DOI: 10.1093/brain/awg061 *Brain* (2003), **126,** 515–530

INVITED REVIEW

Axonal damage: a key predictor of outcome in human CNS diseases

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Summary

Axonal damage has recently been recognized to be a key predictor of outcome in a number of diverse human CNS diseases, including head and spinal cord trauma, metabolic encephalopathies, multiple sclerosis and other white-matter diseases (acute haemorrhagic leucoencephalitis, leucodystrophies and central pontine myelinolysis), infections [malaria, acquired immunodeficiency syndrome (AIDS) and infection with human lymphotropic virus type 1 (HTLV-I) causing HTLV-I-associated myelopathy (HAM)/tropical spastic paraparesis (TSP)] and subcortical ischaemic damage. The evidence for axonal damage and, where available, its correlation with neurological outcome in each of these conditions is reviewed. We consider the possible pathogenetic mechanisms involved and how increasing understanding of these may lead to more effective therapeutic or preventive interventions.

Keywords: axonal damage; neuropathology; cerebral malaria; multiple sclerosis; acute haemorrhagic encephalitis; vascular dementia; HIV encephalitis

Abbreviations: AHLE = acute haemorrhagic leucoencephalitis; AIDS = acquired immunodeficiency syndrome; β-APP = β-amyloid precursor protein; DAI = diffuse axonal injury; HAM = HTLV-I-associated myelopathy; HIV = human immunodeficiency virus; HIVD = HIV dementia; HTLV-I = human lymphotropic virus type 1; ICP = increased intracranial pressure; TAI = traumatic axonal injury

Introduction

Axonal damage and its profound consequences for outcome in human brain and spinal cord injury and disease were first fully recognized in the context of head trauma. Severe diffuse axonal injury caused by mechanical shearing and stretch forces, inflicted particularly in rotational acceleration injuries (Gennarelli *et al*., 1982), underlies the persistent vegetative state in subjects who survive severe head injury (Strich, 1956; Graham *et al*., 1983; Adams *et al*., 1989, 1999). It is also commonly present in those who succumb rapidly to such an injury (Gentleman *et al*., 1993; Oehmichen *et al*., 1998). At the other end of the scale of severity of traumatic injury, axonal tears were the lesions identified by Oppenheimer (1968) as likely to underlie transient loss of consciousness in those who suffer a mild head injury and die of something else.

Adoption of immunocytochemical staining for amyloid precursor protein (β-APP), a new technique that allows more sensitive detection of damage to axons than previous methods have done, has opened the way for axonal damage to be investigated more systematically and in more detail in a greater variety of diseases than hitherto (Gentleman *et al*., 1993; Sherriff *et al*., 1994*b*; Blumbergs *et al*., 1995; McKenzie *et al*., 1996; Geddes *et al*., 1997). Such studies have shown that axon damage occurs in a wide range of CNS disorders. At the same time, imaging studies of the CNS in some of these disorders have reinforced recognition of the serious functional consequences of axonal damage (Matthews *et al*., 1998; Grimaud *et al*., 1999; Fisher *et al*., 2000; Paolillo *et al*., 2000; Pelletier *et al*., 2001). This recognition of the importance of axonal damage has implications both for diagnosis and treatment or prophylaxis of CNS damage in these diseases. In this review we summarize recent findings about axonal damage in a range of different CNS diseases,

and consider its importance for functional disability and recovery and for diagnosis and treatment.

Detecting damaged axons

The traditional stains for visualizing axons, the single, slender processes that emerge from neurons at the axon hillocks and extend with a relatively uniform calibre to the terminals up to a metre or more away, are silver stains that bind to the neurofilaments. With such stains, damaged axons appear swollen because of the interruption in their fast transport system and the proximal accumulation of organelles and fluid.

Immunocytochemical staining for β-APP more sensitively detects axons that have impaired fast axonal transport (Fig. 1). In normally functioning axons, the protein is transported in this way and never builds up to a concentration that allows its detection in tissue sections. However, axons that have this transport system disrupted rapidly accumulate β-APP proximal to the disrupted segment. This occurs before conventional morphological evidence of axonal damage (e.g. in the form of axonal end bulbs) develops, so the immunocytochemical method for detecting β-APP is more sensitive than routine histological methods for detecting axon damage. Other proteins transported by fast axonal transport also accumulate but antibodies to β-APP have been shown to be the most sensitive for detecting this type of damage (Grady *et al*., 1993; Gultekin and Smith, 1994; Ng *et al*., 1994; Sherriff *et al*., 1994*a*; Li *et al*., 1995; Pesini *et al*., 1999). Experimental animal studies of brain trauma have shown that some axonal damage is reversible but it is not known if axonal damage severe enough to be detected with β-APP immunoreactivity in humans is ever reversible. With regard to the timing of damage detectable in this way, the immunoreaction in damaged axons for β-APP becomes positive in head trauma 1–3 h after the insult (Sherriff *et al*., 1994*b*; McKenzie *et al*., 1996; Oehmichen *et al*., 1998) and remains positive for up to 1 month (Geddes *et al*., 2000). However, the possibility that degenerate axons survive in this state for longer is raised by the findings of Blumbergs and colleagues that β-APP immunoreactivity remained detectable 99 days after head trauma (Blumbergs *et al*., 1994). The distal parts of irreversibly damaged axons will undergo Wallerian degeneration, which can be detected by such traditional methods as the Marchi technique on tissue sections and in living subjects by neuroimaging with (Banati *et al*., 2000) or without (Simon *et al*., 2000) novel ligands. An estimate of the scale of long-previous irreversible axonal damage can be obtained by performing estimates of axon numbers, a relatively straightforward task using modern computerized image analysis facilities.

Head and spinal cord trauma

Head trauma

Head trauma encompasses a broad spectrum of focal and diffuse pathologies. Focal brain injury is most often associated with an impact to the head whereas diffuse brain injury results from inertial forces that are commonly produced by road-traffic accidents, falls from a height and, in some cases, from assaults and sports injuries (Gennarelli *et al*., 1982; Geddes *et al*., 2000). Focal brain injury may produce mass effects from haemorrhage, contusion or haematoma, which can induce herniation and brainstem compression. As a result, coma may not be immediate but may develop later. Diffuse brain injury has been demonstrated in animal models to induce an immediate and prolonged post-traumatic unconsciousness in the absence of mass lesions (Gennarelli *et al*., 1982).

Damage to axons is almost a universal finding in cases of mild, moderate and severe head trauma (Gentleman *et al*., 1995) with outcomes ranging from mild concussion (Jane *et al*., 1985; Blumbergs *et al*., 1994; Adams *et al*., 2001) to profound coma and even the vegetative state (Kinney and Samuels, 1994; Fitzpatrick *et al*., 1998; Adams *et al*., 1999). Resultant traumatic axonal injury (TAI) may vary from small foci of axonal injury to the most severe form, diffuse TAI, originally termed diffuse axonal injury (DAI), in which there is widespread axonal injury throughout the brain, including the brainstem (Geddes *et al*., 2000) (Fig. 1A).

