Published in final edited form as:
Exp Neurol. 2013 August ; 246: 35–43. doi:10.1016/j.expneurol.2012.01.013.

Axonal Pathology in Traumatic Brain Injury

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Abstract

Over the past 70 years, diffuse axonal injury (DAI) has emerged as one of the most common and important pathological features of traumatic brain injury (TBI). Axons in the white matter appear to be especially vulnerable to injury due to the mechanical loading of the brain during TBI. As such, DAI has been found in all severities of TBI and may represent a key pathologic substrate of mild TBI (concussion). Pathologically, DAI encompasses a spectrum of effects from primary mechanical breaking of the axonal cytoskeleton, to transport interruption, swelling and proteolysis, through secondary physiological changes. Depending on the severity and extent of injury, these changes can manifest acutely as immediate loss of consciousness or confusion and persist as coma and/or cognitive dysfunction. In addition, recent evidence suggests that TBI may induce long-term neurodegenerative processes, such as insidiously progressive axonal pathology. Indeed, axonal degeneration has been found to continue even years after injury in humans, and appears to play a role in the development of Alzheimer's disease-like pathological changes. Here we review the current understanding of DAI as a uniquely mechanical injury, its histopathological identification, and its acute and chronic pathogenesis following TBI.

Keywords

Axon; Diffuse axonal Injury; DAI; axonal pathology; traumatic brain injury; TBI; neurodegeneration; head injury; rotational acceleration; microtubule

Introduction

Although historically ignored as a major health issue, traumatic brain injury (TBI) is a leading cause of morbidity and mortality internationally, with significant socio-economic implications. In the US alone, over 1.7M individuals suffer a TBI each year (Faul, et al., 2010) at an estimated cost of over $60 billion (Finkelstein E, et al., 2006). Moreover, there is considerable evidence indicating just a single TBI may be associated with the later onset of neurodegenerative disorders, including Alzheimer's disease (AD) (Fleminger, et al., 2003, Graves, et al., 1990, Guo, et al., 2000, Johnson, et al., Molgaard, et al., 1990, Mortimer, et

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al., 1985, Mortimer, et al., 1991, O'Meara, et al., 1997, Plassman, et al., 2000, Salib and Hillier, 1997, Schofield, et al., 1997).

While the neuropathological consequences of TBI are heterogeneous, one of the most common across all severities of closed head injury is diffuse axonal injury (DAI) (Adams, et al., 1989, Adams, et al., 1982, Graham, et al., 1988, Povlishock, 1992, Povlishock and Becker, 1985, Povlishock, et al., 1983, Povlishock and Katz, 2005, Smith and Meaney, 2000), which may reflect the selective vulnerability of white matter axons to damage from mechanical loading of the brain during rapid head accelerations. After TBI, axonal degeneration arising from DAI is conventionally recognized as a progression from disruption in axonal transport leading to axonal swelling followed by secondary disconnection and, finally, Wallerian degeneration. Traditionally this process was thought to be limited to the acute and sub-acute periods following trauma. However, recent evidence has identified axonal degeneration in human brain material years following injury, suggesting TBI may precipitate a progressive, long-term neurodegenerative process, in part reflected in axonal degeneration (Chen, et al., 2009). Of particular note, axonal pathology may have a role in the development of Alzheimer-like pathologies both in the acute phase following injury as well as with longer term survival (Chen, et al., 2009, Chen, et al., 2004, Johnson, et al., 2010, Marklund, et al., 2009, Smith, et al., 2003, Smith, et al., 1999, Smith, et al., 2003, Stone, et al., 2002, Tran, et al.). Here we explore the current understanding of the short- and long-term pathological sequelae of axonal degeneration following TBI.

Historical Perspective of DAI

Classical descriptions of diffuse axonal injury are of a clinicopathological syndrome manifest as a patient unconscious from the time of injury where, on subsequent autopsy examination of the brain, there is widespread axonal injury in the cerebral hemispheres, cerebellum and brainstem. Approaching the middle of last century, the pathological appearances of trauma related axonal injury were first described in human tissue where, in addition to the previously well-known macroscopic focal lesions, microscopic examination revealed TBI could also induce widespread, subtle and yet seemingly important pathological changes throughout the brain parenchyma (Rand and Courville, 1946). Apparently unique to TBI, it was proposed that these pathological white matter changes occur as a direct consequence of the mechanical forces experienced at the time of injury.

This hypothesis evolved from experimental observations by Holbourn who induced rapid rotational forces on gelatin molds of brain sections and observed that rotational accelerations, at levels akin to those calculated for head rotations in human TBI, could induce widely distributed shear and tensile strains throughout the surrogate brain material (Holbourn, 1943, Holbourn, 1945). Following detailed histopathological examination of tissue from severely injured TBI patients, it was proposed that these shear strains at the moment of injury were likely to be responsible for the observed morphological alterations in white matter (Strich, 1956, Strich, 1961).

