Distinctive pattern of interface astroglial scarring in human brain after blast exposure: a postmortem case series

Sharon Baughman Shively,* Iren Horkayne-Szakaly,* Robert V Jones, James P Kelly, Regina C Armstrong, Daniel P Perl

Department of Pathology, F. Edward Hébert School of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD, USA (S B Shively MD, D P Perl MD); **Center for Neuroscience and Regenerative Medicine** (S B Shively MD, R C Armstrong PhD, D P Perl MD); **The Joint Pathology Center, Defense Health Agency, Silver Spring, MD, USA** (I Horkayne-Szakaly MD, R V Jones MD); **Department of Neurosurgery, University of Colorado School of Medicine, Aurora, CO, USA** (J P Kelly MD); **Department of Anatomy, Physiology and Genetics, F. Edward Hébert School of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD, USA** (R C Armstrong PhD); **Henry M. Jackson Foundation for the Advancement of Military Medicine** (S B Shively MD)

Corresponding Author:

Daniel P. Perl, MD
Department of Pathology, F. Edward Hébert School of Medicine
Uniformed Services University of the Health Sciences
Center for Neuroscience and Regenerative Medicine
4301 Jones Bridge Road
Bethesda, MD 20814
Email: daniel.perl@usuhs.edu
Telephone number: 301-295-5534
Fax number: 301-319-0647

*Drs. Baughman Shively and Horkayne-Szakaly contributed equally to this article.

Key words: military traumatic brain injury, human brain, high explosives, blast wave, interface astrogliosis, astroglial scar, glial fibrillary acidic protein, astrocytes

Summary

Background

For most traumatic brain injuries associated with blast exposure, current literature offers no evidence-based guidelines for definitive diagnosis or directed treatment, partially due to unknown pathophysiology. Moreover, few neuropathological studies exist to address whether blast exposure produces unique lesions in the human brain, especially compared to impact traumatic brain injury.

Methods

In this postmortem case series, we investigated multiple features of TBI, using standard clinical histopathology techniques and markers, to identify novel pathology in brain tissues from five service members with remote blast exposures, and analyzed the limited associated clinical case histories. We also evaluated brain tissues from three service members who had died shortly after severe blast exposures. We then compared these results to those of brain tissues from civilian (non-military) cases with no blast exposures, including five cases with remote impact traumatic brain injuries, three cases with no traumatic brain injuries, and four cases with opioid exposures but no traumatic brain injuries.

Findings

All five remote blast exposure cases revealed prominent astroglial scarring that involved the subpial glial plate, penetrating cortical blood vessels, grey-white matter junction, and structures lining ventricles. The acute blast exposure cases showed early phase astrogliosis in the same sites. The civilian cases, with or without history of impact traumatic brain injury, or with history of opioid exposure, did not demonstrate similar neuroanatomical patterns of astrogliosis as the blast exposure cases.

Interpretation

The blast exposure cases showed a distinctive pattern of interface astroglial scarring at boundaries between brain parenchyma and fluids, and at junctions between grey and white matter, providing evidence of novel pathology in these brain tissues. Furthermore, the unique astroglial scarring pattern is consistent with blast biophysics and potentially accounts for clinical sequelae.

Funding

U.S. Army Medical Research and Materiel Command

Introduction

With the invention of high explosives, thousands worldwide have suffered from blast exposures, including traumatic brain injuries (TBIs), especially in times of war whether as civilians or military personnel. Over the past century, most medical literature focuses on military combatants, given the concentration of blast exposures in this population. Since 2001 approximately 2.5 million U.S. service members have deployed overseas, in addition to hundreds of thousands from countries of the European Union and the world. During intense conflicts, coalition troops frequently encountered attacks with high explosives, which caused at least 60% of combat casualties.[1-4] Modern military equipment meritoriously protects the lives of service members, but proves ineffective in preventing TBI, especially the mild form (concussion) that accounts for most cases.[3,4] Even though conventional neuroimaging for mild TBI typically shows no brain abnormalities, military personnel can suffer from persistent postconcussive symptoms, such as headache, sleep disturbance, concentration impairment, memory problems, depression, and anxiety,[5] suggesting structural damage below standard resolution levels. With symptoms but no biomarkers, these TBIs became colloquially termed "invisible wounds."

The medical literature offers few studies characterizing acute or chronic neuropathological sequelae in the human brain. During World War I, Frederick Mott reported acute findings in postmortem brains of three soldiers;[6-9] two of his writings on this subject were published 100 years ago in *The Lancet* in 1916.[6,7] Examination of these brains revealed multiple petechial hemorrhages (mostly within the white matter of the centrum semiovale, corpus callosum, and internal capsule) and extravasation of blood into the subarachnoid space, with no physical evidence of external trauma to the head. From World War II, several publications provided only cursory descriptions of postmortem brains. All these studies were conducted before the introduction of clinical immunohistochemistry techniques in the late 20th century. No other literature emerged until 2011, with the case report of a deceased 27-year-old veteran exposed

to multiple blasts during Operation Iraqi Freedom.[10] At autopsy, the brain displayed neurofibrillary tangles (NFTs) in neuroanatomical areas consistent with chronic traumatic encephalopathy (CTE), a neurodegenerative *tauopathy* associated with repeated mild TBIs from contact sports.[11,12] Shortly thereafter, four additional CTE cases were reported in deceased veterans with blast exposures during deployments.[13,14] Lastly, unlike these other studies, another postmortem brain study of six veterans failed to reveal *tauopathy*, but rather provided evidence of axonal damage.[15]

In this study, we tested the hypothesis that blast exposure produces unique patterns of damage in the human brain, differing from brain damage associated with impact (non-blast) TBI. We examined postmortem brains from combatants with known blast exposures. To detect differences between blast *versus* impact TBI, we also studied postmortem brains from civilians with TBIs (no blast exposure). Finally, we compared the blast-exposed postmortem brains to three civilian cases with no known TBIs and five civilian cases with histories of opioid medications and/or abuse. We analyzed tissue samples from anatomical areas standard for clinical neuropathology and utilized staining and immunohistochemistry methods to investigate multiple features of traumatic brain pathology. The postmortem brains with blast exposures revealed a distinctive pattern of severe astrogliosis in specific neuroanatomical regions in all cases from acute to chronic injuries, demarcating the evolution of astroglial scar formation from the two earliest cases of four days to the latest case of nine years survival.