Recently, Smith and colleagues have explored the anatomical origins of post-traumatic coma using a pig model of inertial brain injury induced by rotational acceleration of the head in the axial and coronal planes (Smith *et al*., 2000). They found that immediate and prolonged coma was produced only by head axial plane rotation, although DAI was produced by head rotation along both planes. However, extensive axonal damage in the brainstem was found only in the pigs injured by head rotation in the axial plane. Furthermore, the severity of coma was found to correlate with both the extent of axonal damage in the brainstem and the applied kinetic loading conditions. No relationship was found between coma and the extent of axonal damage in other brain regions. Thus, two major findings evolved from this work: injury to axons in the brainstem plays a major role in the induction of immediate post-traumatic coma, and DAI can occur without coma (Smith *et al*., 2000).

Even minor head injury is thought to result in structural damage to axons (Oppenheimer, 1968; Jane *et al*., 1985; Blumbergs *et al*., 1994). Minor degrees of axonal injury may provide an explanation for concussion, and for cognitive and behavioural sequelae in less severe head injury (Oppenheimer, 1968; Blumbergs *et al*., 1994). Work in animal models also supports this assertion (Povlishock *et al*., 1983; Jane *et al*., 1985). Brief concussive injury was produced in monkeys by acceleration–deceleration non-impact injury and the presence of degenerating axons was investigated using the Nauta and Fink-Heimer techniques (Jane *et al*., 1985). Degenerating axons were found predominantly in the brainstem but not all of the axonal projections in a given system were destroyed. This led the authors to suggest that the surviving neural elements would replace those that were degenerating, in particular the synaptic connections.

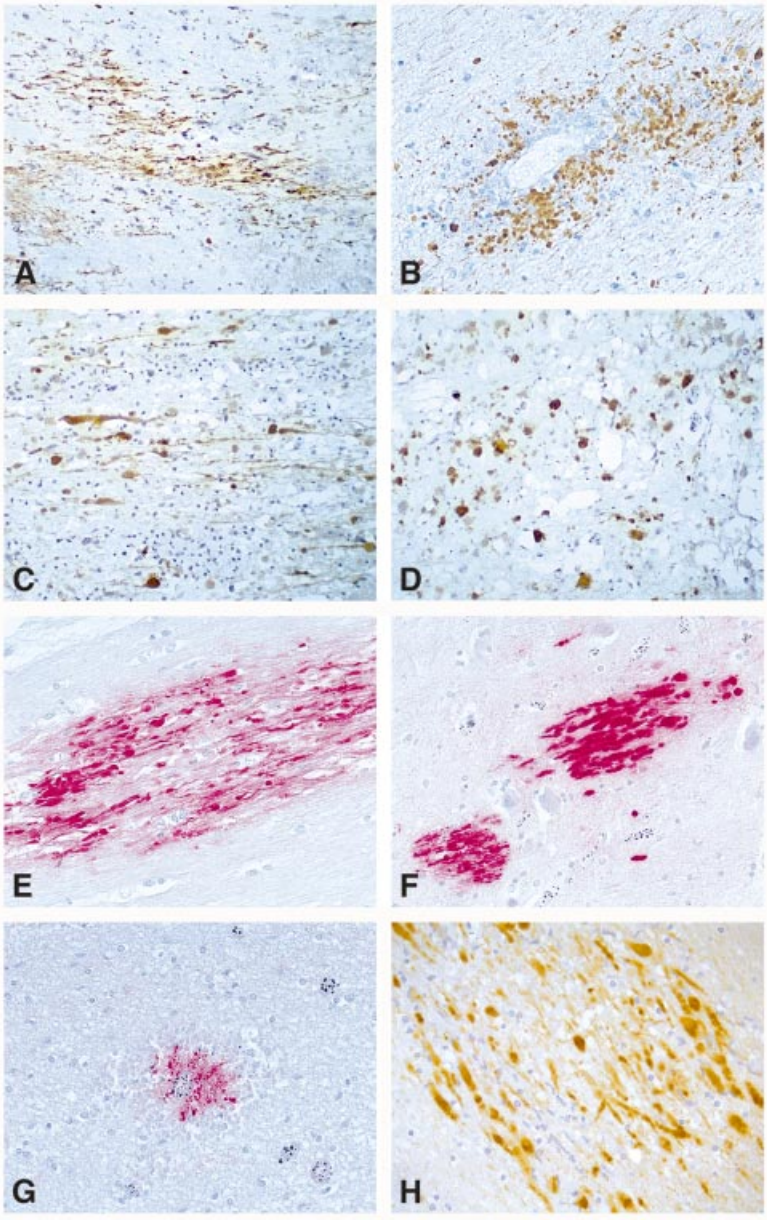

Fig. 1 Brain sections stained for β-amyloid precursor protein (β-APP) (brown immunostain in **A**–**D** and **H**, red immunostain in **E**–**G**) to visualize areas of axonal injury in patients who died with head injury (**A**), acute haemorrhagic leucoencephalitis (AHLE) (**B**), central pontine myelinolysis (CPM) (**C**–**D**), malaria (**E**–**G**) and HIV (**H**). (**A**) Patients who die with head injury often show waves of diffuse axonal staining. (**B**) AHLE is characterized by striking perivenous axon damage as shown here. (**C**–**D**) In CPM, axon damage is seen in the acutely demyelinated base of the pons and affects both transversely running fibres, seen in longitudinal section in **C**, and longitudinally running fibres, seen cut in transverse section in **D**. (**E**–**G**) Axonal damage is a common finding in patients with severe malaria (**E**–**F**). Focal patches of axonal injury are intensely immunoreactive for β-APP, are clearly demarcated, and consist of linear arrays of damaged axonal segments that often appear grossly swollen or club-shaped (**E**–**F**). Injured axons associated with a vessel containing sequestered malaria parasites and a haemorrhage (**G**). The extent and distribution of axonal injury are significantly higher in severe malaria patients with cerebral complications (**F**–**G**). (**H**) Disseminated and swollen axonal segments in cerebral white matter from a patient who died with HIV encephalitis.

However, it was Polvishock and colleagues, using a cat model, who suggested that the damage to axons may be reversible in minor head injury i.e. disruption of axons without physical shearing or tearing (Povlishock *et al*., 1983).

There have been only limited studies of minor head trauma in humans, as patients rarely die from their injuries. One study examined 20 patients with moderate disability after traumatic brain damage who survived for 1–47 years after

injury (Adams *et al*., 2001). DAI was found in 30% of the patients but the diagnosis of axonal injury depended on the microglial reaction rather than the identification of acute abnormalities in axons because of the long period of survival in every case. Blumbergs and colleagues examined five cases of concussive head injury in humans who died of other causes (Blumbergs *et al*., 1994). Multifocal axonal injury in structures thought to be involved in consciousness and memory function was demonstrated using β-APP immunohistochemistry. In particular, there was involvement of the fornices, which are the major hippocampal projection pathways and are thought to be important in memory. Therefore the authors suggested that this may underlie some of the persisting memory deficits that occur in patients after concussion. The mechanisms of TAI have been investigated at the cellular level (reviewed in Maxwell *et al*., 1997) and will be discussed in more detail below.

