OPINION

Traumatic brain injury and amyloid-β pathology: a link to Alzheimer's disease?

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Abstract | Traumatic brain injury (TBI) has devastating acute effects and in many cases seems to initiate long-term neurodegeneration. Indeed, an epidemiological association between TBI and the development of Alzheimer's disease (AD) later in life has been demonstrated, and it has been shown that amyloid-β (Aβ) plaques — one of the hallmarks of AD — may be found in patients within hours following TBI. Here, we explore the mechanistic underpinnings of the link between TBI and AD, focusing on the hypothesis that rapid Aβ plaque formation may result from the accumulation of amyloid precursor protein in damaged axons and a disturbed balance between Aβ genesis and catabolism following TBI.

Traumatic brain injury (TBI) is a common and often devastating health problem[1,2]. Despite its prevalence, TBI has only recently become widely recognized as a major health issue — in part due to the intense media attention on the high incidence of TBI in ongoing military conflicts. In addition, there has been a growing awareness of the epidemiological association between a history of TBI and the development of Alzheimer's disease (AD) later in life[3–12]. This link is supported by the identification of acute and chronic AD-like pathologies in the brains of TBI patients and in animal models of TBI.

There are several possible mechanisms linking an episode of TBI to later development of neurodegenerative disease, such as neuronal loss[13–15], persistent inflammation[16,17] and cytoskeletal pathology[18,19]. However, the pathophysiological link that has received the most attention is the production, accumulation and clearance of amyloid-β (Aβ) peptides following TBI. Here, we will examine the current understanding of how a single TBI can trigger both rapid and insidiously progressive AD-like pathological changes. In particular, we will examine the association between TBI and Aβ turnover.

TBI and AD: epidemiological link

Compelling data from several studies demonstrate that a history of TBI is one of the strongest epigenetic risk factors for AD[3–12,20]. However, there is not a complete consensus, as some epidemiological studies have failed to find such an association[21–28]. A major point of contention has been the retrospective nature of some reports that may have led to recall bias — a systematic error due to inaccuracies in subjects' ability to recall their history of TBI. This is of particular concern when gathering information from patients with cognitive impairments or from secondary informants. Nevertheless, larger, more controlled studies, including level 1 evidence (which requires prospective examination and randomization)[11], has led to a general acceptance that TBI is a risk factor for developing AD[29].

It has also been suggested that a history of TBI accelerates the onset of AD[10,30–32], and that the more severe the injury, the greater the risk of developing AD[9,11]. Indeed, because TBI is a complex and heterogeneous disorder, the type and extent of the acute pathology probably has an important role in determining the risk of developing AD. In addition, the baseline susceptibility of the patient may be predetermined by multiple factors such as age, sex and the interplay of several known or unknown genetic factors. For example, there is evidence that genetic predisposition, as a result of an apolipoprotein E (*APOE*) polymorphism, may influence the likelihood of developing AD after TBI (BOX 1).

TBI and AD: pathological links

Human TBI and Aβ plaques. The first clue indicating a pathological link between TBI with AD was the observation that Aβ plaques, a hallmark of AD[33], are found in up to 30% of patients who die acutely following TBI[34,35]. Notably, these plaques were found in all age groups, even in children. By comparison, in control cases (individuals that died from non-neurological causes), plaques were found almost exclusively in elderly individuals[35]. Plaques have even been observed in peri-contusional tissue surgically excised from survivors of TBI[36,37].

The plaques found in TBI patients are strikingly similar to those observed in the early stages of AD (FIG. 1). However, plaques in AD develop slowly and are predominantly found in the elderly, whereas TBI-associated plaques can appear rapidly (within just a few hours) after injury[35,36]. In addition, plaques were identified following a range of traumatically induced pathologies that resulted from various causes of injury[35].

While TBI-associated plaques largely appear in the grey matter, they have also been identified in white matter[38]. The predominant type of Aβ peptide in the plaques formed after TBI, and in the soluble Aβ found in the brains of these patients, is $Aβ_{42}$, the AD-associated form of Aβ that is prone to aggregation[37,39]. Although the plaques observed following trauma are typically diffuse[40], like those observed in early AD, it is not known whether these plaques mature over time into the denser, neuritic plaques typical of advanced AD.

Although the existence of Aβ plaques following trauma in humans is generally well established[34–36,38,41–43], it is only in recent years that the mechanisms driving plaque development have begun to be elucidated.

Box 1 | **The effects of apolipoprotein E genotype in traumatic brain injury**

As with Alzheimer's disease (AD)[137,138], the lipid transport protein apolipoprotein E (APOE) has been implicated in influencing amyloid pathology and outcome following traumatic brain injury (TBI). A series of studies have found that individuals carrying the *APOE* ε4 allele were more likely to have a poor outcome following injury[139–147]. However, there have also been reports that failed to show any association between *APOE* ε4 carriers and outcome[148–150]. Indeed, a recent prospective study examining 984 cases only found an association with possession of an *APOE* ε4 allele and outcome in younger adults and children, with the association being strongest in patients aged less than 15 years[150]. Thus, despite a general acceptance that possession of an *APOE* ε4 allele worsens outcome after TBI, there is renewed debate in this regard.

Epidemiological data have provided additional information by implicating *APOE4* genotype as a risk factor for the later development of AD following TBI[7,9,11,25,151–153]. However, considerable debate remains over whether APOE and TBI operate in a synergistic manner to increase the risk of AD development or, alternatively, act as independent but additive risk factors.