Methods

Participants

We evaluated brain autopsy specimens from three tissue archives. We obtained most military cases from The Joint Pathology Center (JPC, Department of Defense), most civilian cases from the University of Maryland Brain and Tissue Bank (UMB BTB, National Institutes of Health NeuroBioBank), and the remaining cases from the Center for Neuroscience and Regenerative Medicine Brain Tissue Repository (CNRM BTR, Department of Defense). All cases comprised male patients (table). The blast TBI (bTBI)

cases were divided into two categories: acute/subacute (n=3; military personnel, death 4-60 days following blast exposure, mean age 36 years with range of 28-43 years) and chronic (n=5; military personnel, death >6 months following blast exposure, mean age 33 years with range of 26-45 years). The comparative civilian cases included impact TBIs (n=5; chronic TBIs, 1 also with CTE diagnosis, mean age 30 years with range of 23-38 years, with exclusion of two outliers with advanced ages of 74 and 78 years), opioid use (n=5; 1 also with probable chronic TBI, mean age 38 years with range of 27-44 years), and controls (n=3; no TBIs, mean age 28 years with range of 20-40 years).

Procedures

For each case, we examined formalin-fixed, paraffin-embedded tissue samples from frontal, temporal, cingulate (with corpus callosum), and parietal (when available) lobes and hippocampus, and analyzed the limited associated clinical data. For case 1 (chronic bTBI), we had the entire postmortem brain for extensive sampling and complete medical records. Paraffin-embedded tissue blocks were sectioned at 5 μm thickness. We conducted hematoxylin and eosin (H&E) stains on tissue sections for general morphology/structure and immunohistochemistry with antibodies detecting glial fibrillary acidic protein (GFAP, astrocytes; mouse anti-human monoclonal antibody GA5, Leica Biosystems, PA0026, with Bond heat-induced epitope retrieval, HIER 1:10 for 10 minutes), abnormally phosphorylated tau (AT8; mouse anti-human monoclonal antibody, dilution 1:2000, Thermo Scientific, MN1020, HIER 1:10 for 10 minutes), β-amyloid (4G8; mouse anti-human monoclonal antibody, dilution 1:500, Covance/Biolegend, SIG-39220, HIER 1:10 for 10 minutes), amyloid precursor protein (APP, axonal damage; mouse anti-human monoclonal antibody clone 22c11, dilution 1:10, EMD Millipore, MAB348, HIER 1:10 for 10 minutes), and antigen CD68 (macrophages/activated microglia; mouse anti-human monoclonal antibody clone 514H12, Leica Biosystems, PA0273, HIER 2:20 for 20 minutes). Immunohistochemistry was performed on a Leica Bond III automated immunostainer with a diaminobenzidine (DAB) chromogen

detection system (Leica Biosystems, DS9800). This protocol received Institutional Review Board approvals prior to initiation of study.

Role of funding source

The U.S. Army Medical Research and Materiel Command funded this study. The sponsors had no role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Results

Chronic blast TBI cases

For case 1, we had complete medical records and the entire postmortem brain to conduct clinicopathological and extensive tissue analyses (panel for clinical presentation, table). At autopsy, the brain weighed 1338 grams (within normal limits) with no significant atrophy or ventricular enlargement. No healed cortical contusions were identified.

For all five chronic bTBI cases, prominent astroglial scar, made evident by heightened GFAP immunoreactivity with extensive interdigitations of astrocytic processes, distributed in a unique neuroanatomical pattern, involving tissues adjacent to cerebrospinal fluid, boundaries between grey and white matter, and penetrating cortical blood vessels. In the cortical samples of case 1 (dorsolateral prefrontal, medial orbitofrontal, temporal, anterior insular, anterior and posterior cingulate, entorhinal, parietal, and calcarine), immunohistochemistry to detect GFAP revealed substantial thickening of the subpial glial plate with intense underlying astrogliosis, prominent perivascular astrocytes in the grey matter, and dense astrogliosis at the grey-white matter junction (figure 1B, D). Cases 2-5 showed similar GFAP immunoreactivity patterns; all cortical samples demonstrated prominent astrogliosis in the subpial region and grey-white matter junction, and to variable degrees, GFAP-immunoreactive astrocytes ensheathed penetrating cortical blood vessels (figure 2A-D). In case 1, GFAP immunoreactivity also demonstrated astrogliosis in the hippocampus and dense astrogliosis in tissues lining the lateral

ventricle, including the alveus and fimbria. Other structures lining ventricles that showed intense astrogliosis were hypothalamus, thalamus, corpus callosum, fornix, and amygdala (figure 1E, F). For cases 2-5, brain structures lining ventricles displayed augmented astrogliosis, although analysis was restricted due to reduced tissue availability.

For case 1, immunohistochemistry with abnormally phosphorylated *tau* (AT8) antibody exhibited foci of both NFTs and *tau*-immunoreactive astrocytes in sulcal depths, with perivascular predilection, in frontal and parietal cortices (figure 1G). AT8 immunohistochemistry further displayed few individual NFTs, primarily located in neocortical layers II/III (figure 1H). Scant NFTs were also noted in hippocampus, mammillary body, hypothalamus, thalamus, and amygdala. The mild involvement of the brain with this paradigm of NFTs and *tau*-immunoreactive astrocytes is consistent with a diagnosis of limited CTE.[16] For case 2, AT8 immunoreactivity showed rare NFTs in frontal and temporal cortices; evidence of *tau* pathology was absent in the other three cases.

For all five chronic bTBI cases, β-amyloid (4G8) immunohistochemistry disclosed no plaques. In cortical sections, CD68 immunoreactivity revealed variable amounts of focal perivascular clusters of macrophages in the leptomeninges and macrophages/activated microglia in parenchymal grey and white matter. For assessment of axonal damage, H&E stains did not reveal axonal spheroids, but APP immunoreactivity presented focal axonal varicosities in cases 3 and 5, both of whom had died from methadone overdoses.