Paediatric head trauma

Head injury in infancy and childhood is the single most common cause of death and permanent disability. The type of injury that results depends on the mechanism of trauma and the age of the patient. Young infants are particularly susceptible to head injury because of the thinness and pliability of the skull; the softness of the brain, which is composed primarily of neurons without dendritic connections; the paucity of the myelin sheaths of axons; the relative flatness of the skull base; the underdeveloped neck muscles supporting the large, heavy head; and the nature of the subarachnoid space, which is large in its extent but shallow in depth (reviewed by Case *et al*., 2001). As the child becomes mobile, falls become the number one cause of accidental injury, frequently producing fractures and contusions (Zimmerman and Bilaniuk, 1994).

The most comprehensive neuropathological studies on the structural basis of clinical deficits in fatal paediatric brain damage have been made by Geddes and colleagues (Geddes *et al*., 2001*a*, *b*). These studies involved 53 cases of fatal non-accidental head injury, which included β-APP immunohistochemistry for microscopic damage. The most important finding was a statistically significant pattern of age-related axonal damage. When axonal injury was present in children aged >1 year, the pattern was similar to that of DAI in adults. However, in the infant group, aged <1 year, DAI was rare whereas hypoxia was the predominant neuropathological finding. Axonal pathology appeared predominantly vascular in nature, associated with brain swelling and raised intracranial pressure. Anatomically, 31% showed evidence of localized axonal injury to the craniocervical junction or the cervical cord. This indicated that the craniocervical junction is vulnerable in infant head injury as a result of stretch injury from cervical hyperextension/flexion. The authors proposed that this damage could account for the observed apnoea, which could in turn lead to hypoxic brain damage. Two explanations for this finding given by this group were (i) the unmyelinated axon of the immature cerebral hemispheres is relatively resistant to traumatic damage, and (ii) in shaking-type injuries the brain is not exposed to the forces necessary to produce DAI.

Spinal cord injuries

Spinal cord injury is often caused by mechanical depression from displacement of fractured vertebrae and/or discs extending into the spinal canal (Nashmi and Fehlings, 2001*a*, *b*). The injury often spans several segments and the lesion site is characterized by central cavitation and a subpial rim of surviving axons of small diameter with myelin disruption. However, the spinal cord is rarely totally transected even after severe spinal cord injury associated with complete paralysis (Nashmi and Fehlings, 2001*a*, *b*). The neurological deficits produced by spinal cord injury are predominantly caused by the loss of white matter, particularly the long tracts through which descending and ascending communications occur (Rosenberg *et al*., 1999).

Immediately after fatal spinal cord injury in humans there is complete disruption of a proportion of axons and partial injury to the remaining axons. Injured axons show irregular varicosities, spheroids and β-APP immunoreactivity (Ahlgren and Olsson, 1996; reviewed in Nashmi and Fehlings, 2001*b*). Recently, Nashmi and Fehlings (2001*b*) reviewed the time course of events following spinal cord injury. Eight days to 5 weeks after injury, the injury site is characterized by axonal swelling, fragmentation and increased spacing between axons. Seven to eight weeks after injury, Wallerian degeneration becomes evident in dorsal columns above the injury and lateral columns below the injury, with debris-laden phagocytes intermingled with axonal and myelin debris. Invading macrophages remove the debris at the lesion and scar tissue forms due to astrogliosis. In the chronic stage, the lesions consist of glial-lined, multilocular cysts, nerve root regeneration and a subpial rim of preserved white matter.

The relationship between axonal sparing and neurological outcome has been studied extensively in rat, cat and ferret models of spinal cord injury (Eidelberg *et al*., 1981; Fehlings and Tator, 1995). In these models, <12% of the normal number of axons were shown to be required for recovery of neurological function, such as restoration of locomotion. In human cases it was shown that there was no relationship between neurological function and the extent of preservation of white matter spinal cord tissue (reviewed in Nashmi and Fehlings, 2001*b*). However, retrograde tracing studies in a rat model of spinal cord injury showed that the integrity of certain spinal tracts, namely the rubrospinal, vestibulospinal and raphespinal tracts, correlated with neurological recovery (reviewed in Fehlings and Tator, 1995; Nashmi and Fehlings, 2001*b*).

Several mechanisms have been proposed to explain the dysfunction of surviving axons that traverse the site of spinal cord injury. In the case of experimental contusion injury, the

white matter appears mostly intact. However, over the next 4 h pathology increases, suggesting that secondary injury mechanisms are involved in the loss of white matter (reviewed in Rosenberg *et al*., 1999). Some of these secondary injury mechanisms include: altered activity of ion channels, abnormal myelination, disturbances in mitochondrion function, excitotoxicity, changes in glial–axonal coupling, nitric oxide and anti-ganglioside antibodies (reviewed in Dumont *et al*., 2001; Nashmi and Fehlings, 2001*b*).

Metabolic encephalopathies

There are various pathologies that may contribute to or be the cause of β-APP immunoreactivity in axons. This has implications for medicolegal practice and has inspired a number of groups to investigate the relationship between metabolic disruption and axonal injury. In this context it is particularly important to determine whether the patterns of axonal injury as a result of physical trauma and subsequent trauma-related mechanisms can be dissociated.

Several groups have investigated the role of hypoxia in the induction of axonal injury. Kaur and colleagues have suggested that cardiac arrest *per se* can mimic DAI (Kaur *et al*., 1999). This is disputed by Dolinak and colleagues, who believe that proper sampling and due attention to brain swelling and increased intracranial pressure (ICP) allow the patterns to be distinguished (Dolinak *et al*., 2000*a*, *b*). On the other hand, carbon monoxide poisoning, an example of histotoxic hypoxia that does not result in raised ICP, causes considerable damage in the white matter (Dolinak *et al*., 2000*a*). However, the patterns of β-APP staining can be diverse, ranging from specific foci to a wide distribution.

Dolinak and colleagues have investigated the role of hypoglycaemia in the causation of axonal injury. In this study, extensive neuropathological examination was undertaken in 13 patients in whom coma was attributed to hypoglycaemia. Immunoreactivity for β-APP was present in 11 of the 13 cases. The amount of axonal injury could be attributed to the hypoglycaemia alone, although the amount and the distribution of the axonal injury were altered in the presence of raised ICP. In one case the distribution closely mimicked that seen in microscopic diffuse TAI (Dolinak *et al*., 2000*b*).

