Review

Neurotrauma: 2021 update

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Abstract

Despite the interruptions and restrictions to the progress of science that the COVID-19 pandemic has introduced, 2020 was marked by a number of important advances in the field of neurotrauma. Here, I will highlight what I believe are among the most important contributions. This year there were notable advances towards providing clinically useful information on neurotrauma outcome through the use of fluid biomarkers. I also introduce fascinating approaches to studying the role of microglia in nervous system repair and neuroinflammatory mechanisms leading to dysfunction through the use of colony-stimulating factor 1 receptor inhibitors, especially Plexxikon 5622 (PLX5622). Oral administration of this compound is able to deplete microglial elements and then, following withdrawal from the drug, a new population of microglia then repopulates the brain. Use of this approach in traumatic brain injury experimental models has produced important insights into the pathogenetic role of microglia in responding to this process. Important new data on the nature and distribution of tau involvement of neurons and astrocytes in cases of chronic traumatic encephalopathy (CTE) also appeared suggesting differences and similarities to Alzheimer's disease. Additionally, the use of tau-specific PET scan ligands in at-risk populations has suggested that this approach may be able to identify cases with CTE. Lastly, we note the death in the past year of a major contributor to the field of neurotrauma neuropathology, Professor J. Hume Adams.

Keywords: Traumatic brain injury, Chronic traumatic encephalopathy, Biomarkers, Neuroinflammation, Microglia, Tau

Introduction

For virtually all of us, 2020 will mostly be remembered as the year of COVID-19. With numerous calls for mask wearing, social distancing, partial-to-complete shutdowns of offices and laboratories (including our own) and other similar restrictions, this pandemic has definitely had a dampening effect on everyone's scientific productivity. Nevertheless, by viewing the traumatic brain injury (TBI) literature that appeared during 2020, it is hard to see any significant slowing of progress. I have the impression that with most scientists working within a telecommuting mode, rather than being active in the lab, many took the time to get to accumulated data waiting to be analyzed, write papers waiting to be written, etc., and thus scientific output did not appear to slow, and possibly even increased. It might be predicted that following the achievement of widespread vaccination allowing for a final full return to

our lab benches, there will likely be a lag in output as new experiments need to be put in place and additional data collected. Time will tell. Nevertheless, in surveying the field of neurotrauma, many important contributions were reported in 2020 and here I would like to pick some of what I believe were among the more important advances that were published. This is an individual viewpoint, reflecting my own biases, and I will apologize, in advance, if I have omitted any of your favorites on this year's list. So, among the more than 2,000 publications appearing in the literature on neurotrauma in 2020, here are those that I chose to highlight for you.

Contributions to the development of fluid biomarkers for TBI: Finally some approaches that appear to be useful

Over the past few years, a large number of groups have engaged in efforts to identify useful blood-based biomarkers in the field of TBI that hopefully would be able to reflect outcome, especially with respect to predicting a transition to clinically persistent sequelae. By and large, the results of these efforts have been relatively inconsistent and have not achieved robust clinical validity. However, in 2020 a group reported [1] that increases in exosome and plasma levels of neurofilament light (NfL) chain protein in patients with repeated mild traumatic brain injuries (mTBIs) were predictive of subsequent development of chronic post-concussion syndrome, post-traumatic stress disorder (PTSD) and depression. The study involved a cohort of 195 retired Service Members enrolled in the Chronic Effects of Neurotrauma Consortium Longitudinal Study. The cohort received detailed assessments to document and quantify episodes of prior mTBI and were grouped into repetitive mTBI (3 or more episodes), 1-2 mTBIs and controls with no TBI history. These assessments were obtained through interviews with the subjects. These data have particular importance, because they were obtained from retired Service Members, and thus their TBI exposures mostly occurred years prior to obtaining the biomarker assay.

The results reported suggest that their assays are reflecting a chronic, ongoing pathologic process that extended from the mTBI exposures to the time when the blood was drawn. The nature of that process remains unclear, although in their discussion the authors noted literature pointing towards the elevation of NfL levels in association with axonal injury. The use of isolated exosomes, brain-derived membrane-bound components that cross the blood-brain-barrier to enter the blood stream, is particularly noteworthy. Since these exosomes can be linked back to a central nervous system origin, it suggests that this approach is capable of providing indicators of more specific brain-related pathology and thus more closely reflect relevant biologic processes. Since the results were predictive for the development of depression and symptoms indicative of PTSD, this result represents another piece of evidence for a biologic substrate of the long-term persistent behavioral consequences of TBI.

