clusters categorized based on which brain lobes they connect. Potentially false positive connections were annotated to enable their exclusion.

2.5. Streamline prototyping. Upon parcellation, each cluster's most representative WM streamline was identified using a streamline prototyping approach [4–6]. Briefly, this method can be applied to each cluster to enable the identification of a single WM streamline whose trajectory, torsion and other properties are representative of the cluster's streamlines. Prototyping is needed partly because dMRI tractography can generate spurious streamlines which do not correspond to physical WM connections. Thus, the prototype of a streamline cluster is a more trustworthy descriptor of underlying WM architecture within the cluster and of its properties.

2.6. Streamline projection. Before quantifying WM changes across timepoints, the weighted average FA along the mean trajectory of each cluster's streamlines was computed

as follows. First, a weighted mean FA was calculated based on information provided at each dMRI voxel within the spatial extent of the cluster. The weighting reflected the extent to which streamlines in the cluster shared the trajectory and length of the prototype. Second, the weighted mean FA values were assigned to points along the cluster's prototype streamline; this ensured that FA values at vertices along the prototype streamline were weighted averages of FA values along the trajectories of streamlines in the cluster. Thus, the streamline prototype fiber reflected not only the weighted average of cluster streamline trajectories, but also the weighted average of the FA along these streamlines. Because calculating the weighted average of streamline FA values along the prototype streamline can be conceptualized in terms of 'projecting' the FA values along individual streamlines upon the prototype streamline, we refer to this process as *streamline projection*.

2.6. Curve matching. After streamline projection, the curves associated with each cluster's streamline prototype—as resolved at each time point—were matched. This step was necessary because streamline prototypes' trajectories can vary subtly across time points even within the same subject. Thus, to obtain an accurate calculation of FA differences across time points, vertices along prototype trajectories must be matched across time points. To address this curve matching task, we model streamline prototypes as shapes using square root velocity functions (SRVFs), i.e. as elements in an infinite-dimensional space of curves [7–9]. This space is induced by a suitable Riemannian metric defined on its tangent space. Shape matching between streamlines is enabled by measuring shortest length paths (also known as geodesics) connecting two shapes in the shape space. The geodesic not only measures the length of the path and quantifies the geometric distance between two shapes, but also represents an optimal geometric deformation which highlights the anatomical differences between shapes. Instead of computing maximum density paths of streamline bundles and computing geodesics between them *across* subjects, we compute instead geodesics *within* subjects but across time points. The mathematical details of Riemannian curve matching as related to our contribution are described elsewhere [7–9].

3. RESULTS AND DISCUSSION

All 21 older adults included in the analysis had their WM fasciculi segmented, prototyped and matched across time points. Leveraging the methodological approach proposed here enabled the identification of time-related FA decreases ranging from ~1% to ~17% of their initial values. Across subjects, $12.3\% \pm 3.5\%$ of WM clusters had average FA values which decreased by a mean of $11.8\% \pm 5.3\%$ across the first six months post-injury. FA decreases were identified throughout the WM, particularly in the inferior fronto-occipital, inferior longitudinal and superior longitudinal fasciculi. This is unsurprising because these relatively long