Carriers of the *APOE* ε4 allele were found to be at increased risk of amyloid-β (Aβ) deposition following TBI[154]. Aβ deposition was also significantly increased following head trauma in PDAPP (platelet-derived growth factor promoter expressing amyloid precursor protein) mice carrying the human *APOE* ε4 allele versus those carrying *APOE* ε3 or no APOE[155]. The mechanism by which APOE is able to exert an effect on Aβ deposition remains elusive. In addition, the interplay of *APOE* polymorphism with the microsatellite polymorphism in neprilysin, also shown to contribute to Aβ deposition[112], will be of interest. Indeed, when combined, these polymorphisms could potentially provide useful predictive information.

Human TBI and unaggregated Aβ peptides. In contrast to the well-characterized formation of Aβ plaques after TBI, comparatively little is known about how total brain concentrations of Aβ, including both soluble and oligomeric forms of the peptide, vary following injury.

An initial study reported an increase in the presence of Aβ in ventricular cerebrospinal fluid (CSF) following severe head trauma[44], although it is important to note that control cases in this study were elderly, which makes interpretation of these findings difficult. Levels of Aβ were elevated for the first week after TBI and then declined towards control levels in the subsequent 2 weeks[44]. In addition, the same Aβ peptide that is predominant in plaques following TBI, $A\beta_{42}$, was found in the CSF of these patients[39,44,45]. By contrast, a later study reported a persistent decrease in Aβ concentration in ventricular CSF from days 1–5 following severe TBI[46]. This contradictory finding was supported by a further study[47], although in this case progression over time was not investigated and the collection time points ranged from an acute measurement to more than 9 months after injury. A further caveat of this work was that the ventricular CSF from TBI cases was compared with lumbar CSF in controls[47]. Clearly there is a lack of consensus on this issue. What influences the movement of Aβ from the extracellular space to cerebrospinal circulation is also unknown, particularly following TBI in which blood–brain barrier permeability and vascular integrity may be dramatically altered. Therefore, it is not apparent whether Aβ in CSF, either ventricular or lumbar, reflects Aβ concentrations in the interstitial space within the brain parenchyma.

Recent studies have used invasive intracranial microdialysis to obtain direct measurements of brain parenchymal Aβ concentrations in humans following severe TBI[48,49]. Although no baseline (pre-injury) data were available, one such study found that microdialysate Aβ concentrations steadily increased over time following TBI, and were correlated with improved global neurological status[48]. One interpretation of this finding is that depressed neuronal function following TBI decreases Aβ genesis, which subsequently returns to baseline as recovery ensues. Another study that used similar techniques failed to demonstrate any overt change in post-TBI Aβ concentrations between 27 h and 99 h after TBI[49]. However, they did find that patients with diffuse axonal injury (DAI) had elevated Aβ levels when compared with those with focal injuries, suggesting that the type of injury may be an important influence on Aβ dynamics.

Axonal pathology: a source of Aβ?

Axonal injury after TBI. Although it is likely that multiple sources contribute to Aβ plaque formation after TBI, axonal swellings represented an obvious pathology for examination in initial investigations. Notably, DAI is one of the most common pathologies of TBI, and independently contributes to significant morbidity and mortality[50–52]. A key feature of DAI is interruption of axonal transport due to cytoskeletal disruption. This causes an accumulation of proteins in the axon, including amyloid precursor protein (APP), which can be cleaved to form Aβ[53–57]. These accumulations occur in tortuous varicosities along the length of the axon or at the disconnected axon terminals, known as axonal bulbs. Although originally described as diffuse, the actual distribution of axonal pathology is multifocal, with varicosities and bulbs occurring throughout the deep and subcortical white matter. Swollen axons are particularly common in midline structures such as the corpus callosum[58]. Eventual structural disorganization of the axon can lead to secondary axonal disconnection, which ultimately culminates in a progressive, degenerative axonal pathology[59–61].

Owing to the rapid and abundant accumulation of APP in damaged axons after TBI, APP immunostaining is used for the pathological assessment of DAI in humans[53–58] (FIG. 1). Accordingly, it was suspected that this large reservoir of APP in injured axons might be aberrantly cleaved to rapidly form Aβ.

Acute Aβ formation in rodent TBI models. Following the observation of acute Aβ plaque formation in humans, and speculation that the source of Aβ may be damaged axons, attention was turned to animal models of TBI to explore this process (TABLE 1). Studies using non-transgenic rat models of TBI investigated these pathologies in the acute phase following injury[62,63]. However, although these studies consistently found extensive intra-axonal accumulation of APP, they failed to identify Aβ, via staining, in either plaques or axons[62,63].