Further advances in the blood biomarker field were reported by Okonkwo et al. [2] in a study where the predictive accuracy of determining glial fibrillary acidic protein (GFAP) levels was compared with S100 calcium-binding protein B (S100B) assays in the blood of TBI patients. The U.S. Centers for Disease Control and Prevention reports that each year about 4,800,000 evaluations for TBI occur in emergency departments in the United States. About 80-90% of these patients have mild forms of TBI (mTBI, as defined by a Glasgow Coma Scale score of 13-15) and only 10% of the mTBI patients will subsequently demonstrate abnormalities on computed tomography (CT) studies of the head and thus need further evaluation and monitoring. Accordingly, rapid reliably predictive studies are needed to lighten this huge clinical diagnostic load and better identify which patients need further CT scrutiny.

In the United States, assays of serum GFAP have been approved by the U.S. Food and Drug Administration (FDA) so that they can be used in a clinical setting to determine the need for a head CT within 12 hours of an episode of mTBI. Alternatively, in Europe, approval has been granted for S100B assays that are currently used to serve this clinical assessment function. Importantly, in the study now reported, plasma GFAP was determined using a newly developed point-of-care prototype assay that can be completed within 15 minutes. Serum S100B was determined by a more standard laboratory-based analytic method that requires transfer of the specimen

to the laboratory, adding considerably greater time to provide a report. The endpoint used for the 1,359 TBI patients in the study, who ranged from mild to more severe degrees of injury, was the ability to predict the presence of structural abnormalities on a head CT scan. Using a predetermined cut-off value for serum GFAP of 22 pg/mL, this study found that GFAP levels were an excellent predictor of CT abnormalities and could do so even on samples taken up to 24 hours from the time of trauma. GFAP assays substantially outperformed that of S100B as a CT abnormality predictor. Further, the mean level of GFAP correlated with increasing numbers of distinct lesions identified on head CT, suggesting a dose response. Indeed, patients with severe to moderate TBI (GCS 3-12) had 10-fold greater levels than the levels assayed on patients with mTBI (GCS 13-15).

Overall, the GFAP assay had a predictive sensitivity of 0.987. It should be pointed out that the point-of-care assay, with its rapid reporting time (15 minutes) is much faster than the currently FDA approved lab-based methodology and, by its nature, can be performed in obscure locations without access to laboratory facilities. It is my understanding that this new and faster technology is in the process of being evaluated by the FDA for approval as a clinical diagnostic tool. This promises to become an extremely valuable approach for clinicians faced with a staggering number of TBI patients to evaluate. A rapid point-of-care assay method will also be of great value for military medicine where many TBIs are clinically evaluated downrange in isolated battlefield locations. Assays on the playing field of contact sport events might also find this approach quite attractive for clinicians involved in such evaluations.

The role of microglia in repair of TBI

Microglia are abundant cellular constituents of the brain that play important roles in brain development, maintenance and the pathogenesis of most disease states. We are taught to consider microglia as playing a central role in virtually all nervous system repair processes, including that of TBI. Indeed, although the precise mechanism for their action remains unknown, microglia have been thought to mediate ongoing neuroinflammatory damage to the brain that is responsible for at least some of the long-term functional sequelae of TBI and even aspects of potential subsequent neurodegenerative phenomena. Recently, a series of drugs have been identified that inhibit colony-stimulating factor 1 receptor (CSF-1R), which microglia depend upon for their survival. These drugs penetrate the brain after oral administration and within two to three weeks are able to almost completely deplete the entire microglial cellular compartment of the brain in an exposed animal. Following withdrawal of the drug, there will be a steady repopulation of the brain by microglia (Figure 1). Importantly, the repopulated microglia appear to be a new rejuvenated population of cells without a prior history of antigen exposure. The primary agent used in such microglial repopulation experiments is referred to as Plexxikon (PLX) 5622.

