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Traumatic brain injury in the neonate, child and adolescent human: An overview of pathology

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ABSTRACT

In the middle of the last century it had been thought that a good recovery of function and behavior would occur after traumatic brain injury (TBI) in very young human beings. A recent major change in thinking states that early childhood TBI may result in a severe compromise of normal brain growth and development such that TBI, rather, may compromise later normal development resulting in a need for very long term patient care and management. The mechanisms of injury and pathology within the injured brain are reviewed and compared between when injury occurs at or close to the time of birth, in an infant, in a young child, in a child between ages 5 and 10, in young and older adolescents and in young adulthood. Our understanding of pathophysiological responses by cells of the human central nervous system has recently greatly increased but has really only served to illustrate the great complexity of interactions between different types of cell within the growing and developing CNS. The hypothesis is developed that the outcome for a very young patient differs with the relative state of development of injured cells at the locus of injury. And that the potential for either repair, re-instatement of normal cellular and organ function or for continued normal development is much reduced after an early brain insult (EBI) compared with TBI in a slightly older child or young adult patient. The advent of increasingly sophisticated non-invasive imaging technology has allowed assessment of the influence and time course of brain pathology both early and late after TBI. This has generated greater confidence on the part of clinicians in forecasting outcomes for an injured patient. But our increased understanding has still not allowed development of therapeutic strategies that might ameliorate the effect of an injury. It is suggested that an improved integration of major clinical and scientific effort needs to be made to appreciate the import of multiple interactions between cells forming the neurovascular unit in order to improve any potential for post-traumatic recovery after TBI in neonates and young children.

1. Introduction and definition of childhood TBI

Traumatic brain injury (TBI) occurs when an external mechanical force results in tissue and cellular damage within the brain that may possibly lead to permanent or temporary impairment of cognitive, physical, psychosocial functions, and a diminished or altered state of consciousness. There is a clinical consensus that traumatic brain injury occurs in post-partum children and young adults in the commonest scenarios of a fall, an assault, or being in a motor-vehicle accident (MVA) either as a passenger or a pedestrian. However, maturation of central white matter extends from the third trimester until about 30 years of age (Ballesteros et al., 1993; Blakemore, 2009; Barnea-Goraly et al., 2005; Huang et al., 2006; Giza, 2006; Knickmeyer et al., 2008; Lebel et al., 2008; Tamnes et al., 2010). One tenet of this article is that the relative state of maturation of myelinated axons within central white matter influences the response by that tissue upon injury. It is suggested, further, that a complex interplay occurs between neurons, glial cells and blood vessels; otherwise termed the neurovascular unit, within the brain both at a locus of injury and peripheral to the locus. Some consideration will therefore be paid to responses by glia and their relative maturity particularly when injury occurs in a very young patient and there is damage to capillaries and smaller blood vessels leading to microbleeds or petechial hemorrhages which provide a scenario for secondary edema and swelling of the brain.

However, it is also becoming apparent that a fetal brain may be physiologically stressed shortly before and during the birth process. During parturition, the brain may be transiently exposed to considerable compressive forces. Although not presently considered as a form of TBI, transient compression may compromise the supply of oxygen to the brain and induce a birth related hypoxic-ischemic insult (HII) that may seriously compromise post-natal brain development (Anderson et al., 2009; Iwata et al., 2010). It has long been established that TBI in adults (Graham et al., 1978)

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and children (Graham et al., 1989) has a high incidence of hypoxic or ischemic lesions which may result in edema and an increase in the volume of injured tissue. Acute post-traumatic brain edema may occur both in response to the initial mechanical damage and as more widespread secondary damage as a result of hypoxia or hypovolemia. The influence of factors resulting from damage to blood vessels and evolution of exacerbation of the original locus of injury, for example the greater cerebral blood flow in young pediatric patients (Muizelaar et al., 1989), and more diffuse swelling of the brain in injured children (Graham et al., 1989), may be significant as earlier noted by Gorrie et al. (2001). This review will explore the relationships between cells of the "neurovascular unit" rather than neurons in isolation and illustrate differences in pathology in the injured brain in pre-partum, the neonate, the child, adolescence and young adult humans.

1.1. Outline of growth of the brain

Before considering injury in terms of loss of gray matter and white matter following injury in the developing brain at any time within the first two decades of the human lifespan, it would be instructive to appreciate an overview of normal development.

The human brain is relatively immature at birth and maturation extends over the first 20–30 years of life (Giza, 2006; Groeschel et al., 2010) and is summarized below in Tables 1 and 2.