The five chronic bTBI patients shared antemortem diagnoses of posttraumatic stress disorder (PTSD). Dependent on the individual and available clinical data, other symptoms included headache, anxiety, insomnia, memory impairment, depression, seizure disorder, and chronic pain (table).

Acute/subacute blast TBI cases

We examined brain tissues from three active duty service members, two of whom had died four days and the other two months after blast exposure (cases 6-8, table). H&E stains revealed reactive astrocytes, which indicate sites of acute injury, underlying the subpial glial plate and at the grey-white matter junction in all cortical samples, and in periventricular areas (figure 2), as well as corresponding increased GFAP immunoreactivity. CD68 immunoreactivity showed perivascular macrophages in the leptomeninges and varying degrees of perivascular macrophages/activated microglia in the grey and white matter. In all three cases, H&E stains showed axonal spheroids, and APP immunoreactivity of cortical white matter and corpus callosum demonstrated focal axonal damage. 4G8 and AT8 immunohistochemistry presented no β-amyloid plaques or *tau* pathology, respectively.

Civilian cases

Due to intrinsic uncertainty of negative blast exposure history in military personnel, we selected civilian brain specimens for comparison, comprising five cases with chronic (long-term survival) impact TBIs and three control cases with no known TBIs. In addition, we included five civilian cases with histories of opioid use because two chronic bTBI cases died from methadone intoxications and the three acute/subacute bTBI cases most likely required morphine or an opioid equivalent during treatments (cases 9-21, table).

For all the civilian cases, GFAP immunohistochemistry failed to reveal similar astrogliosis patterns as the blast cases (figures 1, 3). The chronic impact TBI, opioid, and control cases displayed rare, isolated CD68-immunoreactive cells in perivascular locations, with the exception of the CTE case (case 13) which revealed clusters of perivascular immunoreactive cells in the leptomeninges and grey and white matter. The CTE case also exhibited NFTs and *tau*-immunoreactive astrocytes in patterns consistent with the diagnosis. Even though we selected case 17 primarily based on the patient's history of prescription methadone (chronic pain with multiple amputations due to a motor vehicle accident), the frontal cortex

showed NFTs and *tau*-immunoreactive astrocytes particularly favoring sulcal depths and perivascular sites, which fulfills current requirements for a CTE diagnosis, as well as numerous NFTs in the cingulate cortex. In most cases, immunohistochemistry revealed no β-amyloid plaques, except case 12 which displayed both focal diffuse plaques and NFTs in transentorhinal cortex. APP immunoreactivity indicated focal axonal damage with white matter varicosities in the opioid cases 14, 16, and 18.

Discussion

Few studies characterize altered neuroanatomical structure or molecular expression in human postmortem brain after blast exposure, either in acute or chronic phases. In this study, we found a unique neuroanatomical pattern of astrogliosis in persons exposed to high explosives. We first examined brains from five deceased service members with remote history of bTBI and found severe astroglial scarring at the subpial glial plate, penetrating cortical blood vessels, grey-white matter junction, and structures lining ventricles. We then examined brains from three service members who had died shortly after blast exposure and identified reactive astrocytes in similar distributive patterns. In the human brain, astrocytes respond to local damage, detectable within hours with cytoplasmic enlargement, nuclear displacement, and GFAP augmentation (reactive astrocytes), and extend processes that can subsequently intertwine and consolidate to form astroglial scar. The acute bTBI cases with reactive astrocytes in the same neuroanatomical locations as dense astroglial scar of the chronic bTBI cases provides temporal and topographic evidence for a pathophysiologic link to the blast event. Furthermore, damage to these brain structures possibly explains several persistent clinical symptoms of patients with bTBIs, for example, pia and penetrating vessels (disturbed cerebrospinal fluid flow and headache), U-fiber connections at the grey-white matter junction (cognitive dysfunction), and structures lining ventricles that includes fundamental parts of the limbic system (short-term memory impairment) and hypothalamus (sleep disorder). For case 1, astroglial scarring involved ventromedial prefrontal (orbitofrontal) cortex, dorsolateral prefrontal cortex, anterior cingulate cortex, anterior insular cortex,

amygdala, hypothalamus, and hippocampus – neuroanatomical areas associated with PTSD.[17-19] For control cases, we analyzed civilian cases with diminutive likelihood of blast exposure. Because service members sustain impact TBIs, including with blast exposure, and engage in contact sports, we examined postmortem brains of five civilians with primarily chronic impact TBIs, as well as three with no TBI histories and five with opioid histories (one with probable chronic impact TBI); we did not observe a similar pattern of astrogliosis in these non-blast cases.

With blast exposure, a person may suffer brain damage from multiple mechanisms that can occur in rapid succession.[20,21] At detonation, conversion of liquid or solid into gas, with released energy, generates high pressures and temperatures. The rapidly expanding gases compress surrounding air to form a blast wave that moves outward radially from the explosive core at rates surpassing the speed of sound. The blast wave causes primary injury by propagating intense pressure and energy through the body, most likely including the brain.[21-24] The biophysical mechanisms of primary brain injury and subsequent neuropathophysiology remain poorly understood. After the blast wave, a blast wind follows with the potential of reaching hurricane speeds while hurling objects in its path. From the blast wind, debris can cause penetrating trauma, known as secondary injury, and tertiary injury can result from acceleration of the head to impact against another solid object, a component of many bTBIs.[25]