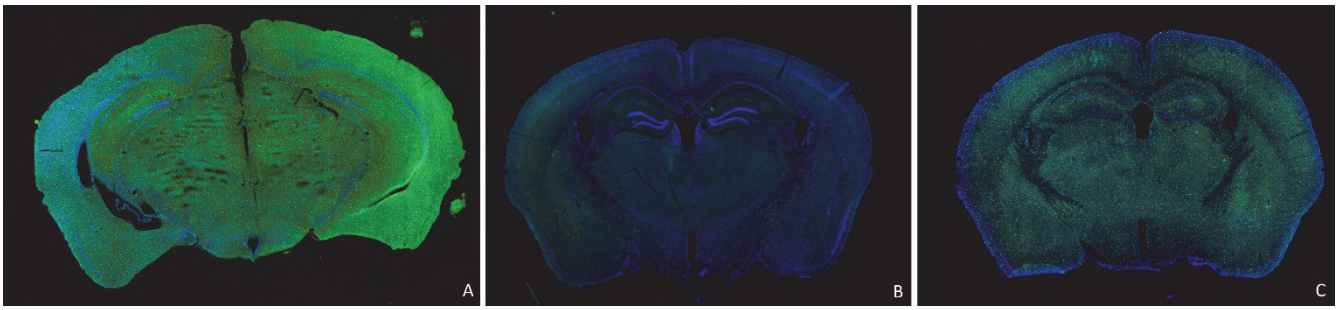

Figure 1. Microglia are depicted in 8 weeks old CX3CR1-GFP mice (microglia – green; DAPI – blue) A) control mouse, normal diet, B) 3 weeks following a diet containing 1200 mg/kg of PLX5622 (note virtual complete absence of microglia) and C) after 3 weeks on the PLX5622 diet then 2 weeks on the control diet (note the return of prominent green staining from the repopulated microglia). From ongoing studies of K. Whiting and Z. Galdzicki of Uniformed Service University - Neurosciences Program.

The use of drugs such as PLX5622 have begun to be used to explore the specific roles that microglia play in the repair processes and sequelae of experimental TBI, such as occurs with controlled cortical impact. Henry and colleagues [3] administered PLX5622 one month after controlled cortical impact in mice and then withdrew the drug to allow for microglial repopulation. By 3 months post-injury, they noticed that the PLX5622 treated animals possessed a smaller cortical lesion, reduced hippocampal neuron cell death and decreased expression of NOX2 and NLRP3 inflammasome-associated neuroinflammatory modulators, when compared to non-treated animals receiving equivalent controlled cortical impact injury. The PLX5622 treatment animals also showed improved long-term motor and cognitive function. These intriguing experiments demonstrated that removal of microglia in the chronic phase of TBI repair reduced subsequent neuroinflammation as well as lessening subsequent motor and cognitive functional deficits. Further, it showed that such inflammatory effects extended far longer than has been traditionally believed.

Willis and coworkers [4] also used PLX5622 for microglial depletion and repopulation in a mouse model of TBI (they too used closed cortical impact), however these workers showed that removal of microglia had little effect on the clinical outcome of their TBI model. Nevertheless, examination of the treated animals showed neuroprotective effects that appeared to aid in recovery. These beneficial effects were mostly modulated through interleukin-6 (IL-6) signaling via the soluble IL-6 receptor and its support of neurogenesis. These authors suggested that the presence of activated microglia associated with neurotrauma may not have a negative effect on outcomes and that it would appear that, as they state, “these cells lack an ability to support endogenous repair processes.” Obviously, more needs to be done to further dissect out the role that microglia play in the brain’s response to TBI. The timing of when the microglial removal and repopulation takes place in these models appears to be critical to the results obtained.

It may be predicted that the use of tools such as PLX5622 will greatly help in unraveling such issues. In the discussion, the authors wonder if PLX5622 might even represent a clinically feasible therapeutic approach to reducing some of the long-term complications of TBI. PLX5622 has received limited FDA approval for clinical use in patients with other non-TBI indications. For those who are interested in pursuing microglial depletion and repopulation, in a companion publication, this group has provided detailed protocols for such experiments in mice [5]. This would appear to be a valuable new tool for the investigation of the role of microglia, both positive and negative, in the pathogenesis of not only neurotrauma but many other disease processes [6, 7].

Studies of the long-term effects of sub-concussive blast exposure in breachers

The effects of blast TBI on the brain have mostly been studied either experimentally in small animal models or in humans following a single significant blast event, typically related to exposure to an improvised explosive device (IED). It should be kept in mind that, in the combat setting, blunt impact TBI also commonly occurs in conjunction with these blast injuries. Breaching represents a process where explosions are used to blast open doors and thus gain entry to buildings (Figure 2). As such, breaching is associated with blast exposure in the absence of impact trauma. Stone and colleagues [8] reported studies comparing a cohort of career breachers (typically breaching instructors) to a matched but minimally exposed control group. Of the 20 experienced breachers studied, they reported having experienced an average of 4,628 breaching blast exposures over their careers, as opposed to the control group (n=14) who experienced an average of 3 exposures. Keep in mind that these exposures are all considered to be sub-concussive in nature.