Further neuropathological assessments emerged in support of this concept, with axonal pathology identified at very early time points post-injury (Nevin, 1967). In addition, it became clear that the presence of swollen axonal profiles could be identified across a range of injury severities (Oppenheimer, 1968, Peerless and Rewcastle, 1967), though in these early studies, the injuries would now be regarded as in the moderate to severe range (i.e. Glasgow coma scales at presentation less than 13).

Subsequently, Adams and colleagues performed extensive characterization of the extent and distribution of axonal pathology in a large series of TBI cases and introduced the now universally recognized term "diffuse axonal injury" (Adams, et al., 1982, Gennarelli, et al.,

1982). In addition to confirming the presence of axonal pathology in patients dying shortly after a moderate to severe injury, DAI was identified following various causes of injury including rapid acceleration/deceleration, such as can occur in motor vehicle collisions, and direct impacts, such as from falls or assaults (Gennarelli, et al., 1982). It was these data that led Adams and colleagues to develop and refine a grading system for DAI based on the extent and distribution of pathology (Adams JH, 1989, Adams, et al., 1982).

Diagnosis of DAI, other than through histopathological examination, has remained a major challenge. As a consequence of its microscopic and disseminated nature, the axonal pathology of DAI is not readily discernable with standard non-invasive techniques, such as conventional CT or MRI. Therefore, this 'stealth' pathology is often missed or regarded as a diagnosis of exclusion based on symptoms in the absence of overt changes with conventional neuroimaging following TBI. To further complicate matters, the term "diffuse axonal injury" is itself somewhat of a misnomer, since technically the distribution is not diffuse, but is instead stereotypically multifocal; preferentially involving midline white matter tracts such as the corpus callosum, internal capsules, brainstem and cerebellar peduncles (Adams JH, et al., 1989, Adams, et al., 1991). Moreover, the term diffuse axonal injury has occasionally been adopted in other fields where widespread white matter pathology may be encountered, such as demyelinating diseases and in reference to hypoxic/ischemic injury. However, while there have been efforts to underline the etiology of the axonal injury in trauma, using terms such as "diffuse traumatic axonal injury (dTAI)" or "traumatic axonal injury (TAI)", the clinical designation in relation to trauma induced diffuse axonal damage remains "DAI", which will be the terminology used in this review.

DAI: Pathological Features and Identification

A primary outcome of dynamic deformation of white matter tracts during trauma is the interruption of axonal transport, resulting in accumulation of transported materials as axonal swellings within just hours of trauma (Christman, et al., 1994, Povlishock and Becker, 1985, Smith, et al., 1999). Commonly, these swellings appear in a periodic arrangement along the length of an axon at the site of injury, classically referred to as "axonal varicosities" (Figure 1a,c-d). A more widely recognized, but not necessarily more common, axonal pathology found shortly after TBI is a large single swelling described as an "axonal bulb" (previously referred to as a "retraction ball"), which likely represents complete axonal disconnection (Figure 1a-b) (Adams, et al., 1989, Adams, et al., 1984, Adams, et al., 1982, Cajal, 1928, Chen, et al., 1999, Povlishock, 1992, Povlishock and Becker, 1985, Povlishock, et al., 1983, Povlishock, et al., 1999, Povlishock and Katz, 2005, Rand and Courville, 1946, Smith and Meaney, 2000, Smith and Meaney, 2000, Smith, et al., 2003, Strich, 1956).

The histopathological identification of DAI is dependent upon the visualization of abnormal axonal profiles as described above. Historically, standard tinctorial stains, such as Hematoxlylin and eosin (H&E), and various silver impregnation techniques, such as Palmgren's, were successfully applied to identify damaged axons. However, the introduction of immunohistochemical methods revealed that such techniques often under-represent the extent of axonal pathology and are limited in their capacity to identify damaged axons in individuals with very short survival times. Following immunohistochemical examination of multiple candidate proteins accumulating in injured axons (Grady, et al., 1993, Gultekin and Smith, 1994, Ng, et al., 1994, Sherriff, et al., 1994), immunoreactivity to amyloid precursor protein (APP) has emerged as a highly sensitive and robust technique for the detection of DAI (Gentleman, et al., 1993, Sherriff, et al., 1994) (Figure 1). Transported by fast axonal transport, APP may be identified accumulating in damaged axons within 2 hours following injury. Moreover, when directly compared to silver impregnation techniques, APP staining reveals far more extensive axonal pathology (Gentleman, et al., 1995). As such, APP

immunohistochemistry remains the gold-standard for the clinico-pathological identification of axonal pathology. However, accumulation of APP in axons is not exclusive to trauma and has been described following other mechanisms of brain injury including hypoxic/ischemic injury (Graham et al 2004; Reichard et al 2005). In this regard, careful assessment of the extent and distribution of pathology, as well as its evolution in relation to survival time, is critical in the assessment of DAI.