For the bTBI cases, the pattern of astrogliosis in tissues adjacent to cerebrospinal fluid, along boundaries between grey and white matter, and around blood vessels may denote neuroanatomical areas particularly vulnerable to damage from high explosives and concomitantly provide further insight into the biophysics of blast wave impinging on the human brain. Classic mechanisms of blast injury in the human body include spallation, implosion, and inertial effect.[26] Spallation results from the pressure wave advancing between media of different densities, with mechanical disruption at the interface. Implosion involves compression of dissolved gas bubbles in liquid medium, which then expand rapidly after the pressure wave passes, thereby damaging surrounding tissues. Inertial effects occur when

lighter objects accelerate at greater rates than heavier objects, causing stress at the boundaries. More specifically, waves with high frequency/low amplitude can disrupt structures with differing densities, such as the border between parenchyma and blood in the brain, whereas low frequency/high amplitude waves can generate local motions that strain regions with elasticity, such as the border between grey and white matter.[20] Another possible mechanism considers energy transfer to the torso, thus propagating pressure waves to the brain through vascular and cerebrospinal fluids.[20,27,28] In blast wave research, controversies exist concerning biophysical mechanisms of injury in human brain, optimal methods to simulate high explosives in theater, scientific approaches to study blast wave reflections from surrounding surfaces, scaling experimental animal models to human brain, among numerous other issues.[20-22] Several animal model studies of blast exposure show increased encephalic GFAP expression; however, possibly in part due to blast research restrictions outlined, none articulates yet an equivalent neuroanatomical astrogliosis pattern as described in this study.[29] Our findings of extensive astrogliosis in specific neuroanatomical areas, with emphasis on structural boundaries between tissues of differing densities and tissues adjacent to vascular or cerebrospinal fluids, generally corroborate current knowledge and theories of blast wave biophysics. In short, we presently hypothesize that the blast wave causes damage at the interface of structural boundaries, to which the human brain responds with astrogliosis, potentially resulting in astroglial scar with persistent structural and functional changes.

Other issues similarly continue to challenge this nascent field of research. Until implementation of in-theater medical evaluation requirements in 2010,[30] U.S. military personnel generally disregarded mild TBI secondary to blast exposure in these contemporary conflicts. Yet recent publications, including reports from survivors, insinuate extensive blast exposures for coalition ground troops, without reliable sources for verification.[2,31-33] Pointedly, a study involving a convenience sample of 34 living U.S. veterans with service in Iraq and Afghanistan revealed approximate numbers of blast exposures for these individuals, ranging from 1 to 100 with 60% reporting more than five incidents.[33] Furthermore, recent

publications indicate an association between combat bTBI and PTSD.[31,33-35] One case control study particularly demarcated the number of combat mild TBIs with loss of consciousness (most due to blast exposure) positively correlating with PTSD severity, neurologic deficits, and cognitive impairment, such that over 90% of those combat veterans with more than five episodes suffered from these medical ailments.[34] Neuroimaging studies show identifiable, biological abnormalities underpinning cases of PTSD,[18,19] but the medical literature lacks detailed neuropathology studies characterizing these abnormalities, particularly cellular and molecular disturbances, in postmortem brains of PTSD patients. Because we often could not verify blast exposures for military personnel with records or patient histories, we relied upon civilian cases as non-blast controls, which ideally accommodates neither the aggressive lifestyles of combatants, nor medical issues pertinent to the military. Specifically, military personnel engage in contact sports or often experience TBIs from motor vehicle accidents, falls, fights, or training, either as civilians or in service, which further complicates analysis of bTBI in these postmortem brains. We chose military cases with at least one known combat blast exposure, but lacked data to account for probable influencing factors, for example, number of exposures, tertiary injury, proximity to detonation and surroundings, power of the explosion, clinical symptoms, impact TBI histories, contact sports participation, and previous mental health. In addition, we evaluated cases from tissue archives, which introduces other inopportune variables such as case and tissue availability. Thus, we only described these initial findings.

Modern neuropathology study of human bTBI lies within recent literature, much focusing on *tau* pathology associated with CTE. Among the five CTE cases with blast exposures, three veterans concurrently had known confounding head impact histories common in life.[10,13,14] Another study of brains from six veterans failed to show abnormal *tau* immunoreactivity, such that the authors proposed axonal damage as contributing to neuropsychiatric symptomatology.[15] Evidence exists to support the hypothesis that blast wave produces axonal injury,[36,37] but this type of injury is classically associated with

impact TBI, which probably includes tertiary injury, and also with substance abuse.[15,38,39] In the current study of bTBI cases, we noted equivalent neuropsychiatric features, including PTSD, in the five chronic cases and identified axonal pathology in the chronic cases with methadone overdoses and in all three acute/subacute cases. Furthermore, we found one chronic case with minimal CTE and another with occasional NFTs, but the other three showed no evidence of *tauopathy*. In addition to the unknown pathophysiology from head injury to CTE onset, we append that other unexplored variables probably affect disease development (type of injury, dose, individual susceptibility), as well as recognize the intrinsic challenge of obtaining comprehensive head injury histories especially in postmortem studies. Hence, we still do not know if blast exposure is truly associated with CTE; however, the astroglial burden possibly exacerbates the disease process. Alterations in the glymphatic system, which depends upon the integrity of subpial and perivascular astrocytes and normal sleep patterns for optimal function, may impede clearance of proteins such as *tau*.[40,41] This raises further concern that susceptible persons with bTBI may be at increased risk for neurodegenerative disease.

For the current military conflicts, bTBI has been called the "invisible wound" since numerous service members suffer from debilitating neuropsychiatric symptoms without the existence of medical knowledge about the underlying pathophysiology. The neuroanatomical pattern of interface astrogliosis (scarring) in these blast cases reveals previously undetected damage to the human brain that persists for years after initial injury and possibly underlies aspects of clinical sequelae. Nonetheless, not only are former military combatants attempting to reintegrate into society with these wounds, but citizens throughout the world are increasingly victim to high explosive attacks, with both components transferring these issues directly to civilian medicine. These initial findings need further expansion with conduction of more comprehensive clinicopathological studies; development of clinical biomarkers to identify lesions and characterize damage to affected brain structures in more detail; advancement of clinical diagnostic criteria; elaboration of mathematical, computational, physical, and animal models;

enhancement of personal protective equipment for present and future service members; and discovery of effective therapeutics. We believe that clinical investigators will find ways to make these injuries not only visible, but also treatable for similarly wounded service members, veterans, and civilians.

Contributors

DP had full access to all the data in the study and had final responsibility for the decision to submit for publication. SBS and DP designed the study concept. SBS, IHS, RJ, JK, RA, and DP acquired, analyzed, and interpreted data for the work. SBS drafted the manuscript. SBS, IHS, RJ, JK, RA, and DP revised the manuscript critically for important intellectual content. RA and DP secured funding for this project. All authors have approved the final version of the report. JK is a board-certified neurologist, and IHS, RJ, and DP are board-certified neuropathologists.