The lack of evidence of Aβ deposition in non-transgenic rats was attributed, in part, to differences in the Aβ peptides found in different species. Accordingly, various transgenic TBI models were utilized in an attempt to replicate plaque pathology. Strains of mice were selected for their predisposition to the eventual development of Aβ plaques with ageing. In a study using mice that overexpress normal human APP, there was an increase in tissue concentrations of $A\beta_{40}$ acutely after injury. However, there was no increase in plaque formation, overall pathology or altered functional outcome[64]. Further studies used PDAPP mice, which overexpress a mutant form of APP and develop Aβ plaque pathology as they age. TBI before plaque formation induced a surge in the tissue concentration of Aβ that was associated with an increase in hippocampal neuronal

death and memory impairment, indicating the potential toxicity of Aβ[65]. However, even in these mice, TBI did not induce acute plaque formation. Furthermore, there was a paradoxical reduction of plaques in the PDAPP mice 8 months after TBI[66]. A similar reduction in plaques was seen when aged mice were subjected to injury, suggesting that regression of plaques is possible[67]. It was thought that the loss of hippocampal neurons after injury actually decreased the overall capacity to generate Aβ. Alternatively, trauma may have induced a surge in Aβ concentrations, which in turn upregulated the mechanisms by which Aβ is cleared.

Acute elevations of hippocampal Aβ levels in the absence of plaques were also found following injury in $APP^{NLh/NLh}$ mice, which have both the Swedish familial AD mutation and the human Aβ sequence knocked in to their endogenous *APP* gene[68,69]. Of note, unlike the PDAPP mice, APP expression in this model is dependant on the endogenous promoter and thus continuous overexpression of APP does not occur. Using this model, it was demonstrated that, by inhibiting caspase-3 activity, injury-induced elevations in Aβ levels could be reduced in association with improved histological outcomes[68]. In addition to providing a potential mechanism of Aβ formation (see below), this study further supported the idea that post-TBI increases in Aβ concentrations without plaque formation may be detrimental. Similarly, mice deficient in the rate-limiting enzyme for Aβ genesis — β-amyloid converting enzyme (BACE) — were found to have significantly improved histological, radiological and behavioural outcomes following injury[70].

Together, these data provide important information regarding the potentially harmful consequences of elevated Aβ levels following TBI. However, they also consistently demonstrate that rodents fail to recapitulate the plaque pathology observed acutely following human TBI.

Acute Aβ formation in a swine TBI model. Why rodent TBI models failed to recapitulate the Aβ plaque pathology found acutely after human TBI was a puzzle. Initially it was thought that differences in the Aβ sequence in rodents might prevent its aggregation into plaques. However, even mice modified to generate the human Aβ sequence failed to develop plaques. It was therefore suggested that Aβ production and deposition after TBI may depend on brain anatomy as well as the type of injury. Notably, rodents have relatively small lissencephalic brains in which white matter is sparse, meaning that only limited axonal pathology can be produced in rodent TBI models. Furthermore, most rodent models utilize impact forces to induce TBI, whereas a common cause of human DAI is rotational acceleration, such as occurs in automobile crashes.

Therefore, to more closely examine the role of DAI on Aβ generation and deposition, a swine model of head rotational acceleration was used. This animal species was selected because of its relatively large gyrencephalic brain with extensive white matter. Notably, the swine Aβ sequence is identical to that of humans. This model produces DAI that is very similar in appearance to that found clinically[71,72]. In addition to the anticipated accumulation of APP in swollen axons, co-accumulation of Aβ was also found[18]. Furthermore, this model also induced the formation of parenchymal Aβ diffuse plaques in both grey and white matter. Although the number of plaques was small compared to those found in human TBI, this model finally enabled Aβ deposition to be induced in an experimental model. Furthermore, the co-accumulation of Aβ and APP in swollen axons hinted that the potential mechanism of Aβ production after TBI is linked to traumatic axonal injury.

Persistence of Aβ formation after TBI. Following the identification and axonal accumulation of Aβ plaques in the swine TBI model, rodent TBI models were re-examined both acutely and with an expanded time frame. Initially, Aβ accumulation was identified in damaged axons shortly after injury in a rat model of TBI, albeit still in the absence of Aβ plaques[73]. Although there was concern about the potential cross-reactivity of the primary