Using detailed neuroimaging approaches, they found, somewhat surprisingly, evidence of a significant degree of cerebral cortical thickening in the breacher group. This change was widespread, throughout the cerebral cortex. The nature of this

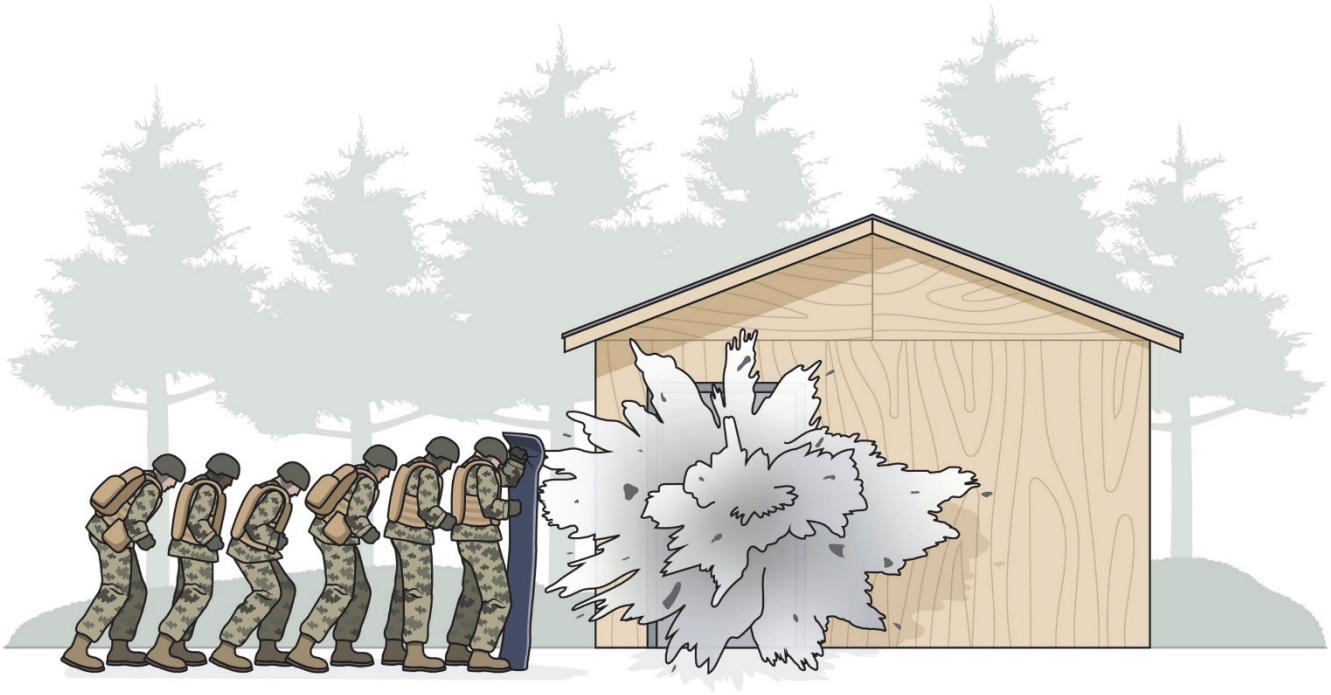

Figure 2. Breaching is a procedure whereby an explosive charge, typically placed on a door, is used by combatants to enter a building and engage the enemy. Service Members participate in breaching in both training exercises and on the field of battle. Repeated training exercises, such as portrayed here, expose participants to numerous sub-concussive blast wave exposures, in the absence of impact TBI. Courtesy of Sofia Echelmeyer.

cerebral cortical enlargement remains unclear, as no neuropathologic studies of deceased career breachers have yet to be reported. The authors also noted differences between the heavily exposed and control groups related to regional blood flow, various neuropsychological assessment results and serum biomarkers, however none of these differences survived Bonferroni correction for multiple comparisons. This suggested that further studies using a larger cohort would be instructive. Service Members carrying out other duties, such as those who spend a career firing high caliber artillery ordinances or explosive ordinance disposal personnel, may also have an equivalent degree of blast exposure, and it would appear that similar studies of these groups are warranted.