Declaration of interests

The authors declare no competing interests.

Acknowledgments

This study was funded by the U.S. Army Medical Research and Materiel Command (MRMC) through a Broad Agency Announcement grant (BAA 11286007), Center for Neuroscience and Regenerative Medicine (CNRM), Uniformed Services University of the Health Sciences (USUHS), and The Joint Pathology Center (JPC), within the U.S. Department of Defense. The authors wish to thank Drs. Charles Rice, Thomas Baker, Arthur Kellermann, and Steven Kaminsky for their continuous encouragement and support. We also acknowledge the excellent histotechnology and administrative support of Patricia Lee and Leslie Sawyers, respectively. Human tissue for most civilian cases was obtained from University of Maryland Brain and Tissue Bank (UMB BTB) which is a Brain and Tissue Repository of the NIH NeuroBioBank. The opinions expressed herein are those of the authors and are not necessarily

representative of those of the Uniformed Services University of the Health Sciences (USUHS), the Department of Defense (DOD); or, the United States Army, Navy, or Air Force.

Panel: case 1 clinical presentation

Case 1 involves a 45-year-old male veteran who committed suicide by gunshot wound. During his distinguished military career of 25 years, he received numerous commendations. Colleagues considered him highly competent, reliable, and emotionally stable. According to surviving members of his team, they routinely experienced blast exposures during training exercises and combat missions with bombs landing or improvised explosive devices (IEDs) detonating in close proximity. With blast exposure, team members described a jolting sensation and noted that these incidents commonly resulted in postconcussive symptoms. After retirement, the patient admitted to multiple mild TBIs during his military service, but had chosen not to report his symptoms at the time for fear of being deemed unfit for duty. He complained of headache and memory problems. He also expressed trouble maintaining mental focus, which he related to severe sleep disturbance. He often lost coherence of thought and jumbled his speech. Likewise, his wife reported cognitive and behavioral changes. For instance, she described his abnormally slow hand movements over the car steering wheel, ignition, and gear shift, as if confused about their functions. He failed to remember family plans. Formerly superior in spatial concepts, he struggled to pack the car for holiday travel. On several occasions, he became uncharacteristically angry with her. Clinicians described poor eye contact, flat affect, and low voice tone and treated him for PTSD, depression, and anxiety. One month before he died, conventional magnetic resonance imaging (MRI, 1.5T) showed no significant brain abnormalities. In addition, no formal report of TBI could be found in his medical records. His wife recounted that he had wrestled and boxed during his school years and experienced three motor vehicle accidents throughout his life. There was no indication of substance abuse by history or postmortem toxicology screening.

Panel: research in context

Systematic review

We searched PubMed in September 2010 to identify previous studies addressing neuropathological sequelae in the human brain after exposure to high explosives. We used numerous terms including "human," "high explosives," and "neuropathology," which yielded no results. In his book *Shell Shock to PTSD: Military Psychiatry from 1900 to the Gulf War*, Edgar Jones reviewed the history of high explosives and medicine in warfare beginning in World War I (Psychology Press, Hove, 2005). We first used Dr. Jones's book and then reference lists in papers to search the sparse literature that mostly appeared in the pre-PubMed era. We found the writings of Frederick Mott, neurologist/neuropathologist, who reported acute findings in the postmortem brains of three soldiers exposed to high explosives during World War I and a few papers from World War II with cursory examinations of postmortem brains, also primarily from acute cases. Since 2011, published studies describe five cases of chronic traumatic encephalopathy (CTE) and six other cases with axonal pathology (and no *tau* pathology) in blast-exposed U.S. veterans. We are aware of no other published neuropathological studies on the brains of patients exposed to high explosives. Furthermore, clinical literature from the past 100 years reveals that a significant percentage of blast-exposed service members suffer from persistent neurologic/behavioral symptomatology; there is an ongoing debate about whether these manifestations are organic or functional in nature. In the absence of any accepted neuroimaging or other biomarkers, brain damage due to blast exposure and related pathophysiology potentially contributing to these clinical features remain unclear.

Interpretation

In this study, we examined postmortem brain tissues from service members exposed to high explosives in combat, both with short-term and more prolonged survival. In all five blast-exposed cases with chronic survival, we found a distinctive, consistent, and unique pattern of prominent astroglial scar situated at the boundaries between brain parenchyma and fluids (cerebrospinal and blood), namely the

subpial zone, penetrating cerebral cortical blood vessels, and ventricles, and between grey and white matter in cortices. The brain tissues from blast-exposed service members with survival of only four days showed evidence of early-phase astroglial scar formation (reactive astrocytes) in the same locations, providing temporal and topographic evidence that this astroglial pattern relates to the blast event. Identical analysis of brain tissues from civilians with remote histories of impact TBI did not demonstrate similar astrogliosis as the blast cases, which further suggests the astroglial pattern unique to high explosive exposure. Review of the literature on the interaction between blast wave and the human body revealed that the astroglial scarring pattern in the blast cases is consistent with general knowledge of blast wave biophysics and predictions of damage patterns in the human brain. In addition, the neuroanatomical locations of the interface astroglial scarring support the concept that persistent symptoms of blast-exposed individuals may correlate with damage to particular structures with potential interference or alteration of their functions. Our findings suggest, for the first time, that there may be a predictable pattern of physical damage to human brain following blast exposure, which resolution capabilities of standard clinical neuroimaging techniques currently cannot detect. We anticipate reconsideration about pathophysiology underlying the neuropsychiatric sequelae that follow blast exposure and also innovative approaches to diagnosis and treatment.

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Table legend

***Table*. Case histories**

Traumatic brain injury (TBI), blast traumatic brain injury (bTBI), improvised explosive device (IED), motor vehicle accident (MVA), posttraumatic stress disorder (PTSD), loss of consciousness (LOC), computed tomography (CT), chronic traumatic encephalopathy (CTE), not applicable (NA).