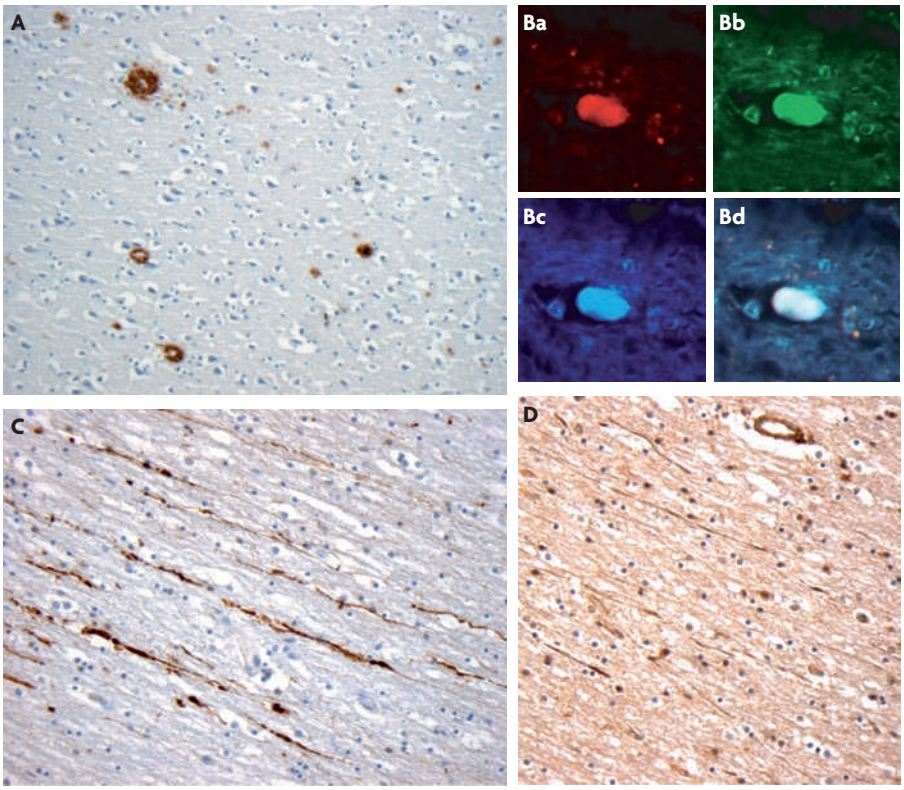

Figure 1 | **Immunohistochemical findings exploring mechanisms of amyloid-β plaque formation following traumatic brain injury. A** | Representative amyloid-β (Aβ) plaques (brown) found acutely following a single incidence of traumatic brain injury (TBI) caused by a fall in an 18 year old male. The survival time from injury was just 10 hours. Plaques were identified using an antibody specific for Aβ. **B** | Representative immunohistochemistry showing amyloid precursor protein (APP) (**Ba**), β-site-APP-cleaving enzyme (BACE) (**Bb**) and presenilin-1(PS-1) (**Bc**) co-accumulating (**Bd**) in the disconnected terminal of an axon, known as an axon bulb. **C** | Demonstration of axonal pathology using APP immunohistochemistry. APP (brown) accumulates within the tortuous varicosities along the length of damaged axons. **D** | Increased neprilysin immunoreactivity (brown) is also observed in damaged axons following TBI. Panel **B** is reproduced, with permission, from REF. 42 © (2009) International Society of Neuropathology.

Aβ antibody with APP in this study, another study using a different rat model of TBI used multiple specific antibodies to confirm the axonal accumulation of Aβ[74]. However, in this case only limited axonal Aβ was found acutely, whereas greater axonal accumulations were found 1 month after injury and persisted for at least 1 year. Increased Aβ presence could also be seen within the neuronal somata of these animals[74]. Notably, these chronic increases in Aβ levels were not associated with increased expression of APP and no plaque formation was found at any post-injury time point[74]. These findings clearly indicated the potentially chronic nature of the trauma response with ongoing development of axonal pathology and accompanying Aβ accumulation.

Interestingly, when the swine model of head rotational acceleration injury was examined up to 6 months after injury, evidence of ongoing axonal pathology was also revealed[75]. Again, this was characterized by APP accumulation, often with co-accumulation of Aβ. Although it was generally thought that axonal pathology is only observed in the acute phase of injury and is cleared within a few months of trauma, these results demonstrated that axonal degeneration may chronically persist[42,74,75]. Moreover, the continued presence of damaged axons offers a potential mechanism by which Aβ is chronically generated. However, despite

Table 1 | **Animal models of traumatic brain injury and amyloid pathology**

Species (strain)	Injury	Summary of findings	Ref.
Mouse (APP–YAC)	Controlled cortical impact	• No difference in neuronal loss, cognition or motor function following injury versus wild-type controls • Decrease in total tissue levels of $A\beta_{40}$ but not $A\beta_{42}$ after injury	64
Mouse ($APP^{NLh/NLh}$)	Controlled cortical impact	• Suppression of injury-induced elevations in caspase-3 by administration of a pan-caspase inhibitor • Both caspase-cleaved APP and Aβ were reduced in association with improved histological outcome	68
Mouse ($APP^{NLh/NLh}$)	Controlled cortical impact	• Administration of simvistatin 3 h after injury resulted in decreased hippocampal Aβ levels, decreased hippocampal tissue loss and preserved synaptic integrity • Behavioural outcome also improved	69
Mouse (*BACE*$^{-/-}$)	Controlled cortical impact	• Improved histological, radiological, behavioural and motor outcomes following injury versus *BACE*$^{+/+}$ mice • Administration of a γ-secretase inhibitor (DAPT) in non-transgenic mice also improved outcomes	70
Mouse (PDAPP)	Controlled cortical impact at 4 months old	• Levels of $A\beta_{40}$ and $A\beta_{42}$ in tissues increased following injury, peaking at 2 h • Associated with increased hippocampal neuronal death and memory impairment • No Aβ plaques were observed up to 2 months after injury	65
Mouse (PDAPP)	Controlled cortical impact at 4 months old	• Decrease in Aβ plaques at 5 and 8 months after injury versus uninjured PDAPP mice (who normally demonstrate abundant Aβ plaques at these time points)	66
Mouse (PDAPP)	Controlled cortical impact at 2 years old	• Regression in Aβ plaque burden observed in the ipsilateral hippocampus of injured PDAPP mice 16 weeks after injury versus the contralateral hippocampus or uninjured PDAPP control mice	67
Mice (PDAAP, expressing *Apoe3* or *Apoe4*, or *Apoe*$^{-/-}$)	Controlled cortical impact	• PDAPP mice expressing *Apoe4* had increased Aβ deposition compared with those expressing *Apoe3* • Both groups displayed deposition at an age at which it is not observed in uninjured controls • Mice with *Apoe4* demonstrated Aβ deposition that stained positive for thiaflavin-S in the molecular layer of the dentate gyrus	155
Rat (Sprague Dawley)	Weight drop (open skull)	• Extensive APP accumulation in damaged axons (1, 3 and 21 days following injury), and later in cortical neuropil • No accumulating Aβ observed intracellularly or in plaques	62
Rat (Sprague Dawley)	Lateral fluid percussion	• APP accumulation in damaged axons up to 2 weeks following injury • No Aβ observed at any time point intracellulary or in plaques	63
Rat (Sprague Dawley)	Weight drop (closed skull)	• Axonal accumulation of APP observed from 6 h to 10 days following trauma • Aβ identified in damaged axons 12 h after injury • Although APP and Aβ were persistently found in axons for up to 10 days after injury, immunoreactivity reduced over time • No plaques observed at any time	73
Rat (Sprague Dawley)	Lateral fluid percussion	• Low levels of Aβ accumulated in axons, emerging at around 2 weeks after injury • More profound immunoreactivity demonstrated at 1 month and persisted up to 1 year • Extent of Aβ production was dependent on the maturity of the injury, but was uncoupled from the gene expression of *APP*	74
Swine	Rotational acceleration (model of DAI)	• Accumulation of intra-axonal APP and Aβ observed 3–10 days following injury • Sparse, diffuse Aβ plaques observed in the grey and white matter over the same time course • First animal model to replicate human Aβ plaque pathology observed after traumatic brain injury	18
Swine	Rotational acceleration (model of DAI)	• Aβ observed in axons, co-accumulating with APP, BACE and presenilin-1 • This was observed acutely (3 days and persisted up to 6 months after injury) • Sparse Aβ plaques were observed both acutely and at 6 months following injury, but did not increase in number over this time	75