Multiple sclerosis

Since the earliest descriptions of the pathology of multiple sclerosis, it has been characterized as a disease in which there is multifocal demyelination with relative preservation of axons. The relative axonal preservation serves to distinguish a plaque of demyelination from a white-matter infarct. The early investigators of multiple sclerosis were well aware of axonal damage in multiple sclerosis plaques (reviewed by Kornek and Lassmann, 1999) and of Wallerian degeneration secondary to this. However, relatively few attempts were made to quantify axonal loss (Greenfield and King, 1936; Putnam, 1936) and relate it to clinical disability. Two recent developments have served to put axonal damage under greater scrutiny in multiple sclerosis. The first is the availability of the β-APP immunoreaction, which provides a much better means of detecting damaged axons in tissue sections, as mentioned above. The second is non-invasive MRI and spectroscopic methods that provide accurate means of assessing axon loss in living patients and allow longitudinal clinicopathological studies to be undertaken in multiple sclerosis for the first time. These latter studies have shown, on the one hand, that demyelination on its own does not account for chronic functional impairments, and on the other that magnetic resonance spectroscopy (MRS) and MRI measures of axonal loss show a strong correlation with disability in multiple sclerosis (Matthews *et al*., 1998; Grimaud *et al*., 1999; Fisher *et al*., 2000; Paolillo *et al*., 2000; Pelletier *et al*., 2001). In consequence comes the important realization that if disability is to be prevented we need to understand when, where and how axon damage arises in the disease.

There is now considerable consensus that axon damage occurs early in the course of multiple sclerosis. The two initial studies of β-APP immunoreactivity in multiple sclerosis showed that axonal damage occurs in acute plaques (Ferguson *et al*., 1997; Trapp *et al*., 1998) and this has been confirmed by later studies (Bitsch *et al*., 2000; Kornek *et al*., 2000; Kuhlmann *et al*., 2002). Acute plaques and the actively demyelinating borders of chronic active plaques display numerous β-APP-immunoreactive damaged axons, while chronic plaques contain few, if any (Ferguson *et al*., 1997; Kornek *et al*., 2000; Kuhlmann *et al*., 2002) (Fig. 2). The greatest abundance of damaged axons is seen in the acute plaques occurring in the early years of disease, as detected in biopsy specimens taken before the clinical diagnosis was certain (Kuhlmann *et al*., 2002). In several ways this early occurrence of axonal damage in multiple sclerosis is counterintuitive to established views of the disease. A primarily demyelinating disease might be expected to show demyelination followed by axon loss rather than simultaneous axon and myelin destruction. Furthermore, the initial relapsing–remitting pattern of disease in the early stages in most cases does not fit well with early axon damage, which is presumed to be largely irreversible. Axon loss occurring much later would have fitted better with secondary progression of disease. However an initial ability to repair at least some damage to axons and a normally substantial reserve followed by a progressive failure of repair would be compatible with the clinical course.

The overwhelming majority of damaged axons are found in or at the borders of plaques, and very few occur in normal-appearing white matter. The damage to axons in plaques results in Wallerian degeneration, which is readily detectable as reduced axon density or total number in normal-appearing white matter (reduced by ~50%) as well as in plaques (reduced by 50–82%) (Mews *et al*., 1998; Ganter *et al*., 1999;

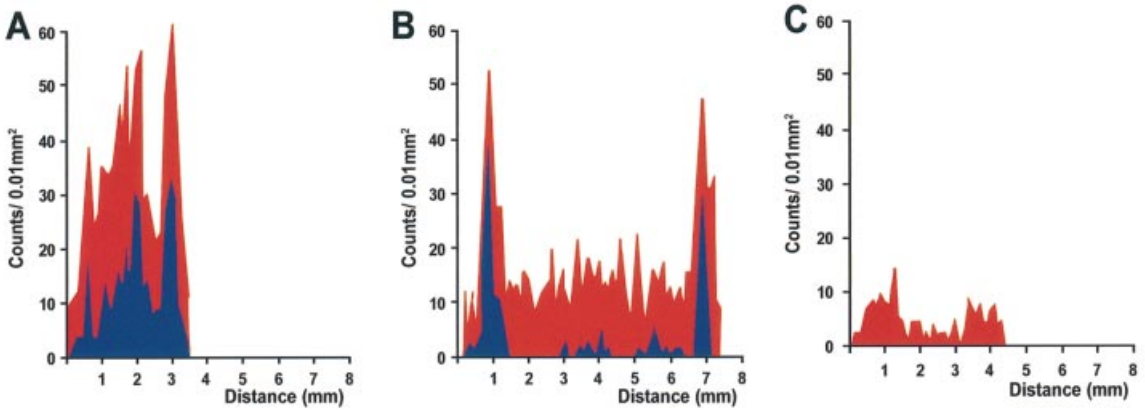

Fig. 2 Graphs illustrating the density of macrophages (blue) and APP+ axons (red) at distances from the centre of an acute plaque (**A**), a chronic active plaque (**B**) and a chronic inactive plaque (**C**) taken from autopsy material from cases of multiple sclerosis. (From an original figure published in Ferguson *et al*., 1997. Reproduced with permission of Oxford University Press.)

Evangelou *et al*., 2000*a*, 2001; Lovas *et al*., 2000). That damage to axons in plaques is responsible for axon loss in normal-appearing white matter is supported by the finding that axon density in the corpus callosum strongly correlates with the volume of plaques in the cerebral hemispheres (Evangelou *et al*., 2000*b*).

The question of how axon damage occurs in multiple sclerosis is harder to answer. Is it secondary to the loss of myelin or is it a direct effect of other changes, such as inflammation, that are taking place in acute plaques? Obviously, clarification of this point has important implications for trying to prevent disability in multiple sclerosis. If axon damage is secondary to demyelination, vigorous and early promotion of remyelination might be helpful, but if the damage is directly inflicted remyelination may have little effect. One interesting feature of the axonal loss in multiple sclerosis is that it is remarkably specific for small nerve fibres and spares large fibres, though whether this reflects selective damage of small fibres or selective inability of these fibres to recover from damage is not clear (Ganter *et al*., 1999; Lovas *et al*., 2000; Evangelou *et al*., 2001). That axonal damage has a reversible element in acute disease is suggested by the MRS observation of a temporary decrease in *N*-acetyl aspartate, a neuronal marker, in acute relapses of multiple sclerosis (Davie *et al*., 1994).

Inflammatory mediators are attractive candidates for axon-damaging agents in acute multiple sclerosis plaques. Nitric oxide and its synthetic enzyme nitric oxide synthase, which is detectable in increased amounts in multiple sclerosis plaques (Bo *et al*., 1994; Bagasra *et al*., 1995; De Groot *et al*., 1997; Bitsch *et al*., 2000), has been shown to damage axons irreversibly *in vitro*, small fibres being particularly at risk (Smith *et al*., 2001; Garthwaite *et al*., 2002). Inducible nitric oxide synthase mRNA in multiple sclerosis plaques was found to correlate with axon density (Bitsch *et al*., 2000). Matrix metalloproteinases, which are released in acute multiple sclerosis plaques (Anthony *et al*., 1997; Gveric *et al*., 2001; Leppert *et al*., 2001; Lindberg *et al*., 2001), are also known to be capable of damaging axons *in vitro* (Gijbels *et al*., 1993; Chandler *et al*., 1995). Autoimmune encephalitis in experimental animals appears to mimic the disability-related axonal loss seen in multiple sclerosis and may afford a convenient opportunity to study axon-damaging mechanisms of relevance to multiple sclerosis (Kornek *et al*., 2000; Wujek *et al*., 2002). More general aspects of the mechanics of axon damage are discussed below.