Figure legends

***Figure 1*. Detailed neuropathological analysis of brain tissues from chronic bTBI case 1**

Panels A and C show normal GFAP immunoreactivity in neocortical tissue without TBI (case 19, civilian control with no known TBI history). For chronic bTBI case 1 in panels B and D, GFAP immunoreactivity in neocortical tissue shows substantial thickening of the subpial glial plate with intense underlying astrogliosis (double-headed arrow) and prominent perivascular astrocytes in the grey matter (arrows);

panel B also shows dense astrogliosis at the grey-white matter junction. Panel E shows GFAP immunoreactivity in structures lining the wall of the third ventricle, with dramatic astrogliosis of the hypothalamus; the mammillary body is relatively spared. In panel F, GFAP immunoreactivity in hippocampus with temporal pole of lateral ventricle shows astrogliosis of hippocampus and dense periventricular astrogliosis including the alveus and fimbria. In panels B, D, E, and F, augmented GFAP immunoreactivity with dense astrocytic process interdigitations indicates astroglial scar. Panel G shows AT8 immunoreactivity for abnormally phosphorylated *tau* in neocortical tissue with a sulcal depth encompassed by neurofibrillary tangles (NFTs) and astrocytic tangles. The solid arrows point to individual NFTs, and the empty arrow points to a perivascular *tau*-immunoreactive focus. Panel H shows AT8 immunoreactivity of isolated NFTs in superficial neocortical layers (arrows). Subpial glial plate (SGP), grey matter (GM), grey-white matter (GM-WM) junction, third ventricle (3rd V), hypothalamus (HT), mammillary body (MB), hippocampus (H), alveus (A), fimbria (F), lateral ventricle (LV), sulcal depth (SD). Scale bars A, B 500 µm; C, D, G 200 µm; E 5 mm; F 2 mm; H 100 µm.

***Figure 2*. Additional neuropathological analyses of brain tissues from chronic and acute bTBI cases**

In panels A, B, and C, GFAP immunoreactivity in neocortical tissue shows substantial thickening of the subpial glial plate with intense underlying astrogliosis, prominent perivascular astrocytes in the grey matter, and dense astrogliosis at the grey-white matter junction in chronic bTBI cases (panel A, case 5; panel B, case 4; panel C, case 5). Panel D shows GFAP immunoreactivity of astrocytes ensheathing a blood vessel in neocortical grey matter (case 3, chronic bTBI). In panels E and F, hematoxylin and eosin (H&E) stains show numerous reactive astrocytes, with prominent eosinophilic cytoplasm and nuclear displacement (arrows), which indicate acute injury sites at the subpial zones of frontal cortices (cases 8 and 6, acute bTBIs). In panel G, H&E stain of corpus callosum shows axonal spheroids (arrows), indicative of axonal injury (case 8, acute bTBI). In panel H, amyloid precursor protein (APP) immunoreactivity in

corpus callosum shows varicosities, also indicative of axonal injury (case 7, subacute bTBI). Scale bars A 3 mm; B, C 500 µm; D, E, H 50 µm; F, G 20 µm.

***Figure 3*. Comparison of GFAP immunoreactivities among chronic impact TBI, chronic substance abuse, acute bTBI, and chronic bTBI cases**

Panels A and E show minimal GFAP immunoreactivity in neocortical tissue with chronic impact TBI (case 11, civilian with remote history of impact TBI and no blast exposure). Panels B and F show minimal GFAP immunoreactivity in neocortical tissue with chronic substance abuse (case 15, civilian with no known history of impact TBI or blast exposure). In panels C and G, GFAP immunoreactivity shows neocortical tissue of acute bTBI with initial stages of GFAP deposition, indicating early repair response (case 6, acute bTBI). In panels D and H, GFAP immunoreactivity in neocortical tissue with chronic bTBI shows substantial thickening of the subpial glial plate with intense underlying astrogliosis, prominent perivascular astrocytes in the grey matter, and dense astrogliosis at the grey-white matter junction (case 5, chronic bTBI). Scale bars A, B, C, D 500 µm; E, F, G, H 200 µm.

Table							
Case number	**Age gender**	**Cause of death**	**Blast exposure history**	**Impact TBI history**	**Time interval between incident and death**	**Substance abuse history**	**Medical history**
Chronic blast TBI cases							
1	45 years male	suicide by gunshot wound	bombs, IEDs, breacher exercises	wrestler, boxer, MVA x 3 (5 years old, 2x in military)	>2 years after military retirement, 27 years after contact sports, 30 and 14 years after MVAs (3rd interval unknown)	no	PTSD, headache, anxiety, depression, insomnia, memory and concentration impairments, chronic pain
2	31 years male	not determined	high explosives	unknown	9 years	unknown	PTSD, anxiety, seizure disorder, lower extremity amputation
3	26 years male	methadone overdose	IED	unknown	15 months	prescription drugs	PTSD, depression, postconcussion syndrome, seizure disorder, gunshot wound in neck, chronic pain
4	37 years male	multidrug toxicity	IED	unknown	7 months	unknown	PTSD, chronic pain
5	26 years male	methadone overdose	multiple IEDs	assault	1 year after IEDs, 2 months after assault	alcohol, multiple drugs	PTSD, memory impairment
Acute/subacute blast TBI cases							
6	43 years male	blast injuries	IED	unknown	4 days	no	closed head injury, right subarachnoid hemorrhage, multiple facial fractures, bilateral pulmonary contusions, pneumomediastinum, diabetes insipidus
7	38 years male	blast injuries	IED	unknown	2 months	unknown	burns to head, face and scalp, right eye enucleation, pulmonary edema
8	28 years	blast injuries	IED	unknown	4 days	unknown	subarachnoid & parenchymal hemorrhages