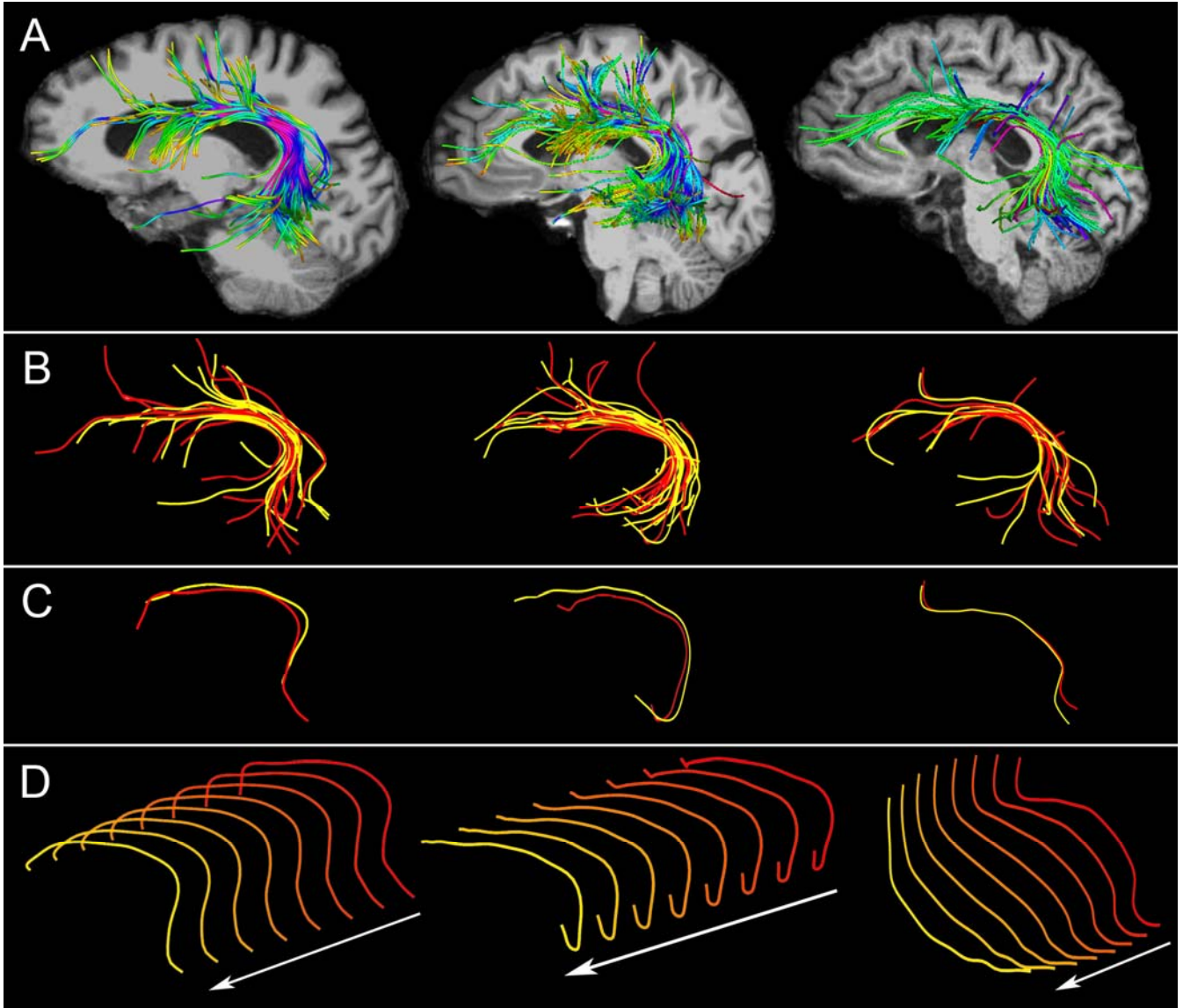


Figure. Longitudinal comparison and quantification of WM streamlines using DTI and Riemannian matching of elastic curves. Each column displays results from a different subject. (A). Automatically segmented streamlines of the arcuate fasciculus (AF) are superimposed to a skull-stripped, T_1 -weighted sagittal slice of the brain. Color encodes streamline orientation at each location (red: left to right; green: anterior to posterior; blue: inferior to superior). (B) Streamline prototypes are calculated for AF clusters both at the acute time point (red, within two weeks post-injury) and at the chronic time point (yellow, ~6 months post-injury). (C) Separate depictions of sample pairs of prototype streamlines from each subject highlight inter-subject variability in prototype length and trajectory. (D) Riemannian matching of elastic curves facilitates the process of identifying a point-to-point correspondence between the prototype streamlines associated with the acute (red) and chronic (yellow) time points, respectively. Intermediate steps of the matching process are drawn using different colors from a palette ranging from red to yellow (see arrow directions). Curves may not look identical when comparing (C) to (D) because the angle from which they are viewed in (D) is chosen to facilitate visualization of all intermediate steps of the curve matching process with minimum overlap.