TBI-related neuroinflammatory markers

Neuroinflammatory response to TBI is a subject that has received considerable attention in both experimental models and clinical settings. Clinically, studies of cytokine expression have mostly focused on moderate to severe TBI patients which have demonstrated slightly elevated plasma cytokine levels in the acute phase post-injury. Post-mortem studies by Johnson and colleagues [9] have demonstrated that activated microglia can persist for decades after the initial traumatic incident. A recent study by Chaban and colleagues [10] published in 2020 looked at plasma levels of 12 different cytokines in a cohort including 207 patients with mTBI and 82 matched uninjured community controls. Plasma samples were drawn at admission for evaluation of the TBI, as well as 2 weeks, 3 months and 12 months following the injury. Brain magnetic resonance imaging (MRI) was also performed on all the participants. Comparing the mTBI group with the controls, they found significant elevations in plasma interferon gamma, IL-8, macrophage inflammatory protein-1 beta, monocyte chemoattractant protein-1, IL-17A, IL-9, tumor necrosis factor, and basic fibroblast growth factor at all time points, whereas a number of the other cytokines remained unchanged. The presence of persistent cytokine levels did not correlate with the MRI findings, suggesting that the cytokine changes were not related to the

extent of tissue damage. This is one of the few longitudinal studies of plasma neuroinflammatory markers of patients with mTBI that showed evidence of persistent systemic inflammation lasting up to a year.

The nature of intracellular *tau* accumulations in cases of chronic traumatic encephalopathy (CTE)

TBI, especially repeated impact TBI, constitutes a risk factor for the development of chronic traumatic encephalopathy (CTE), a tauopathy associated with neurofibrillary tangles (NFTs) and *tau* accumulation in astrocytes, primarily in the form of what are referred to as thorn-shaped astrocytes (TSAs). Arena and colleagues [11] reported a detailed immunohistochemical study on the nature of *tau* accumulations in NFTs and TSAs in cases of CTE and compared them to staining results obtained in several other forms of tau-related neurodegenerative disorders (Alzheimer's disease, frontotemporal dementia, Pick's disease) and other tau-related conditions seen in the elderly (age-related *tau* astrogliopathy, or ARTAG, and primary age-related tauopathy, or PART). This study showed that the NFTs of the CTE cases contained both 3R and 4R isoforms that are also classically seen in association with the tangles of Alzheimer's disease and PART. The TSAs of CTE stained virtually entirely for 4R *tau*, similar to what is seen in cases of ARTAG but distinct from what has been observed in Alzheimer's disease.

The use of antibodies directed towards various post-translational modifications of *tau* (primarily related to the presence of specific phosphorylation residues) also showed consistent similarities between the TSAs of CTE and those of Alzheimer's disease and ARTAG. Further, the authors employed recently developed anti-*tau* antibodies that are configuration-dependent [12] (GT-7 and GT-38) which showed that the NFTs in the depths of sulci in the cases of CTE were strongly immunoreactive, which is consistent with the staining reaction that is seen in the NFTs of Alzheimer's disease. However, in all but a few of the CTE cases, the astrocytes failed to stain with these antibodies. In their discussion, the authors noted the similarity between the *tau* immunophenotype seen in the NFTs of CTE and that of Alzheimer's disease and PART. In contrast, the *tau*-positive astrocytes of CTE were indistinguishable from those of ARTAG but distinctly different from the Alzheimer's disease results. This led to the suggestion that CTE and ARTAG may share pathogenetic mechanisms that separate these two diseases from that of Alzheimer's disease. Clearly, these are concepts that need to be explored further and may be of importance in sorting out the complex and confusing nosology and diagnostic criteria for these various neuropathologic entities. Obviously, additional work needs to be done and neuropathologists involved in this area will follow it with interest.

Animal models of CTE and the use of PET ligand for tau for clinical diagnosis of CTE

Further on *tau* and its association with repeated TBI, Dickstein and colleagues [13] investigated various biomarkers in rats that were experimentally exposed to repeated low-level blast overpressures as well as military Service Members who had been exposed to IEDs on the battlefield and subsequently suffered persistent behavioral, cognitive and/or memory complaints.[1] In the rats, six weeks following blast exposure, by Western blot, abnormally phosphorylated *tau* (Thr181, *p-tau*) was shown to be increased in the right anterior cortex and right hippocampus but not in contralateral locations. By ten months post-exposure, *p-tau* levels had further increased and were now more likely to be encountered bilaterally. Using immunohistochemistry, involved animals primarily showed fine dendritic staining although some did show perikaryal accumulations, a most unique observation among repeated TBI experimental rodent models.