	male						
Impact TBI cases							
9	23 years male	blunt trauma due to MVA	no	yes	remote	unknown	unknown
10	28 years male	epilepsy	no	MVA	remote	unknown	seizure disorder secondary to TBI
11	38 years male	status epilepticus	no	yes	remote	unknown	seizure disorder secondary to TBI
12	78 years male	respiratory failure	no	MVA at age 12 years, multiple falls at age 77 years	66 years after MVA	no	dystonia secondary to TBI from MVA, neurologic examination after fall with facial trauma (current medication diazepam, cognitive deficits, CT unremarkable with possible mild atrophy)
13	74 years male	cardiac arrest	no	football and rugby player, moderate TBI with LOC at age 27 years, MVA (age early 50s)	40 years after contact sports, 47 years after moderate TBI, 20 years after MVA	no	CTE, dementia, personality changes, bipolar disorder, myocardial infarction, chronic atrial fibrillation
Opioid cases							
14	27 years male	methadone overdose, cocaine use	no	no	NA	methadone, heroin, cocaine	unknown
15	38 years male	heroin overdose	no	no	NA	oxycodone	oxycodone prescription for back pain after work incident leading to chronic abuse, patient in treatment center for substance abuse, no previous suicide attempts
16	39 years male	multidrug toxicity	no	no	NA	hydrocodone, carisoprodol	prescription drug abuse, smoker
17	40 years male	atherosclerotic cardiovascular disease	no	probable	remote	unknown	current medication methadone, chronic pain, amputations (left below knee and lower arm) and skin graft (right leg) after MVA (no TBI documentation), smoker
18	44 years	heroin overdose,	no	no	NA	unknown	current medications alprazolam and albuterol,

	male	cocaine use					asthma
Control cases (no TBI history)							
19	40 years male	hypertensive cardiovascular disease	no	no	NA	unknown	hypertension, hyperlipidemia, smoker
20	24 years male	reactive airway disease	no	no	NA	no	asthma, non-smoker
21	20 years male	dilated cardiomyopathy	no	no	NA	unknown	morbid obesity, arrythmia