While diagnostic confirmation of DAI is currently only possible with histopathological examination of post-mortem brain tissue, increasing evidence suggests novel advanced neuroimaging techniques may be useful in the assessment of white matter tracts in vivo. In particular, diffusion tensor imaging (DTI) has emerged as a promising technique for assessing white matter integrity via the measurement of the anisotropic diffusion of water molecules (for review see (Hunter, et al., 2011). Indeed, early evidence indicates DTI may be useful in assessing patients with even mild TBI (Bazarian, et al., 2007, Bazarian, et al., Mayer, et al., Miles, et al., 2008, Wilde, et al., 2008). However, considerable work remains before DTI can be reasonably included as part of the routine clinical diagnosis of TBI. Notably, it remains unclear what the neuropathological substrates are for changes observed using DTI, and thus whether findings are representative of the axonal pathology of DAI.

DAI Genesis and the Link with Coma and Transient Loss of Consciousness

Animal models have been instrumental in confirming that the principal mechanical force responsible for DAI is rotational acceleration of the brain, resulting from unrestricted head movement inducing dynamic shear, tensile, and compressive strains within the tissue (Gennarelli, et al., 1982, Meaney, et al., 1996, Ommaya and Hirsch, 1971, Smith, et al., 1997, Thibault, et al., 1990). As such, the size of the human brain plays an important role in the development of DAI, as the substantial mass effects occurring during injury can result in high shear strains between regions of tissue (Smith and Meaney, 2000, Smith, et al., 1996). Few clinically relevant models of DAI in gyrencephalic animals have been characterized. This reflects the difficulty of developing a model system that replicates the dynamics of diffuse injury, such as the inertial loading conditions produced in automotive crashes or at the moment of head impact (Smith, et al., 1996, Smith, 2003).

The first model of DAI was developed at the University of Pennsylvania in the 1980s by Gennarelli and colleagues (Gennarelli, et al., 1982). In landmark studies, they demonstrated that head rotational acceleration in non-human primates could induce extensive axonal pathology throughout the white matter with identical characteristics to human DAI. Moreover, Gennarelli and colleagues found that DAI was responsible for immediate and prolonged post-traumatic coma, independent of a mass lesion. Non-human primates were originally chosen due to their large brain mass with extensive white matter domains, similar to the human brain. Nonetheless, despite their relatively sizable brains (approximately 95g), far greater rotational accelerations were necessary to generate the same tissue deformations calculated for the larger human brain in TBI (Holbourn, 1943, Margulies, et al., 1990). These data were the first to conclusively link dynamic mechanical deformation of the brain during trauma with the selective pathology of DAI. In addition, the concurrence of DAI with immediate and prolonged coma in this animal model was directly extrapolated to transform the clinical diagnosis of DAI in TBI patients.

Subsequently, using the same injury device, a similar model was developed using miniature swine which have gyrencephalic brains of similar size to non-human primates (Meaney, et al., 1995, Smith, et al., 1997). Studies using this model demonstrated that the plane of head rotational acceleration in reference to the brainstem is critical in determining the induction and duration of loss of consciousness following injury. Specifically, rotation, transverse to

the brainstem, was associated with coma, whereas equivalent rotation circumferential to the brainstem was not (Smith, et al., 2000). Notably, even at mild parameters of injury, loss of consciousness was induced following rotations transverse to the brainstem (Browne, et al., 2011). The duration of loss of consciousness or coma following injury was directly related to the extent of axonal pathology in the brainstem, indicating brainstem damage is a key anatomic substrate for immediate loss of consciousness in TBI (Smith, et al., 2000). However, when the brainstem was relatively spared, even extensive axonal pathology throughout the hemispheric white matter produced little or no loss of consciousness. Thus, while there is a link between DAI and immediate post-traumatic loss of consciousness, it appears that the distribution, rather than the overall extent, of axonal pathology is important in determining consciousness immediately following TBI (Browne, et al., 2011).

Potential Primary Mechanical Damage due to Axonal Trauma

With evidence that mechanical damage to axons can directly account for clinical symptoms, the biomechanical nature of TBI was increasingly recognized as an important and unique feature. Specifically, the viscoelastic properties of the brain emerged as a potential liability during the rapid mechanical loading conditions of TBI. White matter axons appear especially vulnerable to injury under such circumstances, potentially as a result of their highly anisotropic arrangement and/or their inherent structural design. In normal circumstances, axons are compliant and ductile under stretch, readily relaxing back to their original length when the stretch is removed. However, with rapid application of tissue strain, such as at the moment of head impact, axons behave differently, essentially becoming brittle (Smith and Meaney, 2000, Smith, et al., 1999). Nonetheless, disconnection of axons at the moment of injury, known as “primary axotomy”, is considered a relatively rare occurrence. Instead, in the majority of cases, the swelling that follows cytoskeletal disruption can induce “secondary axotomy” (Christman, et al., 1994). Due to the critical role of mechanical damage in the development of DAI, evaluation of the effects of dynamic tissue deformation on the axonal cytoskeleton during trauma has been the subject of various avenues of investigation both from a structural and functional perspective (Chung, et al., 2005, Jafari, et al., 1997, Jafari, et al., 1998, Maxwell, et al., 2003, Maxwell and Graham, 1997, Maxwell, et al., 1999, Maxwell, et al., 1995, Pettus and Povlishock, 1996, Povlishock and Pettus, 1996, Staal, et al., 2009, Staal, et al., 2010).