The human military cases were studied by PET scanning using [^{18}F]AV45 (flortaucipir), a PET ligand that is reported to be selective for *tau*. Half of the ten blast-exposed Service Members receiving PET scans showed excessive accumulation of the ligand at gray-white matter junctions in frontal, parietal and temporal regions, a pattern the authors interpreted to be "a typical localization of CTE tauopathy."

[1] The first author on this paper, Dr. Dara Dickstein, works with me at the Uniformed Services University and several of the other authors have been colleagues of mine on other TBI-related studies. While I am happy to recognize their contribution here, I don't believe this seriously affects my decision that this paper deserves to be discussed among the more important publications to appear in the neurotrauma literature in 2020.i

In addition, levels of plasma NfL were elevated in the blast-exposed subjects who showed excess [^{18}F]AV45 retention on PET analysis.

This study suggests the potential value of the rat as an experimental model of *tau* accumulation following repeated blast exposure. This stands in direct contrast to the mostly negative findings in mice exposed to repetitive impact TBI. The reported results also point to the potential for further use of *tau* PET ligands in the study of human subjects at risk for CTE. Of note, since in the human studies they engaged living patients, no opportunity for neuropathologic confirmation of the proposed diagnosis of CTE could be made.

Follow-up from last year's Neurotrauma 2019 report

In my contribution last year, I highlighted a paper suggesting that the use of tranexamic acid treatment showed a significant lowering of mortality in a very large randomized placebo-controlled clinical trial in patients with more severe forms of acute TBI [14]. I felt this was important in view of the dismal record, despite numerous attempts, for introducing therapeutic agents with evidence-based positive results in the treatment of TBI patients. At the time, I was encouraged by the size of the study and their positive results. Since tranexamic acid appears to be quite safe and is a very inexpensive drug, I was further encouraged by this approach. I also used this publication to highlight the importance of vascular pathology and bleeding in TBI pathophysiology, morbidity and mortality. Despite my optimism over the positive results of prehospital administration of tranexamic acid in TBI patients, in 2020, Bossers et al. [15] reported the results of a multicenter cohort study of using the drug to treat 1,827 severe TBI patients. This new study showed that prehospital administration of tranexamic acid produced increased mortality in those patients receiving the drug. Of course, one negative study does not settle matters here, but these results suggest that care must be taken before proceeding in this direction. It would appear that, once again, those who seek an effective therapy for TBI patients are left with few, if any, therapeutic approaches showing efficacy. The toolbox for clinicians dealing with the effects of TBI continues to remain virtually empty of drugs that carry evidence of improved clinical outcome.

Finally

For those of us with an interest in the neuropathology of neurotrauma, there is one additional event that occurred in 2020 that I felt was important to note. On April 12, 2020, Professor James Hume Adams died [16]. Professor Adams was a pioneering, major contributor to our understanding of the neuropathology of neurotrauma, spending virtually all of his career studying the disorder. He trained in neuropathology at the Institute of Psychiatry at the Maudsley Hospital in London, where he initially came in contact with several people with an interest in neurotrauma, namely Sabina Stritch, Peter Daniel and J.A.N. Corsellis. It is clear that the seeds for his life-long interest in neurotrauma were planted at this time. He subsequently took a position in Glasgow at the Department of Pathology, Western Infirmary, eventually moving to the new facility at the Southern General Hospital. While in Glasgow, he developed and ran the Glasgow Database of Human Head Injury, and its brain bank repository has represented a major resource for the study of the effects of impact TBI on the human brain. Importantly, this unique facility represents one of the only available collections in which one would be able to characterize the long-term effects of impact TBI. Over the years, this facility has been extensively used by the Glasgow group, now under the direction of Dr. Willie Stewart. Professor Adams was best known for his seminal contributions in describing and then further characterizing diffuse axonal injury (DAI), both clinically and experimentally. Much of this work was carried out in conjunction with colleagues in Glasgow and at the University of Pennsylvania. The critical concepts related to the effects of trauma on the brain that he first proposed continue to be understood and used to this day. Although in more recent years he had been in retirement as an Emeritus Professor, the influence of this work continues and according to his obituary, the 60 papers he published with the University of Pennsylvania group on neurotrauma have been cited more than 9,000 times. In 2020, we lost a towering figure who contributed greatly to the field of neurotrauma.

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