Other demyelinating diseases

Axon damage in other demyelinating diseases has been studied in much less detail than in multiple sclerosis but, as in that disease, it has long been recognized that axon loss occurs both in chronic diseases, such as the leucodystrophies (Schaumburg *et al*., 1975; Lake, 1997; Powers and Moser, 1998), and acute diseases, such as acute haemorrhagic leucoencephalitis (AHLE). In the latter para-infectious disease there is striking perivenous axon damage demonstrable with β-APP immunocytochemistry (Fig. 1B), whereas in the other acute para-infectious disease, acute perivenous encephalitis, very little axon damage is demonstrable (unpublished observations, Ghosh *et al*.). Axon damage is also a prominent feature of central pontine myelinolysis, another acute demyelinating condition with a high mortality rate seen in severely debilitated or alcoholic subjects who undergo rapid correction of hyponatraemia (N.Ghosh, G.C.De Luca, M.M.Esiri, unpublished observations) (Fig. 1C and D).

Infections

Malaria

Impairment of consciousness and other signs of cerebral dysfunction are common complications of severe falciparum malaria. The majority of neurological complications are transient but a significant minority of patients develop

sequelae (Newton *et al*., 2000). The mechanism by which malaria infection induces severe but potentially reversible neurological dysfunction remains elusive. The malaria parasite invades and develops within erythrocytes, which sequester in the cerebral microvasculature by adhesion to specific endothelial receptors (Turner, 1997). However, it is not known how malaria parasites, which remain within the vascular space and do not infect brain cells, influence parenchymal brain function to induce coma and possibly death.

Cerebral white matter lesions have been associated with neurological complications in malaria infection (Davis *et al*., 1992; Kochar and Makkar, 1994; Dugbartey *et al*., 1998; Lewallen *et al*., 1999; Medana *et al*., 2002). In our recent pathological studies of cerebral malaria in Vietnamese adults (Medana *et al*., 2002) we have identified impairment of transport within axons as a possible cause of neurological dysfunction that has the potential either to resolve or to progress to irreversible damage. β-APP immunocytochemistry was performed on brain sections from the cortex, internal capsule, pons and cerebellum of 54 adult Vietnamese patients with falciparum malaria to determine whether defects in axonal transport would reflect cerebral impairment in this group. The extent and distribution of axonal damage was found to distinguish the groups of patients infected with *Plasmodium falciparum* with and without cerebral complications during life. This is the only finding, in our series, of positive quantitative associations with clinical manifestations of cerebral malaria with the exception of parasite sequestration. There were significant associations between axonal damage and important clinical and biochemical parameters, including lactate, CSF protein and Glasgow coma score. β-APP staining was also found in 'pure cerebral malaria' patients without other organ complications. However, the mechanisms of axonal injury remain elusive because (i) axonal injury could be found independently of oedema, haemorrhage and glial responses, (ii) there were no associations between axonal injury and ICP or systemic hypoglycaemia, (iii) there was no association with impairment of vital organ function, such as renal failure, jaundice and shock, and (iv) there was no exacerbation of axonal injury with increasing numbers of criteria of severity, which may imply that although these patients are increasingly ill and more likely to die, the extent of axonal injury within the CNS is determined independently or early in the disease (Medana *et al*., 2002). These findings suggest that axons are vulnerable to a broad range of cerebral insults that occur during falciparum malaria infection. Disruption in axonal transport may represent a final common pathway leading to neurological dysfunction in cerebral malaria (Medana *et al*., 2002).

These pathological findings are consistent with studies of cerebral malaria patients during life. Impairment of somatosensory discrimination and conduction has been found in patients with a history of cerebral malaria (Kochar and Makkar, 1994; Dugbartey *et al*., 1998). Also, cotton wool spots have been found in retinas of patients with cerebral malaria (Davis *et al*., 1992; Lewallen *et al*., 1999). In both cases the findings are likely to be manifestations of axonal disruption or obstruction of axonal flow (McLeod *et al*., 1977). Thus, discerning the mechanisms of axonal injury is likely to be an important step in understanding the specific neurological complications associated with cerebral malaria.

Kochar and Makkar (1994) recorded somatosensory evoked potentials by median nerve stimulation in 10 adult patients from India with cerebral malaria. They observed abnormalities in 80% of patients, of which the commonest problem was prolongation of central conduction time. It was not possible for the authors to elucidate the exact pathogenetic mechanisms responsible for the electrophysiological changes. However, they suggested that a phase exists in cerebral malaria which causes delay in the conduction of travelling impulses in the brain due to damage in the white matter and changes in volume conduction at the partition of geometric boundaries.

Dugbartey and colleagues also have investigated, using neuropsychological tests, neuropathological changes in paediatric patients with a history of cerebral malaria (Dugbartey *et al*., 1998). They pointed out that the corpus callosum is the largest neocortical fibre tract in the brain that facilitates efficient communication between the cerebral hemispheres. They reasoned that if cerebral malaria shows a predilection for cerebral white matter, interhemispheric transfer inefficiencies should be demonstrable. Bimanual tactile roughness discrimination was found to be significantly impaired in the cerebral malaria group. In contrast, intrahemispheric processing of tactile information was intact. This group suggested that inefficiency in the integrity of the callosal fibres could account for these findings, although damage to alternative subcortical pathways involved in transfer across the cerebral hemispheres could not be ruled out entirely.

Cotton wool spots have been found in the retinas of patients with cerebral malaria (Davis *et al*., 1992; Lewallen *et al*., 1999). The intense retinal whiteness of small cotton wool spots represents gross localized axonal distensions secondary to the cessation of axoplasmic flow (McLeod *et al*., 1977). Davis and colleagues found that cotton wool spots were observed much more frequently than retinal haemorrhages in adult Thai patients with severe malaria (Davis *et al*., 1992). Furthermore, all but one of the patients with cotton wool spots at presentation had an impairment of consciousness. In these cases the cotton wool spots were found in association with capillary non-perfusion. However, two comatose patients had neither cotton wool spots nor non-perfusion. The lack of cotton wool spots in these patients was thought to reflect the marked variability in the sequestered parasite biomass among patients with severe malaria as well as the variations within individuals and tissues. Cotton wool spots are less common in African children, occurring in 5% of those with cerebral malaria (Lewallen *et al*., 1999).