Direct evidence of primary mechanical damage of axons has been shown using an *in vitro* model of dynamic stretch injury of micropatterned axons spanning two populations of cortical neurons (Iwata, et al., 2004, Smith, et al., 1999, Stys and Jiang, 2002, Tang-Schomer, et al., 2010, Wolf, et al., 2001). Within seconds of dynamic axonal stretch, axons temporarily become undulated and misaligned due to loss of elasticity and underlying cytoskeletal damage (Smith, et al., 1999). Notably, axonal undulations are also a common feature of acute TBI in humans, suggesting primary cytoskeletal failure due to mechanical trauma (Tang-Schomer, et al., 2011).

Recently, in vitro studies have shown that primary breaking of axonal microtubules underlies the observed posttraumatic axonal undulations. Specifically, twisting and misalignment of broken microtubules at multiple sites along injured axons appears to impede relaxation of axons back to their original straight orientation. Although subsequent depolymerization of the microtubules from the break points can allow gradual relaxation of the axons, it comes at a cost, by interrupting axonal transport and inducing progressive swellings and degeneration (Tang-Schomer, et al., 2010). These observations may explain the apparent loss of axonal microtubules previously found in a feline model of TBI and a guinea pig model of dynamic optic nerve stretch injury (Maxwell and Graham, 1997, Pettus and Povlishock, 1996). Notably, by manipulating microtubule stability in the in vitro stretch-

injury model using the microtubule-stabilizing drug Taxol, subsequent axonal degeneration post-injury could be mitigated (Tang-Schomer, et al., 2010).

In addition to the inherent vulnerability of white matter axons to damage in TBI, there is a differential sensitivity of axon subtypes. Indeed, several studies indicate that myelinated fibers are more tolerant to mechanical strains compared to unmyelinated fibers, with both in vivo and in vitro TBI models (Reeves, et al., 2007, Reeves, et al., 2005, Staal and Vickers, 2011). Specifically, in vivo, smaller unmyelinated axons were found more likely to suffer irreversible dysfunction of conduction pathways, while an in vitro model of axon stretch-injury showed that non-myelinated axons were more prone to secondary disconnection when compared to myelinated axons. However, it remains unknown how these differences are related to structural variation between these populations of neurons.

These observations provide a glimpse of the mechanical genesis of selective axonal pathology following trauma leading to cytoskeletal failure and disconnection. Ultimately, it is thought that disconnected axons undergo Wallerian degeneration. However, the possibility that many swollen or otherwise damaged axons may undergo repair remains an intriguing concept. Conceivably, axonal repair could range anywhere from restoration of simple ionic homeostasis through direct replacement of the damaged cytoskeleton such as turnover of microtubules or neurofilaments (Chen, et al., 1999, Tang-Schomer, et al., 2011). For example, it was recently demonstrated that the periodic swellings that comprise axonal varicosities represent a form of “partial interruption of axonal transport” resulting from a staggering of break points between microtubules within axons. This damage at the level of individual microtubules induces only limited derailment and accumulation of transported cargoes at periodic regions of the axon, thereby creating the varicose appearance (Tang-Schomer, et al., 2011). Thus, if none of the swellings grow to the point of inducing disconnection, repair of the microtubule lattice may provide an opportunity for injured axons to contend with the residual protein accumulation. Examination of the mechanisms of axonal repair after trauma will be important for future considerations of therapeutic interventions.

Secondary Chemical Cascades Following TBI

During TBI, all axons within a white matter tract are thought to suffer relatively similar dynamic deformations. Yet, even in severe TBI, only a small percentage of axons within a given tract undergo transport interruption as classically identified by accumulation of transported cargoes in swellings. For the remaining axons that do not display appreciable interruption to transport following dynamic deformation, they may nonetheless suffer important pathophysiological changes capable of contributing to axonal dysfunction, such as the altered conduction velocities observed in animal models, even in mTBI (Baker, et al., 2002, Reeves, et al., 2007, Reeves, et al., 2005). Such secondary cascades may result in degeneration of axons or, conversely, render axons viable yet functionally impaired.