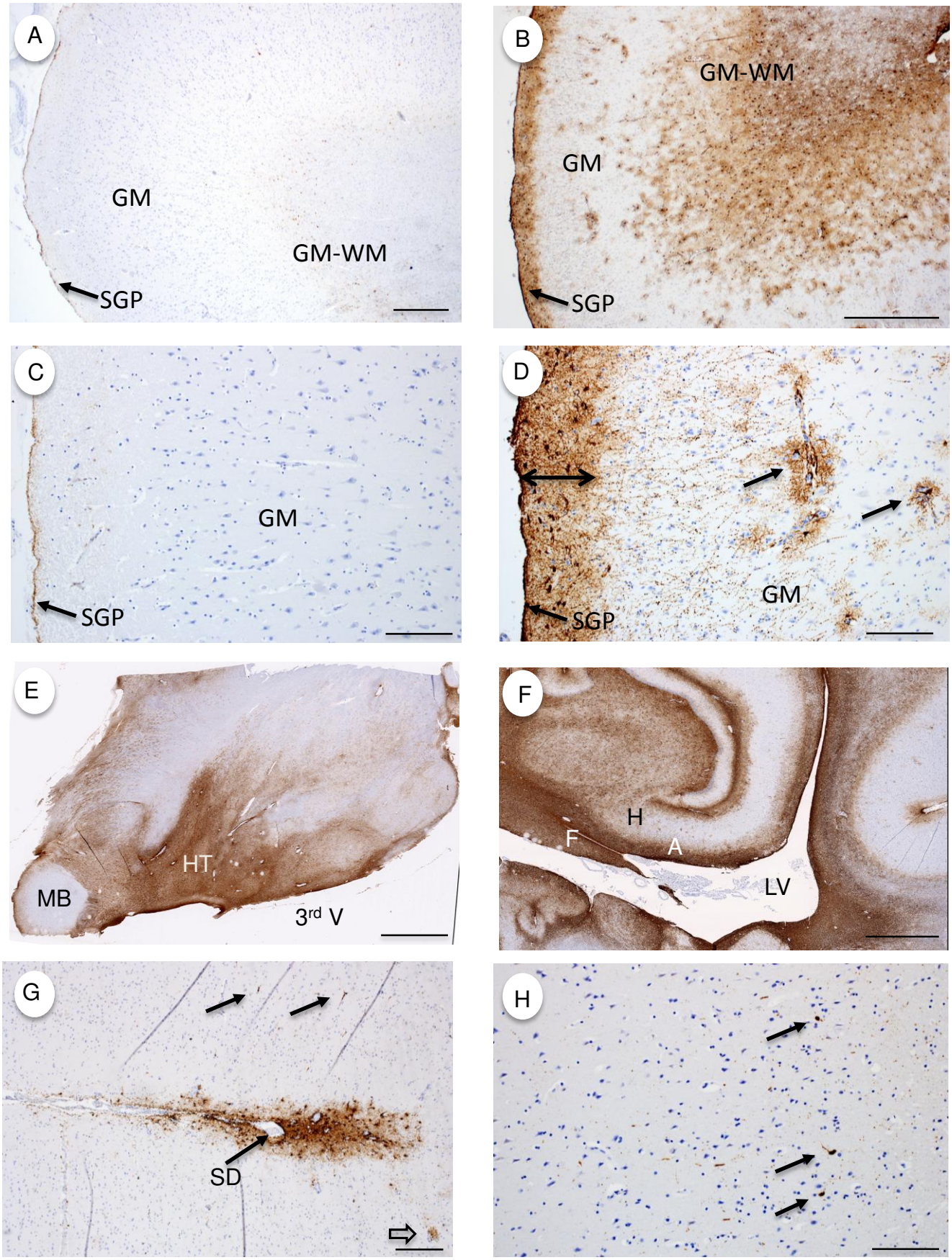
A
GM
GM-WM
SGP
B
GM-WM
GM
SGP
C
GM
SGP
D
GM
SGP
E
HT
MB
3rd V
F
H
F
A
LV
G
SD
H

Figure 1

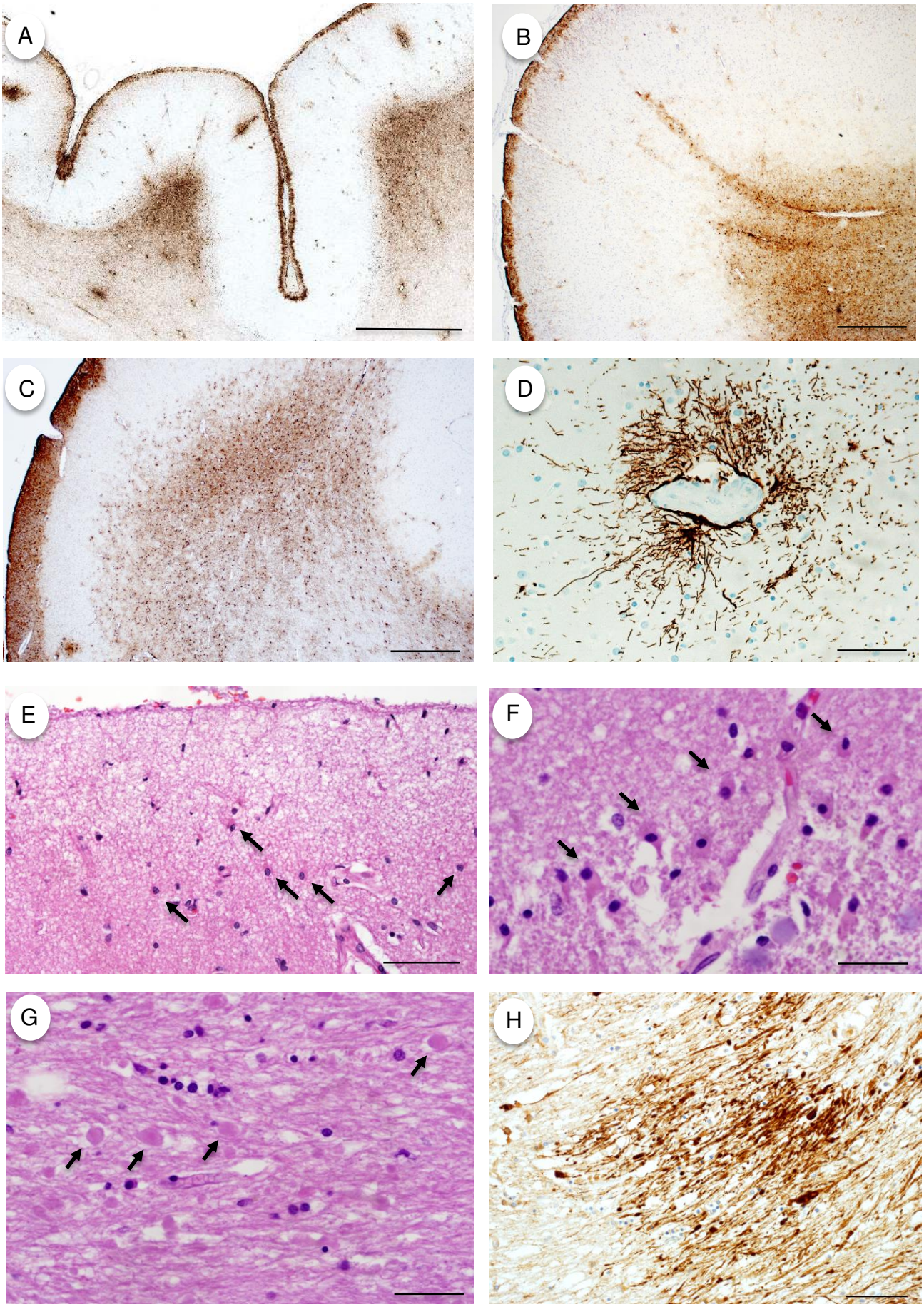

Figure 2

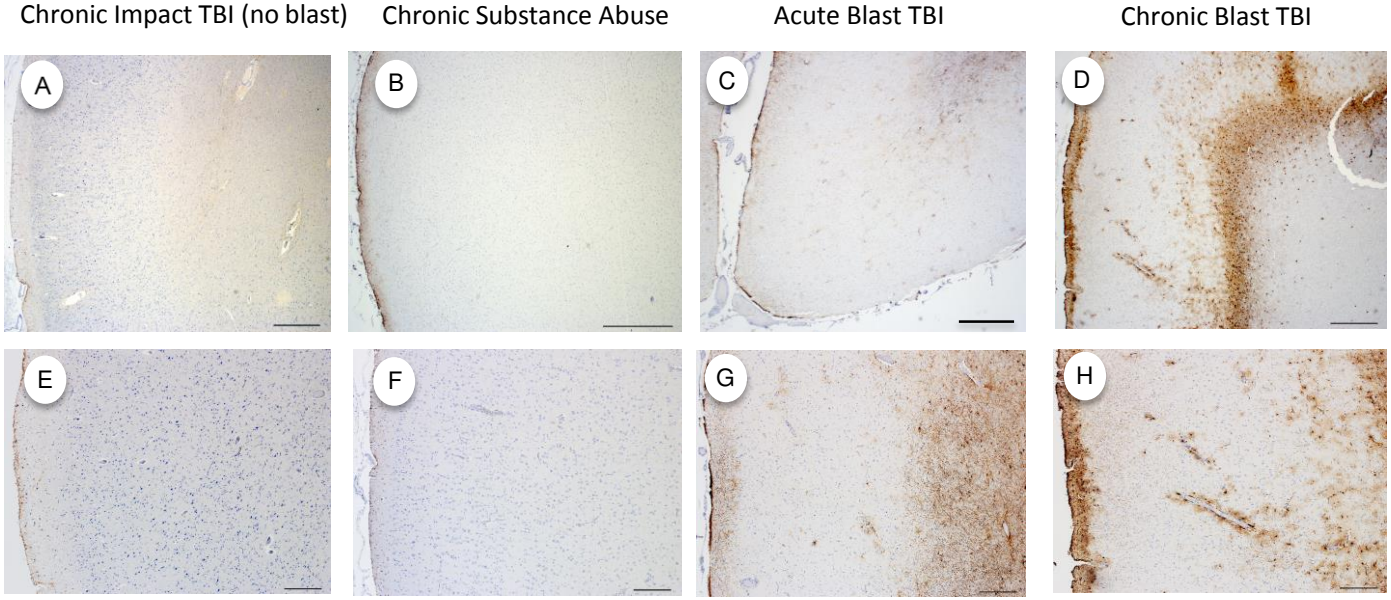
Chronic Impact TBI (no blast)
Chronic Substance Abuse
Acute Blast TBI
Chronic Blast TBI
A
B
C
D
E
F
G
H

Figure 3