Aβ, amyloid-β; APP, amyloid precursor protein; *BACE*, β-site APP-cleaving enzyme; DAI, diffuse axonal injury; DAPT, *N*-[(3,5-difluorophenyl)acetyl]-L-alanyl-2-phenyl] glycin e-1,1-dimethylethyl ester; PDAPP, platelet-derived growth factor promoter expressing amyloid precursor protein; YAC, yeast artificial chromosome.

this long-term production of Aβ, the quantity of Aβ plaques in the tissue observed at 6 months following trauma in swine had not increased compared to that observed following acute injury[75].

Axon pathology and Aβ in humans. Examination of human brains also confirmed the accumulation of Aβ in swollen axons shortly after TBI[38]. More recently, long-term progressive axonal degeneration and intra-axonal Aβ accumulation was also identified following human TBI, and persisted for years following the initial trauma[42]. These findings demonstrate that TBI can trigger long-term neurodegenerative processes in humans. This process may account for the progressive selective atrophy of white matter found after TBI in humans[76]. However, the mechanisms governing this protracted disconnection and degeneration of axons are unknown. It is possible that injured axons that do not degenerate in the acute phase are nonetheless prone to later degeneration with a secondary mild insult.

Aβ genesis and plaque formation in TBI. Immunohistochemical analyses revealed that the enzymes necessary for Aβ cleavage also accumulate in injured axons after TBI. Both presenilin-1 (PS-1) and BACE were found in swollen axons in the swine model of DAI[75] and in humans[42,43] (FIG. 1). It seems that trauma creates a unique situation whereby all the necessary substrates for Aβ formation are forced to coexist in the same place at the same time. It has been postulated that the eventual lysis and breakdown of these damaged axons may allow the expulsion of Aβ into the parenchyma where it aggregates to cause plaque formation[18,75] (FIG. 2). Interestingly, at a much slower pace, this general process of axonal transport failure has been implicated as a mechanism of plaque formation in AD[77].

Although there is strong evidence to support the idea that axons are a source of post-traumatic Aβ, it remains unknown why plaque formation is more prevalent in the grey matter following TBI. It is clear from the multiple studies discussed above that Aβ is not only present in white matter, but may undergo a post-TBI surge at these sites. In addition, increased soluble Aβ has been found in CSF following injury. Nevertheless, there seems to be a predilection for grey matter deposition, which also seems to be the case in AD. There may be specific extracellular attributes of the grey matter that selectively promotes aggregation of Aβ into plaques. Alternatively, it is possible that Aβ genesis also occurs via a different mechanism in the grey matter. Observations of APP accumulation in synaptic terminals led to the suggestion that this may be another potential site of Aβ genesis, leading to deposition in the grey matter[53]. In addition, there may be multiple mechanisms driving increased Aβ production following trauma at any location. It has been postulated that elevated APP production in the neuronal soma following TBI may saturate the normal α-secretase processing pathway, resulting in increased β-secretase processing and Aβ genesis[53,78]. Furthermore, studies of hypoxia–ischaemia suggest that Aβ genesis can be increased via oxidative-stress-mediated upregulation of BACE[79,80]. This BACE upregulation was later shown to be mediated by γ-secretase activity, which was also enhanced due to oxidative stress[81]. As oxidative stress is a well-established consequence of TBI[82], its role in promoting Aβ genesis after injury warrants exploration.

Aβ dynamics in the various compartments, and their relative contributions to plaque formation and pathogenicity, are largely unknown. It is possible that TBI can result in distinct Aβ dynamics as a result of different mechanisms within the various intracellular and extracellular compartments. Elucidating these potential complexities in neuronal Aβ dynamics will be an important consideration for future studies.