A multitude of diverse secondary cascades detrimental to axons have been investigated following TBI. Specifically, alterations to mitochondria, including mictochondrial permeability transition are potentially detrimental to normal energy metabolism and thus axonal integrity (Buki, et al., 1999, Maxwell, et al., 2003, Okonkwo and Povlishock, 1999). In addition, oxidative stress and lipid peroxidation have long been implicated in the pathophysiology of TBI and have been associated with both mitochondrial dysfunction and cytoskeletal degradation in vivo, a process that can be mitigated via treatment using free radical scavengers (Deng, et al., 2007, Deng-Bryant, et al., 2008, Fujita, et al., Mustafa, et al., Mustafa, et al.). Increasing evidence suggests that neuroinflammation and microglial activation in the white matter may also contribute to cellular damage (Loane and Byrnes,

2010) and remarkably, can persist for even years after injury in humans (Chen, et al., 2009, Gentleman, et al., 2004).

Of particular note, ionic imbalance after axonal trauma has been thought to play a central role post-injury in both axonal degeneration and the persistent dysfunction of otherwise intact axons. Specifically, it was long-suspected that elevated intra-axonal Ca2+ levels ([Ca2+]i) play a pivotal role in the secondary damage to axons following mechanical deformation (Banik, et al., 1987, Benz, et al., 1997, Buki, et al., 2003, Buki, et al., 1999, Gitler and Spira, 1998, Ichiya, et al., 1991, Maxwell, et al., 1999, Maxwell, et al., 1995, Maxwell, et al., 1991, Povlishock, 1992, Povlishock, et al., 1999, Wolf, et al., 2001). Maxwell and colleagues found indirect evidence of post-traumatic calcium influx into axons via changes in calcium-ATPase activity after optic nerve stretch injury (Maxwell, et al., 1999, Maxwell, et al., 1995, Maxwell, et al., 1991). Subsequently, using an in vitro axon stretch model, direct visual evidence of calcium entry into axons was seen immediately following trauma (Wolf, et al., 2001). Surprisingly, this post-traumatic rise in [Ca2+]i was found to be dependent on entry of sodium via voltage-gated channels and reversal of the Na +/Ca2+ exchanger (Iwata, et al., 2004). Recent work using another in vitro axon stretch injury model indicates that acute increases in [Ca2+] may also, in part, originate from release of intracellular stores of [Ca2+](Staal, et al., 2010). In addition, both in vitro and in vivo models have shown that increases in the [Ca2+] - activated protease, calpain, was demonstrated to cause a range of subtle to catastrophic damage to the axonal cytoskeleton and ion channels (Buki, et al., 1999, Huh, et al., 2006, Iwata, et al., 2004, Kampfl, et al., 1996, Kupina, et al., 2001, McGinn, et al., 2009, Saatman, et al., 2003, Saatman, et al., 1996, Saatman, et al., 1996, von Reyn, et al., 2009). For example, activated calpain appears responsible for degradation of the inactivation gate of sodium channels, which can cause a deleterious feed-forward process of unmitigated sodium influx, in turn, inducing progressively increasing intraaxonal calcium levels and related pathogenesis (Iwata, et al., 2004, von Reyn, et al., 2009). Inhibition of either calpain and another calcium-activated enzyme, calcineurin was found to mitigate axonal degeneration following injury in vivo (Marmarou and Povlishock, 2006, Reeves, et al., 2007, Saatman, et al., 2000, Singleton, et al., 2001), the latter of which may additionally promote recovery via the promotion of axonal sprouting observed following stretch injury in vitro (Staal, et al., 2010).

Limited clinical and experimental evidence suggests that demyelination may also play an important role in the pathophysiology of TBI. Experimental studies demonstrate disruptions of the myelin sheath acutely following stretch-injury of the guinea pig optic nerve (Maxwell, et al., 2003, Maxwell, et al., 1999). In addition, histochemical analysis of the rat brain following fluid percussion injury showed a loss of myelin staining as indicated by luxol fast blue in association with progressive white matter atrophy up to 1 year post injury (Bramlett and Dietrich, 2002). Similar histochemical analysis of DAI in humans revealed acute myelin globoids in association with axonal pathology (Ng, et al., 1994). However, it is unclear whether damage to the myelin sheath occurs only as a direct consequence of axonal degeneration. Interestingly, apoptotic oligodendrocytes have been observed acutely and chronically following TBI clinically (Shaw, et al., 2001). Such a loss of oligodendrocytes may result in insufficient myelination and potentially compromise the integrity or function of axons. Examination of the time course and mechanistic basis of demyelination following TBI will be an important future consideration.

Evidence for persistent Axonal Degeneration following TBI

Although both overt and subtle pathological changes to axons may play a role in the immediate loss of consciousness and/ or cognitive dysfunction that characterizes TBI, the relative contributions of differing forms of axonal pathologies over time have yet to be

determined. Using APP as a marker of DAI, axonal pathology is observed to increase to a peak in the initial 24 hours following injury, thereafter leveling off (Gultekin and Smith, 1994). Although this represents the peak of pathology, damaged axons have been observed weeks to months and, in a small number of cases, even years following TBI (Blumbergs, et al., 1989, Chen, et al., 2009), perhaps heralding a persistent white matter degeneration instigated by TBI in a proportion of cases. In support of these observations, neuroradiological evidence indicates selective white matter loss post-injury (Gale, et al., 1995). Notably, in the limited descriptions of axonal pathology in long-term survivors thus far, damaged axons displayed the phenotype of axonal bulbs, potentially representing complete disconnection, rather than that of varicose, connected axons (Chen, et al., 2009), indicating a distinct mechanism. However, appropriately extensive characterisation of long-term axonal pathologies has yet to be performed.