HIV

Neurological disease is a common occurrence in patients with acquired immunodeficiency syndrome (AIDS), causing clinical symptoms ranging from cognitive impairments, motor disturbances, behavioural changes, headache and peripheral neuropathy (reviewed by Lawrence and Major, 2002). It has been estimated that 10% of AIDS patients will have a CNS lesion as the first clinical manifestation and 40% will have some type of neurological complication during the course of the disease, and that >70% of AIDS autopsies will demonstrate neuropathological findings (reviewed by Gonzalez *et al*., 1998). A proportion of neurological disease will be associated with opportunistic infections as a result of immunodeficiency. However, in the absence of these infections 20–30% of patients will develop neurocognitive defects: human immunodeficiency virus (HIV)-1-associated cognitive motor complex, also termed HIV dementia (HIVD). At autopsy, the brains of patients with HIVD show numerous disseminated foci composed of microglia, macrophages and multinucleated giant cells (Gray *et al*., 1998), termed HIV encephalitis (Masliah *et al*., 1992). The presence of subclinical decline in cognitive performances in HIV patients before significant immunodeficiency is controversial. However, it has been shown that HIV is present in the CSF and brain during the early asymptomatic phases of HIV infection (An *et al*., 1997).

Although the neuropathological correlates of the clinical manifestations remain unclear, several candidates have been proposed, including viral load, myelin pallor and neuronal loss. As with cerebral malaria, an intriguing problem is that neurons are not the target of infection. In addition, there is some evidence supporting the reversibility of neurological complications. An example highlighted by Lawrence and Major (2002) is that some patients may improve after treatment and further deterioration may be delayed, suggesting that irreversible nerve cell loss is unlikely to be involved. It is likely that HIVD reflects neuronal dysfunction resulting from several pathological mechanisms, which will be discussed in more detail in later sections.

With the introduction of APP immunohistochemistry, widespread axonal injury in the white matter of AIDS patients and to a lesser degree in pre-AIDS cases has been revealed (An *et al*., 1997; Giometto *et al*., 1997; Raja *et al*., 1997; Gray *et al*., 1998; Adle-Biassette *et al*., 1999). APP$^+$ axons have been found predominantly in the subcortical white matter, basal ganglia (An *et al*., 1997; Giometto *et al*., 1997; Raja *et al*., 1997; Gray *et al*., 1998; Adle-Biassette *et al*., 1999) and the brainstem, including pontocerebellar fibres (Gray *et al*., 1998; Adle-Biassette *et al*., 1999). APP$^+$ axons are commonly found in association with microglia/macrophages and multinucleated giant cells. These results have led some authors to hypothesize that areas of disturbed axonal injury in clinically active regions of the brain may contribute to the appearance of neuropsychological symptoms (Giometto *et al*., 1997).

Associations between axonal injury and other neuropathological findings have been made to try to define the possible mechanisms of axonal injury in HIV infection. Diffuse myelin pallor is a common autopsy finding in HIV infection and is more frequent in those who show features of encephalopathy (reviewed in Raja *et al*., 1997). In the majority of reports there is a parallel between APP$^+$ axons and myelin pallor. However, axonal injury is also present in the absence of pallor. This led Raja and colleagues to suggest that β-APP staining is a more sensitive marker of some forms of white matter damage in HIV infection (Raja *et al*., 1997). The link between myelin pallor and axonal injury is less clear in some reports of HIV$^+$ individuals without AIDS (An *et al*., 1997) and is clearly not involved in a featured case of AIDS with a relapsing course with neurological signs (Gray *et al*., 1998). In the former study, of 29 HIV-1$^+$ asymptomatic patients, pallor was minimal in three cases and absent in the others. Axonal injury in the absence of myelin pathology has also been described in a patient who died after a relapsing course of neurological signs. In this case axonal damage was an extremely frequent finding without other neuropathological changes or evidence of productive HIV infection of the brain (Gray *et al*., 1998). Further, in a monkey model of HIV using simian immunodeficiency virus-infected macaques, significant increases in β-APP were found in the white matter in the absence of myelin pallor (Mankowski *et al*., 2002). From these results it is clear that axonal injury can be a primary manifestation of HIV infection in the brain.

The association of axonal injury with vessels has also been a topic of interest, and has produced variable findings. In the report by Raja and colleagues, APP$^+$ foci showed an approximately perivascular distribution (Raja *et al*., 1997). Similar results were found in the case report of Gray and colleagues (Gray *et al*., 1998). In both cases the authors emphasized the potential role of systemic vascular-related factors in the pathogenesis of the axonal damage. In contrast, the study of HIV$^+$ individuals without AIDS showed that the majority of β-APP did not colocalize with vessels (An *et al*., 1997). Similar findings were made in another study by the same group (Giometto *et al*., 1997). In this study the authors made a distinction between β-APP$^+$ structures—ballooned structures representing chronic axonal damage, and bundles of parallel formations representing acute axonal damage. They reported that, with few exceptions, acute axonal damage was not related to blood vessels.

Thus, although this is not conclusive, axonal injury remains an attractive candidate to explain the functional impairment in HIV$^+$ patients and discerning the mechanisms of axonal injury is strongly warranted.

Human T lymphotropic virus type I (HTLV-I)

HIV is not the only viral disease that indirectly leads to axonal injury in the CNS. Another example is HTLV-I, which is associated with adult T-cell leukaemia and a chronic progressive disease of the spinal cord termed HTLV-I-

associated myelopathy (HAM)/tropical spastic paraparesis (TSP) (Umehara *et al*., 2000). Neuropathological studies have revealed infiltration of T lymphocytes and macrophages and increased expression of cytokines. However, demyelination and axonal loss have been described as the histological hallmarks of inflamed lesions of HAM/TSP and the pathological correlates of persistent disability that characterize spastic paraparesis and urinary disturbance.

How the CNS damage develops is unclear but, as in HIV, it is unlikely to be the result of direct infection with virus (Levin *et al*., 2002). Other findings similar to those with HIV are the association with myelin pallor and degeneration, although axonal injury can be found in the absence of myelin pathology. Furthermore, APP^+ axons tend to be located in relation to blood vessels, particularly veins, with or without inflammatory cell infiltrates. Again, these findings have led to the conclusion that alteration in the blood–brain barrier and seepage of neurotoxins into the CNS might relate to the primary axonal changes in HAM/TSP (Umehara *et al*., 2000). Unlike HIV, the neurotoxic mechanisms are more clear, and evidence points to an autoimmune aetiology (Levin *et al*., 2002) involving autoantibodies and cytotoxic T cells. The role of autoimmunity in axonal injury will be discussed in a later section.