WM bundles encompass much of the anteroposterior axis of the brain. The bundles' association with FA changes might reflect coup-contrecoup effects of fronto-occipital TBI, which is known to be relatively frequent [10]. The figure provides a conceptual summary of the procedures utilized to implement (A) automatic WM segmentation, (B) streamline prototyping, (C) the alignment of individual prototype streamlines, and (D) the calculation of a point-to-point correspondence using Riemannian matching of elastic curves. The figure shows results for three representative subjects: a

56-yo male, a 56-yo female, and a 70-yo male (first, second and third columns, respectively). The arcuate fasciculus (AF) is selected for illustration in (A) due to its characteristic shape, whose streamlines curve together in the vicinity of the temporo-parieto-occipital junction to connect Broca's and Wernicke's areas. In (B), the individual variability in AF trajectories across subjects is made apparent. Prototype streamlines are displayed in red for the acute time point and in yellow for the chronic time point. The number of prototypes is equal to the number of automatically segmented

WM clusters assigned to the AF by the streamline labeling procedure. Visual inspection of (C) underscores the fact that, even within the same WM cluster, streamline prototype trajectories vary across time points; this highlights the need for implementing curve matching to calculate a point-to-point correspondence. (D) visually captures the curve matching process by displaying not only the two curves which are matched (red and yellow), but also intermediate steps of the matching process (see Figure).

4. CONCLUSION

Because TBI is a highly heterogeneous neurological condition, WM structures are differentially impacted by injury. Although mTBI patients' neurocognitive sequelae can be strikingly similar, hardly any patients experience identical injury patterns. Using a combination of (A) automatic, atlas-informed labeling of WM streamline clusters, (B) streamline prototyping and (C) Riemannian matching of elastic curves, we have quantified within-subject changes in WM structure properties and found substantial WM changes in a subset of WM bundles. Our results not only support the hypothesis that mTBI is associated with FA decreases, but also provide an illuminating demonstration of how our unique combination of quantitative approaches can be used to identify WM degradation after brain injury.

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6. REFERENCES

- [1] X. Wu, I. I. Kirov, O. Gonen, Y. Ge, R. I. Grossman, and Y. W. J. R. Lui, "MR imaging applications in mild traumatic brain injury: an imaging update," vol. 279, pp. 693-707, 2016.
- [2] C. Eierud *et al.*, "Neuroimaging after mild traumatic brain injury: review and meta-analysis," vol. 4, pp. 283-294, 2014.
- [3] F. Zhang *et al.*, "An anatomically curated fiber clustering white matter atlas for consistent white matter tract parcellation across the lifespan," *Neuroimage*, vol. 179, pp. 429-447, Oct 1 2018.
- [4] K. A. Rostowsky, A. S. Maher, and A. Irimia, "Macroscale white matter alterations due to traumatic cerebral microhemorrhages are revealed by diffusion tensor imaging," *Frontiers in Neurology*, vol. 9, 2018.
- [5] L. J. O'Donnell and C. F. Westin, "Automatic tractography segmentation using a high-dimensional white matter atlas," *IEEE Transactions on Medical Imaging*, vol. 26, pp. 1562-1575, 2007.
- [6] L. J. O'Donnell, C. F. Westin, and A. J. Golby, "Tract-based morphometry for white matter group analysis," *Neuroimage*, vol. 45, pp. 832-44, 2009.
- [7] S. H. Joshi, E. Klassen, A. Srivastava, and I. Jermyn, "A novel representation for Riemannian analysis of elastic curves in R^n ," *2007 IEEE Conference on Computer Vision and Pattern Recognition*, pp. 1643-+, 2007.
- [8] A. Srivastava, E. Klassen, S. H. Joshi, and I. H. Jermyn, "Shape Analysis of Elastic Curves in Euclidean Spaces," *IEEE Transactions on Pattern Analysis and Machine Intelligence*, vol. 33, pp. 1415-1428, 2011.
- [9] G. Prasad *et al.*, "Automatic clustering and population analysis of white matter tracts using maximum density paths," *Neuroimage*, vol. 97, pp. 284-295, 2014.
- [10] A. Irimia, J. D. Van Horn, and P. M. Vespa, "Cerebral microhemorrhages due to traumatic brain injury and their effects on the aging human brain," *Neurobiology of Aging*, vol. 66, pp. 158-164, 2018.