It is true that there may be many other subtle pathological changes that affect functional outcome, such as dendritic alterations, imbalances in neurotransmitters, or changes in brain metabolism, as previously suggested (Monnerie, et al., 2010, Yuen, et al., 2009). These considerations highlight potential difficulties in designating all subtle pathological changes following traumatic injury as “DAI”. Further investigation of the role that chronic axonal pathologies play in neurodegeneration is of particular interest in light of emerging observations between TBI and syndromes of cognitive impairment including Alzheimer’s disease (AD).

TBI and the Link with Neurodegenerative Diseases

The association between TBI and chronic progressive neurodegeneration emerged from observation of boxers at the turn of last century when the term “punch drunk syndrome” was first used to describe a progressive dementing disorder in participants of the sport (Martland, 1928). Now called dementia pugilistica, the disorder is believed to occur as a consequence of repetitive mild TBI and is neuropathologically characterized by abnormal intracellular accumulations of hyperphosphorylated forms of the microtubule associated protein tau, known as neurofibrillary tangles (NFTs) and neuropil threads (NTs). (Braak and Braak, 1991, Corsellis, et al., 1973, Dale, et al., 1991, Forman, et al., 2004, Geddes, et al., 1999, Roberts, et al., 1990, Selkoe, 2001, Tokuda, et al., 1991). Extracellular AD-like beta-amyloid (Aβ) plaques and vascular Aβ deposits later emerged as a potentially important finding (Roberts, et al., 1990, Tokuda, et al., 1991). While much of this work has focused on boxers, recent evidence suggests that repetitive TBI experienced from playing professional American Football can result in increased rates of late-life cognitive impairment (Guskiewicz, et al., 2005). Furthermore, small selected studies examining the pathological studies of the brains of former players in the National Football League in the USA revealed NFTs and, in some cases, Aβ plaques (McKee, et al., 2009, Omalu, et al., 2006, Omalu, et al., 2005).

In addition, several studies have identified a history of just a single TBI as an epigenetic risk factor for the later development of clinical syndromes of cognitive impairment such as AD (Fleminger, et al., 2003, Graves, et al., 1990, Guo, et al., 2000, Molgaard, et al., 1990, Mortimer, et al., 1985, Mortimer, et al., 1991, O’Meara, et al., 1997, Plassman, et al., 2000, Salib and Hillier, 1997, Schofield, et al., 1997). Moreover, recent data indicate TBI may accelerate the onset of dementia (Gedye, et al., 1989, Nemetz, et al., 1999, Schofield, et al., 1997, Sullivan, et al., 1987). The first pathological link between the pathologies of a single TBI and AD was the observation that Aβ plaques are present in up to 30% of patients dying acutely following injury (Chen, et al., 2008, DeKosky, et al., 2007, Gentleman, et al., 1997, Huber A, 1993, Ikonomovic, et al., 2004, Roberts, et al., 1991, Roberts, et al., 1994, Smith, et al., 2003, Uryu, et al., 2007). Notably, plaques following TBI were observed rapidly,

within hours of injury, and across the age spectrum, even in children[35]. It was also found that genetic polymorphisms of both the apolipoprotein E and neprilysin genes are associated with acute Aβ plaques following TBI, suggesting certain individuals may be at increased risk for the development of acute post-traumatic AD-like pathologies (Johnson, et al., 2009, Nicoll, et al., 1995). Moreover, while plaques were observed to diminish in the months following injury (Chen, et al., 2009), with survival of more than a year, plaques re-emerged being present to a greater extent than in uninjured controls (Johnson, et al., 2011). While NFTs, were not observed acutely following TBI, recent work indicates NFTs can be found at an increased extent and frequency, even in young individuals, long-term (>1 year) following just a single moderate – severe TBI in humans (Johnson, et al., 2011).

Injured Axons as a Source of Aβ

Although it is possible that multiple sources contribute to Aβ-plaque formation after TBI, the rapid accumulation of the precursor of Aβ, APP, in damaged axons, represents an intriguing potential source of Aβ for further investigation. Indeed, upon close examination of axonal bulbs in DAI, APP is seen to co-accumulate with the enzymes necessary for its cleavage to Aβ peptides, including presenelin-1 and beta-site APP-cleaving enzyme. Observed in both the pig model of DAI (Chen, et al., 2004) and, subsequently, in humans (Chen, et al., 2008, Uryu, et al., 2007), these data suggest trauma may create a situation whereby the substrates for Aβ formation are forced to co-exist in the same place and at the same time (Figure 2.). Indeed, abundant Aβ was confirmed within these axon bulbs, again both in humans and in animal models (Chen, et al., 2008, Chen, et al., 2004, Ikonomovic, et al., 2004, Smith, et al., 2003, Smith, et al., 1999, Uryu, et al., 2007).