Intra-axonal Aβ formation. Cleavage of APP to form Aβ within the axonal membrane compartment is not consistent with the classical description of Aβ genesis in AD. As APP is a transmembrane protein,

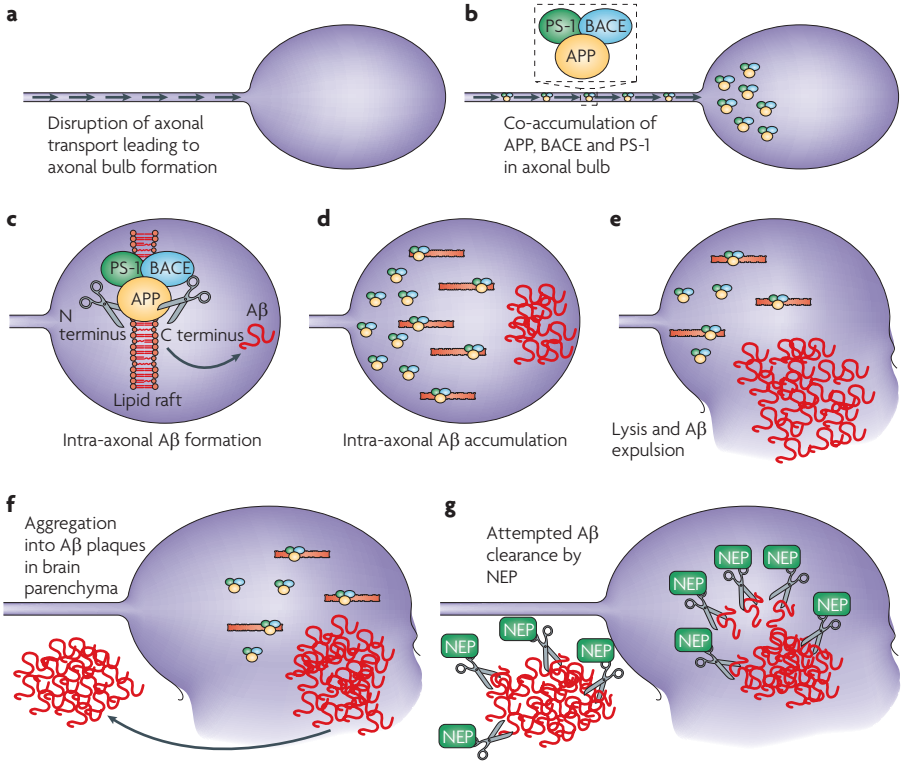

Figure 2 | **Potential mechanisms of post-traumatic amyloid-β formation and clearance.** **a** | The mechanical forces that axons are subjected to during a traumatic event can damage axons by directly altering their structure or by initiating detrimental secondary cascades. Failure of axonal transport in these injured axons results in accumulation of multiple proteins that form swellings at their disconnected terminals known as axon bulbs. **b** | Such protein accumulation has been demonstrated to include the enzymes necessary for the cleavage of amyloid precursor protein (APP) to amyloid-β (Aβ), including presenilin-1 (PS-1) and β-site APP-cleaving enzyme (BACE). **c**–**d** | Although the precise intracellular mechanism of Aβ genesis remains unclear, lipid rafts have been suggested to be important in allowing APP processing and thus Aβ accumulation within the axonal compartment. **e**–**f** | Injured axons that go on to degenerate and lyse will expel the accumulated Aβ into the brain parenchyma where it is at risk of aggregating into plaques. **g** | The enzyme that clears Aβ, neprilsyin (NEP), also accumulates in damaged axons and probably mitigates the effects of enhanced Aβ production. The balance of genesis versus catabolism will ultimately determine Aβ build-up. NEP may potentially act to clear Aβ within the axonal compartment or in the extracellular space.

it has long been assumed that amyloidogenic processing results in the extracellular deposition of Aβ. However, increasing evidence suggests that intracellular accumulation of Aβ is possible and potentially pathogenic[83].

Recent studies have described Aβ production by BACE and PS-1 in the axonal membrane compartment of peripheral nerves in mice[84,85]. The authors suggested that APP, β-secretase and PS-1 are transported within the axonal compartment through a direct interaction between kinesin-1 and APP[84,85]. The observation of Aβ within the axonal compartment led to the suggestion that amyloidogenic cleavage of APP can occur during transportation[84]. However, these findings have been directly challenged by another study that failed to demonstrate co-transportation of either PS-1 or BACE1 with APP in the same murine peripheral nerve[86]. Furthermore, they were unable to detect Aβ peptides within this nerve. The authors suggest that, at least within the peripheral nervous system, intraxonal transport of the machinery of Aβ genesis is unlikely. Whether this is also true for the CNS is unknown.

Intra-axonal processing of membrane-bound APP may involve lipid rafts — cholesterol-rich microdomains of the plasma membrane that are thought to compartmentalize cellular processes[87,88]. Indeed, there is evidence indicating that lipid rafts may be important in amyloidogenic processing of APP in neurons[89]. Axons are also abundant with lipid-rich invaginations of plasma membrane known as caveolae, which may provide another intra-axonal site of APP processing. It is possible that mechanical damage to axons due to trauma may cause changes in linear lipid rafts or caveolae that promote abnormal APP processing. A recent study using $APP^{NLh/NLh}$ mice showed that administration of the cholesterol-lowering drug simvastatin diminished increases in Aβ levels following injury[69]. Associated histological and behavioural outcomes were also improved. Although the mechanisms by which simvastatin modulates Aβ dynamics may be complex, further exploration of its possible role in post-traumatic Aβ processing will be of interest.