Subcortical ischaemic disease

Subcortical ischaemic disease is extremely common in the elderly. It was found in 78% of a recent community-acquired sample of 209 brains obtained from subjects dying aged >65 years (Esiri *et al* 2001). It is also a common substrate for vascular dementia (Esiri et al., 1997; Vinters *et al*., 2000; White *et al*., 2002). It takes the form of lacunes (small infarcts in subcortical grey and white matter) and multifocal areas of subtotal infarction or pallor of staining in myelin-stained sections of white matter associated with hyaline thickening of the walls of small arteries and arterioles and widening of perivascular spaces. A few cases with this pattern of pathology suffer from the inherited condition CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy), in which, in addition to the features outlined above, there are granular periodic acid–Schiff (PAS)-positive deposits in the media of small subcortical arteries that are associated with an immunoreaction for the Notch 3 receptor (Joutel *et al*., 2000). People suffering from vascular dementia with this type of pathology usually show a neuropsychological profile of cognitive impairment more suggestive of subcortical than of cortical disease, and this impairment has been largely attributed to the demyelination and/or axon loss that occurs in the white matter (Skoog *et al*., 1996; Inzitari *et al*., 2000). There have been limited numbers of studies examining β-APP immunoreactivity as a marker of axon damage in vascular dementia, and these have described patchy axonal damage (Suenaga *et al*., 1994; Teahan *et al*., 2002). However, use of this marker is likely to underestimate the extent of axonal damage in this disease because it only discloses relatively recently damaged axons while the clinical disease itself often lasts years. Therefore, as in multiple sclerosis, the extent of axon *depletion* is likely to be a better measure of axon damage over the course of the whole disease. Estimates of the severity of axon depletion in vascular dementia are, however, complicated by the fact that most cases of vascular dementia show some degree of Alzheimer-type pathology as well, and this disease can also be associated with axonal depletion, e.g. in the corpus callosum. The axonal depletion in Alzheimer's disease is thought to represent Wallerian degeneration secondary to neuronal loss from the association cortex (Weis *et al*., 1991; Bozzali *et al*., 2002).

Mechanisms of axonal injury

Selective axonal injury can occur in the absence of focally related somatic or dendritic alterations. Axons often extend for great distances from their cell bodies of origin, and are therefore susceptible to ischaemic or toxic damage in several different vascular territories, without the death of the parent neuron. Regardless of injury mode, axonal injury is associated with a broadly similar pattern of disruption, involving increased axonal membrane permeability, deleterious intracellular cascades and disturbance of axonal transport and possibly degeneration (Fig. 3). In most cases the injury mechanisms can occur in neuronal compartments other than the axon.

Increased axonal membrane permeability

Physical deformations (Ziv *et al*., 1995; Rosenberg *et al*., 1999; Wolf *et al*., 2001), energy deficits (Fink *et al*., 1994), acidosis (Hsu *et al*., 2000) and neuroinflammatory disorders (Kornek *et al*., 2001) are some of the causes of changes in the activity of ion channels leading to unphysiological increases in intra-axonal Na^+ and Ca^{2+} (reviewed in LoPachin and Lehning, 1994). Some of the proposed mechanisms for the change in activity are increased Na^+ channel permeability (Rosenberg *et al*., 1999; Wolf *et al*., 2001), decreased ATPase activity (Fink *et al*., 1994; Tavalin *et al*., 1997; Ahmed *et al*., 2000), ectopic distribution of channels (Kornek *et al*., 2001) and reversal of gradient-dependent ion channels (Stys, 1998; Wolf *et al*., 2001).

Deleterious intracellular cascades

Changes at the axolemma are usually followed by excess Ca^{2+} influx into the axon. Ca^{2+} is pivotal in mediating axonal injury and degeneration. Mitochondrial perturbations are consistent with calcium overloading and opening of the mitochondrial membrane permeability transition (MPT) pore, which permeabilizes the mitochondrial membrane for molecules <1.5 kDa. The process leads to the uptake of water, mitochondrial swelling and ultimate mitochondrial rupture (reviewed in Büki *et al*., 2000). Abnormal/pathological

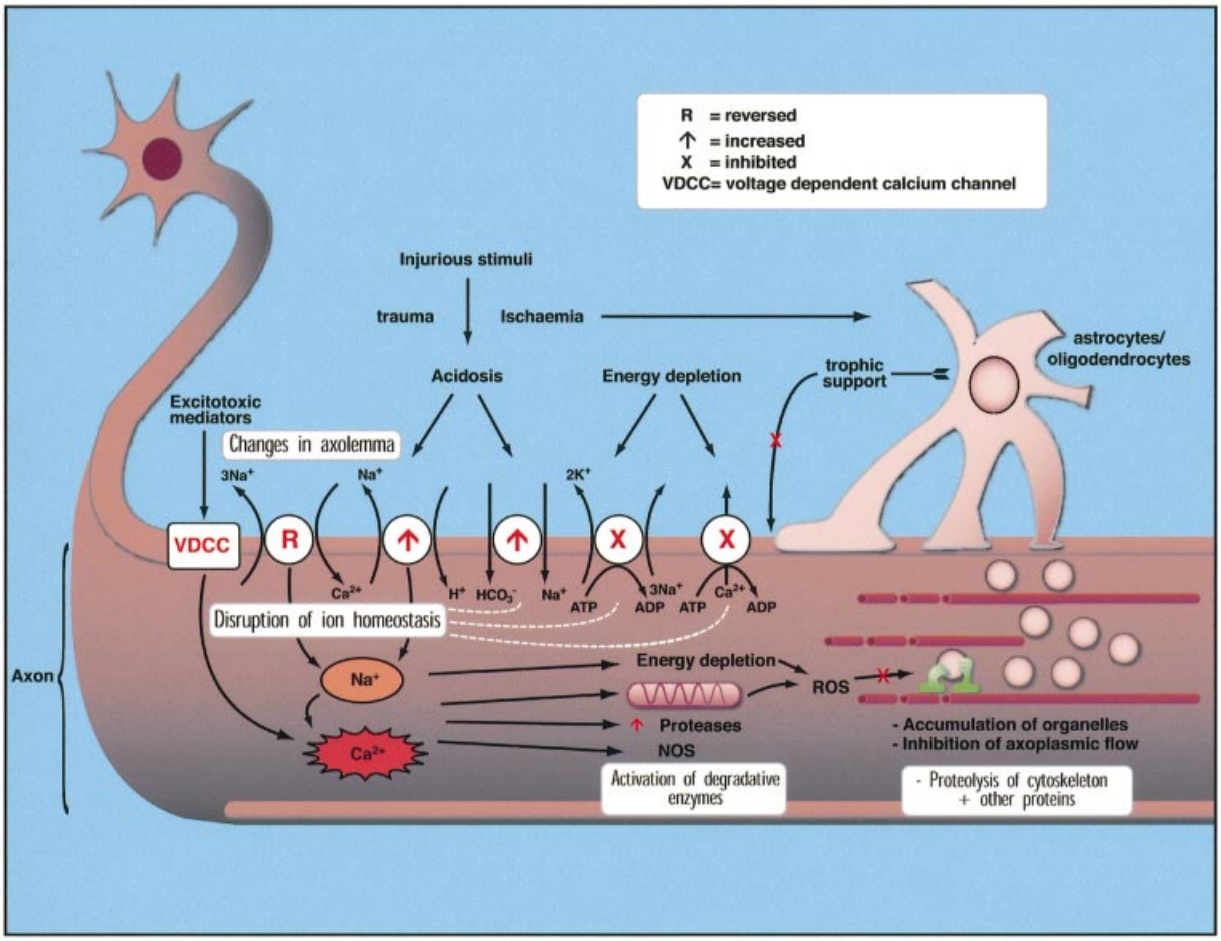

Fig. 3 Diagram illustrating mechanisms of axonal injury. Regardless of injury mode, axonal injury is associated with a broadly similar pattern of disruption: (i) increased axonal membrane permeability with excess Na^+ and/or Ca^{2+} influx into the axon; (ii) deleterious cascades involving activation of intracellular proteases, failure of the mitochondria and cytoskeleton breakdown; and (iii) disturbance of axonal transport and possibly degeneration. See text for more details.

mitochondria, indicative of some form of subcellular perturbation, are associated with cytochrome *c* release into the perimitochondrial domain. Cytochrome *c* release is associated with concomitant caspase activation in the axon (Büki *et al*., 2000).