Interestingly, interruption of axonal transport has also been implicated in APP processing and Aβ formation in AD. Specifically, studies have described Aβ genesis via BACE and PS-1 within the axonal membrane of murine peripheral nerves (Kamal, et al., 2001, Kamal, et al., 2000). The authors suggested that APP, β-secretase and PS-1 are transported within the axonal compartment via interaction with kinesin-1(Kamal, et al., 2001, Kamal, et al., 2000). The observation of Aβ within the axonal compartment led to the suggestion that amyloidogenic cleavage of APP can occur during transportation (Kamal, et al., 2001). However, these findings have been refuted by another study which failed to detect Aβ peptides within the same murine peripheral nerve (Lazarov, et al., 2005). However, more recent data using a well-established AD mouse model, indicates disruption of axonal transport via decreased kinesin-1 can result in increased axonal pathology with associated elevations in intraneuronal Aβ accumulation as well as extracellular Aβ deposition (Stokin, et al., 2005).

The eventual lysis and breakdown of damaged axons following trauma may permit the expulsion of accumulating Aβ into the parenchyma where it can aggregate to form plaques (Chen, et al., 2004, Smith, et al., 1999). In addition to providing a mechanism for the acute formation of Aβ following trauma, the possibility of persistent axonal pathology observed even years after injury may offer a potential mechanism of chronic Aβ genesis (Johnson, et al., 2011). Moreover, given that even mild TBI appears capable of inducing axonal injury (Blumbergs, et al., 1995, Browne, et al., 2011), the mechanistic role of axonal pathologies following single and repetitive mild TBI and associated disorders such as dementia pugilistica and chronic traumatic encephalopathy would be of interest to examine, both with regard to Aβ plaque formation and the accumulation of the microtubule associated-protein tau.

Elucidating the magnitude of persistent axonal degeneration after TBI and uncovering the mechanisms governing the protracted disconnection and degeneration of axons may provide

a direct means of slowing the production of AD-associated proteins following injury, perhaps also providing insight into possible therapies more widely applicable to neurodegenerative disease.

Conclusions

Recently, there has been mounting evidence of the substantial pathological consequences of DAI due to TBI. Occurring as a direct consequence of mechanical injury, DAI has been identified as responsible for immediate and persistent coma following injury and is independently a significant cause of morbidity and mortality. However, more recently it has emerged that DAI may induce a multitude of functionally detrimental effects over a far greater range of severity than previously considered. Specifically, the classical understanding of DAI as morphologically altered axons due to impaired transport may represent just one pathological subset of damaged axons. In contrast, morphologically intact axons with disrupted physiology may contribute to the pathological milieu leading to clinical dysfunction across a wide range of injury severities, including mild TBI. Investigation of these pathways may represent important therapeutic targets in the treatment of TBI and potentially the mitigation of chronic neurodegeneration.

Acknowledgments

This work is supported by the National Institute of Health grants: NS056202, NS038104 (all DHS).

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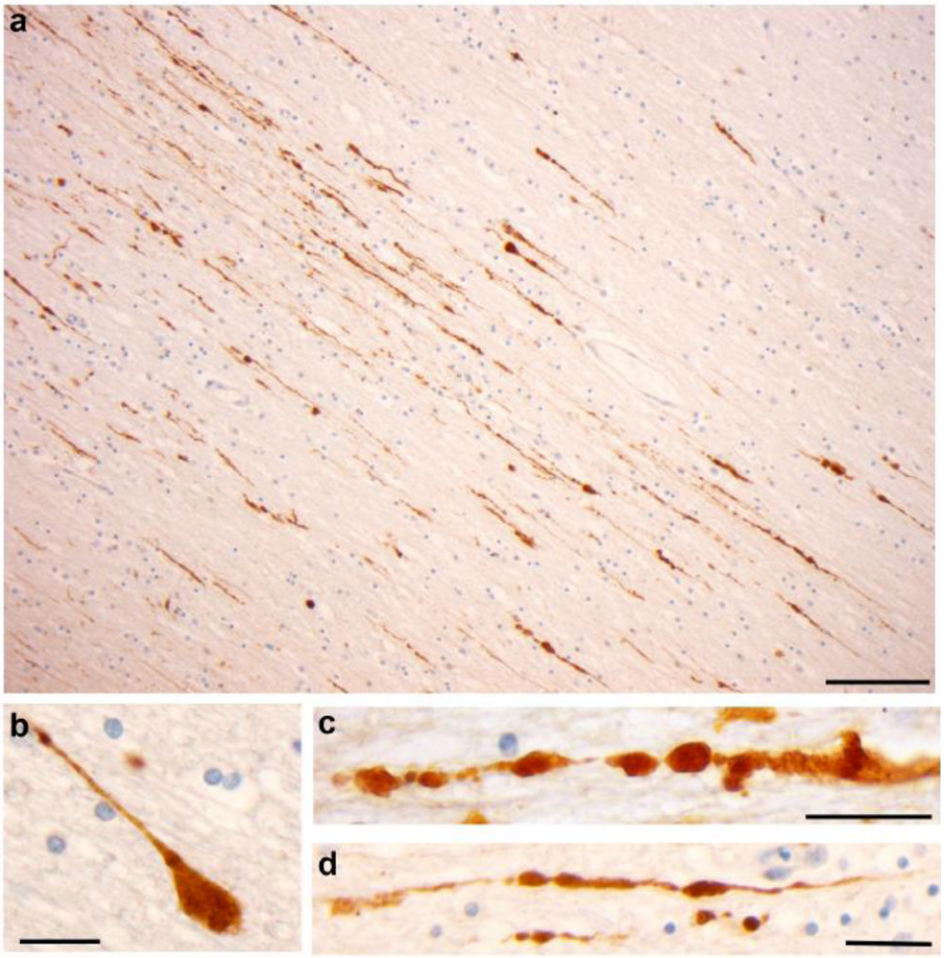