Caspases, TBI and Aβ processing. Cysteine-dependent aspartate-specific proteases (caspases) have been identified as important in Aβ genesis[90]. This finding is of particular interest with regards to TBI, in which increased caspase activation has been described in humans and in animal models[75,91–94].

Following controlled cortical impact in APP mice, pharmacological inhibition of injury-induced caspase-3 activation using a pan-caspase inhibitor (Boc-Asp(OMe)-CH_2F) reduced both caspase-3-mediated APP processing and acute elevations in Aβ concentrations[68]. In addition, there was decreased neuron degeneration and tissue loss. A further study demonstrated caspase-3-mediated APP proteolysis in traumatically injured axons following head impact in a rat[73].

Although the precise mechanisms by which caspases act to increase Aβ levels are not clear, recent work indicates that caspase-3 may increase APP processing by increasing the availability of BACE via an adaptor molecule (GGA3) that interrupts BACE trafficking and prevents its degradation[95]. Elevated BACE levels and activity would promote amyloidogenic processing of APP, potentially leading to increased Aβ genesis. Interestingly, elevated BACE levels and activity have been found following injury in a rat model of TBI[96].

This work highlights the need for better understanding of the mechanisms of Aβ genesis in the post-trauma situation. Little is known about how, or if, β-secretase and γ-secretase activity are altered by trauma. Furthermore, how this influences the role of the non-amyloidogenic α-secretase pathway is largely unknown.

Aβ catabolism in TBI

The observed variations in plaque pathology in humans and in animal models of TBI provided valuable clues regarding potential mechanisms of plaque genesis. They suggested that some species, and perhaps certain individuals, are able to clear Aβ more efficiently than others, potentially because of differences in the extent or activity of Aβ degrading mechanisms. This revelation turned attention to potential mechanisms of Aβ clearance after TBI.

Neprilysin, a membrane zinc metalloendopeptidase, has emerged as a significant Aβ degrading enzyme *in vivo*[97,98], although there are others including insulin degrading enzyme[99]. Neprilysin is transcribed in a tissue-specific manner[100,101], operates as a transmembrane glycoprotein[102,103] and is capable of degrading monomeric and potentially oligomeric forms of Aβ[104]. Furthermore, neprilysin knockout mice have been shown to accumulate $Aβ_{40}$ and $Aβ_{42}$ in a gene dose-dependant manner[105]. Neprilysin has been increasingly implicated in the pathogenesis of AD[106]. Indeed, patients with sporadic AD were found to have up to a 50% reduction in neprilysin mRNA in areas associated with plaque pathology[107].

Neprilysin following human TBI. It has recently been observed that immunoreactivity to neprilysin increases for many months following TBI in humans[42]. Extensive neprilysin immunoreactivity was found in the neuronal soma and axonal bulbs of long-term survivors of TBI (FIG. 1). Interestingly, these cases comprised the same group that have a virtual absence of Aβ plaques. These findings suggest that these individuals may have a long-term upregulation of neprilysin that continually clears both intracellular and/or extracellular Aβ, thereby promoting plaque regression. Moreover, the findings suggest that neprilysin may have an important role in mitigating chronic Aβ production induced by trauma. Although the site at which neprilysin acts to achieve this has not been identified, the presence of neprilysin within axons suggests that intra-axonal clearance is one possible mechanism. However, it remains to be determined whether neprilysin is able to function extracellulary to clear plaques directly or whether plaque turnover is regulated by some other means.

Notably, microglia containing Aβ have been found in association with plaques after TBI[42], suggesting that phagocytic clearance of plaques may occur. It is possible that the extent of the microglial response following injury may influence plaque burden. Like neprilysin, this could potentially contribute to variations in plaque pathology between individuals and indeed animal species. In support of this idea, recent work suggests that increased microglial activation is the mechanism by which passive Aβ immunization therapies act to clear plaques in AD models[108]. Interestingly, microglia also appear to utilize neprilysin to process Aβ[109].

Neprilysin polymorphisms and Aβ plaques. Neprilysin expression is probably regulated by multiple mechanisms, including the production of Aβ itself, which has been suggested to trigger a positive-feedback loop[110,111]. Genetic variation may also influence the extent of expression or activity of neprilysin, which potentially accounts for the presence of Aβ plaques in only 30% of TBI patients. Indeed a recent study identified a relationship between a neprilysin polymorphism and Aβ plaque pathology acutely following TBI[112], although whether this association is functionally

significant or a result of genetic linkage is unknown. Further studies may be of value in determining how this polymorphism affects both short-term and long-term clinical outcome. As such, a genetic screening test of this neprilysin polymorphism could potentially help to identify individuals at risk of plaque formation, which may be an important consideration for those involved in military action or contact sports (BOX 2).

APP and Aβ in TBI pathophysiology

It is unclear whether the accumulation of large quantities of APP in damaged axons after TBI serves a mechanistic role or is simply an epiphenomenon. It has been suggested that APP and its non-amyloidogenic processing have beneficial effects with respect to neuroprotection, neurite outgrowth and synaptogenesis[113–120]. In addition, the administration of soluble APPα (the product of non-amyloidogenic processing via the α-secretase pathway) improved functional outcome and reduced neuronal cell loss and axonal injury following TBI in rats[120].