The activation of calcium-activated cysteine proteases and/or caspases can continue to devastate intra-axonal cytoskeletal and organelle alterations, leading to the ultimate demise of the axon. Caspases can irreversibly cleave intra-axonal spectrin (Wang *et al*., 1998) with ultimate destruction of the cytoskeleton, an event considered to be a major factor in the neuronal degeneration seen in various CNS disorders. Caspase-3 has also been reported to cleave calpastatin, an inhibitor of calpain that might further enhance the ongoing axonal demise (Wang *et al*., 1998; Büki *et al*., 2000). Calpain acts on several substrates, many of which are cytoskeletal proteins, such as neurofilament proteins, microtubule-associated proteins and spectrin (Saido *et al*., 1994; Banik *et al*., 1997; Springer *et al*., 1997; Schumacher *et al*., 1999).

Structural disorganization and/or dissolution of the cytoskeleton are likely to result in severe functional derangements in the axon and that would ultimately affect the neuron as a whole (Fitzpatrick *et al*., 1998).

Indirect effects on axons

There are several indirect mechanisms that can lead to axonal injury. Neuronal–glial interactions are important in the maintenance of brain homeostasis and are vital for neuronal survival after brain injury. Death of glia or loss of function of glia, e.g. acidosis inhibiting glutamate transporters, could affect the outcome of injured axons (Liu *et al*., 1999).

Glia and invading haematogenous cells may be a source of neurotoxic factors produced in response to infectious agents and/or damage to the neural tissue (Giulian *et al*., 1993). Cytotoxic T cells (Manning *et al*., 1987; Medana *et al*., 2001; reviewed in Neumann *et al*., 2002), macrophages and microglia have been implicated in the induction of axonal injury (Brück *et al*., 1996; Bitsch *et al*., 2000). Similarly, autoantibodies (Sloviter *et al*., 1996; Rawes *et al*., 1997; Genain *et al*., 1999), metalloproteinases (Newman *et al*., 2001) and other inflammatory mediators, such as tumour

necrosis factor and nitric oxide, have also been implicated (Brück *et al*., 1996; Giovannoni *et al*., 1998).

Treatments

The question remains as to which processes associated with axonal injury constitute the best target for potential therapeutic intervention. The answer probably lies in the spatial and temporal progression of events. In addition, the interplay of multiple factors including Ca^{2+} buffering capacity, global versus selective nature of the injury, energy demands and production, duration of axonal injury and the resulting magnitude of the Ca^{2+} burden will influence outcome (LoPachin and Lehning, 1997). Potentially beneficial therapy would most likely act on a target that is activated before irreversible structural damage of the axon has occurred. Na^{+} channel blockers have been shown to improve functional outcome and reduce axonal pathology in models of spinal cord crush injury (Teng and Wrathall, 1997) and nitric oxide-induced damage (Kapoor *et al*., 2002), and may have application in treating diffuse axonal injury. Blockade of voltage-gated calcium channels has also been suggested as a potential therapeutic strategy for traumatic axonal injury.

Early phases of TAI involving calcium-activated neutral proteases, such as calpain, have been discussed as rational therapeutic targets. Several experimental studies have reported the potential efficacy of calpain inhibitors in traumatic brain injury (Schumacher *et al*., 2000; Ray *et al*., 2001). The mitochondrial permeability transition pore has also been suggested as an appropriate target for therapeutic intervention. This has been supported by the finding that the use of inhibitors of the mitochondrial permeability transition, which preserve mitochondria, also translate into significant axonal protection (Büki *et al*., 2000).

Conclusion

This review has considered the evidence that axonal damage is of crucial importance in determining the outcome in a remarkably wide range of diseases from the acutely fatal (cerebral malaria, severe brain trauma, acute haemorrhagic leucoencephalitis) through those with an intermediate time of survival (HIV encephalopathy), to the long-standing (multiple sclerosis, vascular dementia). The severity of axon damage in these diseases helps to determine outcome in terms of death or disability. However, the question needs to be asked: is it the axon damage and loss itself that determines outcome, or something else that is closely linked to axon damage? Is it reasonable to attribute death in the acute diseases and disability in the chronic ones to axon damage *per se*? In the rapidly fatal diseases axon damage is widespread and includes axons in the brainstem that are necessary to maintain connections to and from nuclei whose function is essential for life, such as the respiratory and circulatory centres in the medulla. In young infants, for example, it is proposed that a mechanism of fatal non-accidental head trauma involves a stretch injury at the craniocervical junction, resulting in apnoea from interference with respiratory function, hypoxic brain swelling and death as a consequence of brainstem compression. Some cases of immediately fatal head trauma in adult life are associated with traumatic separation of the pons and medulla (Lindenberg and Freytag, 1970; Simpson *et al*., 1989), but lesser tears of axons in the brainstem are found commonly in those dying shortly after a head injury, particularly those involved in road-traffic accidents, though there may also be other lesions that account for death.

In fatal AHLE the degree of cerebral swelling is usually sufficient to account for the fatal outcome, though this does not seem to be true of cerebral malaria. In the more chronic diseases, the best studied of which is multiple sclerosis, the extent and distribution of axon loss correlates well with the clinical disability. Thus, the small fibres in the optic nerves which subserve colour vision are selectively lost and defective colour vision is the most prominent visual symptom in multiple sclerosis (Evangelou *et al*., 2001). Likewise, axon loss in the corticospinal tracts of the spinal cord well explains the common spastic paraparesis seen in multiple sclerosis (Ganter *et al*., 1999; Lovas *et al*., 2000). More detailed study of fatal neurological diseases associated with axonal injury and loss will be needed to clarify other factors that may be involved and that may be amenable to therapeutic interventions. All in all, the recognition of the importance of axonal damage in these diseases opens up new avenues for exploration in attempts to improve the management and treatment of these all-too-common conditions.

Acknowledgements

I.M.M. is supported by the Lloyd's of London Tercentenary Foundation and the Wellcome Trust. I.M.M. wishes to thank Drs N. Day, J. Farrar, C. Newton, G. Turner and N. White for helpful discussions.

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Received July 24, 2002. Revised September 20, 2002.
Accepted September 21, 2002