Figure 1. Representative Images of Axonal Pathology Following TBI in Humans Identified Using APP Immunohistochemistry

(a) Extensive axonal pathology with classic varicosities and axonal bulb formation in a region of the corpus callosum of an young male who died 10 hours following blunt force trauma to the head. Scale bar: 100μm **(b)** High magnification of a single axon immunoreactive for APP displaying the classic morphology of an axonal bulb. Scale Bar 15 μm. **(c-d)** High magnification of a single axon accumulating APP. Axons are morphologically varicose, exhibiting multiple points of transport interruption to give the appearance of beads on a string. Scale bars: 30μm.

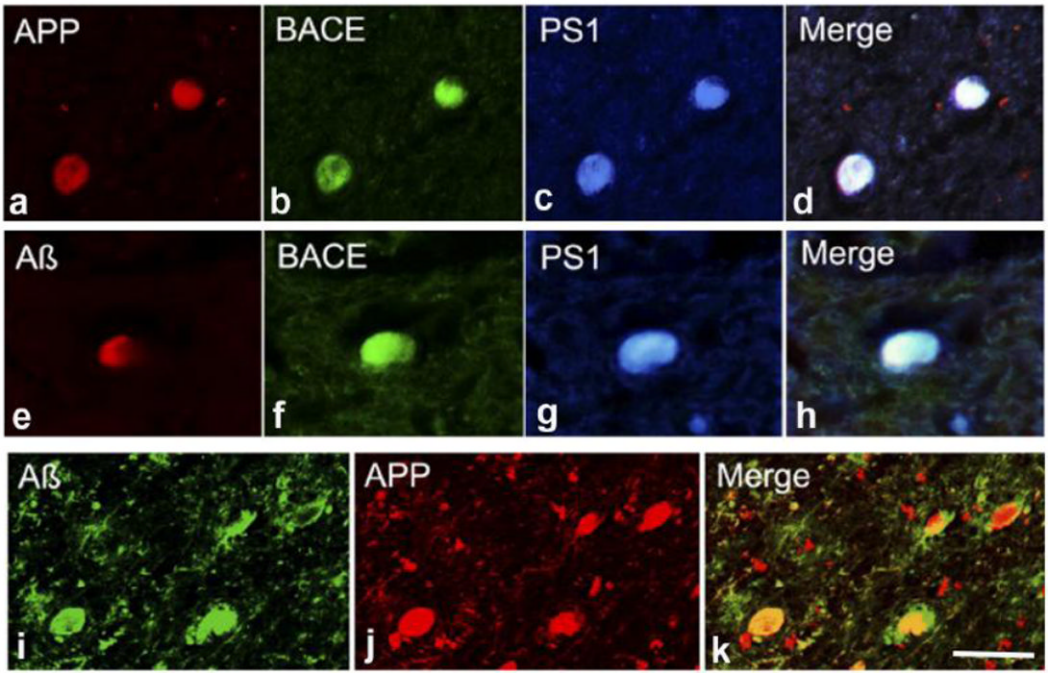

Figure 2. Accumulation of Aβ and Associated Proteins in Axonal Bulbs Following TBI in Humans

(a-c) Double immunofluorescent labeling showing co-accumulation of APP with Aβ in multiple axonal bulbs following TBI. Further immunohistochemical analyses showing co-accumulation of BACE and PS-1 with APP **(d–g)** and Aβ **(h–k)** in axonal bulbs. Scale bar: 50 μm.