The role of Aβ plaques in TBI outcome has also not yet been established. Even in AD, considerable debate remains over the pathogenicity of Aβ plaques (as opposed to its unaggregated soluble forms)[121–123]. Indeed, a recent investigation in which apparent Aβ plaque clearance followed Aβ immunization in patients with AD failed to demonstrate altered clinical outcome[124]. Furthermore, recent evidence suggests that unaggregated oligomeric forms of Aβ may contribute to toxicity[125–130]. As such, rapid aggregation of Aβ into plaques may be a protective event. An evaluation of the role of oligomeric Aβ following TBI will therefore be an important future consideration. So far, only one study has implicated unaggregated Aβ toxicity in neuron death after TBI. As described above, TBI in PDAPP mice resulted in extensive hippocampal neuron death and associated neurocognitive impairment in association with a local surge in unaggregated Aβ levels in the hippocampus[65]. This may suggest that the toxic properties of Aβ only emerge when levels exceed a certain threshold[131].

Unaggregated Aβ could have other deleterious effects in TBI. Solubilized Aβ injected into the brains of rodents has profound effects on cognition and long-term potentiation — an electrophysiological correlate of some forms of learning and memory[132–134]. Thus, after TBI an acute surge in Aβ concentrations, as well as its continued production for years, may influence functional recovery.

Box 2 | Repetitive traumatic brain injury

In 1928, the term 'punch drunk syndrome' was introduced to describe a disorder of progressive dementia that develops after repetitive traumatic brain injury (TBI) from boxing[156]. Now termed 'dementia pugilistica'[157], this syndrome can present many years after retiring from boxing[158], affecting as many as 17% of retired boxers[159]. The incidence of dementia pugilistica increases with duration of time spent boxing[159], number of knockouts experienced[160] and apolipoprotein E (*APOE*) genotype[161]. Although this work has been centred on boxers, increasing evidence suggests that repetitive TBI experienced from playing professional American football can result in increased rates of late-life cognitive impairment[162]. Furthermore, recent pathological studies of the brains of former players in the National Football League in the United States of America revealed extensive Alzheimer's disease (AD)-like neurofibrillary tangles (NFTs) and, in some cases, amyloid-β (Aβ) plaques[163–165].

Although the predominant pathology of dementia pugilistica is the formation of AD-like NFTs, Aβ pathologies later emerged as a potentially important finding. As with single TBI and AD, diffuse Aβ plaques are found in the brain[158,166]. Deposits of Aβ have also been observed in both leptomeningeal and cortical blood vessels[166]. More recently, accelerated Aβ deposition was observed following a model of mild repetitive TBI in Tg2576 mice (known to develop Aβ plaques with ageing)[167,168]. This increased deposition was found in association with evidence of increased lipid peroxidation and could be reversed by pretreatment with oral vitamin E[168].

NFTs and neuropil threads are an important feature of both AD and dementia pugilistica[19,158,165,169–173]. These intracellular structures contain abnormal (hyperphosphorylated) forms of the microtubule-associated protein tau. One study found NFTs and neuropil threads in the neocortex of five cases of repetitive, mild head trauma in young patients[19]. In addition, the molecular profile and ubiquitylation of tau in dementia pugilistica was similar to that observed in the filamentous tau inclusions seen in AD[173,174].

In contrast to well-developed tau pathology in dementia pugilistica, NFTs were not found to be acutely increased after a single TBI[175]. Although tau has also been observed accumulating in axons following trauma, this was only found in a small subset of patients[36,43]. In a study in which pigs received single experimental brain injuries, accumulations of tau were observed in a limited number of neuronal perikarya[18]. Rats also demonstrated phosphorylated tau accumulation in neurons at 6 months after injury[176].

Conclusions and future directions

The link between trauma and the later development of neurodegenerative diseases such as AD is likely to be extremely complex, and work in this field remains in its infancy. One recurring issue in this field of research is the involvement of axons and the pathological accumulation of multiple proteins within axons damaged by trauma. This parallels the increasing emphasis on axonal involvement in the pathogenesis of AD, particularly axonal transport defects.

TBI is a common, devastating disorder and is the leading cause of death in children and young adults[135]. The later development of AD or neurodegenerative disease comes not only at a human cost, but also constitutes a considerable socio-economic burden. Hence, this is an avenue of research of potential importance to almost everyone, and may have particular significance to those at high risk of TBI, such as those involved in contact sports players or in the military.

A mechanistic understanding of what drives the risk of developing AD following TBI will be imperative for the development of post-trauma interventions aimed at halting the onslaught of such debilitating neurodegeneration. Furthermore, the advancement of drug discoveries in the field of AD, such as BACE and γ-secretase inhibitors[70,136] or neprilysin replacement strategies, may have potentially important roles in both the acute and chronic management of TBI.

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doi:10.1038/nrn2808
Published online 10 March 2010

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Acknowledgements
This work was supported by US National Institutes of Health grants NS038104 and NS056202.

Competing interests statement.
The authors declare no competing financial interests

DATABASES

Entrez Gene:
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APOE
OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
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APP | BACE1 | caspase-3 | neprilysin | PS-1

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