Immunocytochemical markers of maturation of neural cells (neuronal nuclear antigen (NeuN), and microtubule-associated protein-2 (MAP-2)) have provided insight of the relative development of cells in different parts of the CNS (Sarnat et al., 1998). NeuN only labels the nuclei of neurons as they approach maturity (Sarnat et al., 1998) and neurons at different regions or locations of the brain differ in the age at which they approach maturity as summarized in Table 1. Major features of the post-natal maturation of the human central nervous system are an increase in numbers of neurons, a transient increase in depth or thickness of the developing cerebral cortex and the spatial extent of myelination of nerve fibers within developing central tracts. Appreciation of the complexity of growth of gray matter has developed rapidly over the last decade. Gilmore et al. (2007) reported that the total volume of gray matter increased rapidly in the first several weeks post-partum with total brain size increasing in the first year of life to circa 72% of adult size, to 83% by the end of the second year (Gilmore et al., 2007; Knickmeyer et al., 2008), to 90% of adult size by 6 years of age (Giedd, 2004) and achieves maximum volume at 13.30 years in females and 18.88 years in males (Giza, 2006; Groeschel et al., 2010). From then on brain volume begins to fall.

The total volume of gray matter in the cerebral hemispheres increases by 149% in the first year, by 14% in the second year (Knickmeyer et al., 2008) and peaks at different ages in males (12.30 yoa) and females (4.36 yoa) (Groeschel et al., 2010). Furthermore, the age at which the maximal volume of gray matter occurs differs between frontal, parietal, occipital and temporal lobes of the cerebrum as summarized in Table 2 (after Blakemore, 2009; Giedd et al., 1999). The volume of gray matter in the frontal and parietal lobes increases until about 12 years of age (yoa) in males and 11 yoa in females (Table 2) (Giedd et al., 1999). In the temporal lobe of males and females the peak volume of gray occurs at the same age at 16.6 yoa (Table 2) (Giedd et al., 1999; Blakemore, 2009). While in the occipital lobe the peak volume of gray matter occurs at 20.00 yoa in females but not until 25.30 yoa in males (Table 2). In the parietal, frontal and temporal lobes the volume of gray matter thereafter follows a slow decline until about 30 years of age when the total volume of the cerebral hemispheres is −15.4% of the peak value. Changes thereafter are beyond the remit of this article. Lastly, although peak cortical thickness occurs by 8–10 years of age in cortical association areas there is a gradual decline during adolescence to a stable thickness at around 25 years of age (Shaw et al., 2008; Tamnes et al., 2010). It has been suggested that gray matter matures faster in sensory and motor regions reflecting the rapid maturation of visual and motor functions relative to the executive functions of the prefrontal cortex in the early postnatal period (Shaw et al., 2008; Tamnes et al., 2010).

Sophisticated magnetic resonance imaging (MRI) has shown that the full integrity of myelinated tracts within central white matter as indicated by fractional anisotropy (FA) may extend over three

Table 1
A summary table of the reported gestational age (GA) of a human embryo or fetus when the named cell types or cell groups listed in the left column achieve maturity as indicated by their capacity to bind to Neu-N antibodies – center column. The first author and date of the relevant publication are listed in the right hand column.

Cell type or identity	Gestational age (GA) of first recognition via NeuN reactivity	Reference
Spinal motor neurons	8–12 weeks GA	Sarnat et al. (1998)
Dorsal root ganglion cells	Weak at 8–12 weeks GA Strongly labeled 20 weeks	Sarnat et al. (1998)
Deep stratum of the cortical plate – future layers 4–6	22 weeks GA	Sarnat et al. (1998)
Cerebral cortical plate		
Deep and superficial strata of the cortical plate – but not in future layers 2 and 3	24 weeks GA	Sarnat et al. (1998)
Layers 2 and 3 of cortical plate	Many neurons do not express NeuN even up to term	Sarnat et al. (1998)
Cerebellum		
External granule cells of cerebellum	20–22 weeks GA but a reduced labeling thereafter	Sarnat et al. (1998)
Purkinje cells	Negative throughout fetal period	Sarnat et al. (1998)
Internal granule cells of cerebellum	24 weeks GA	Sarnat et al. (1998)
Brainstem		
Basal pontine nuclei	20 weeks GA	Sarnat et al. (1998)
Hypoglossal nucleus	20 weeks GA	Sarnat et al. (1998)
Nucleus ambiguus	20 weeks GA	Sarnat et al. (1998)
Nucleus tractus solitarius	20 weeks GA	Sarnat et al. (1998)
Dorsal motor vagal nucleus	20 weeks GA	Sarnat et al. (1998)
Descending trigeminal nucleus	22 weeks GA	Sarnat et al. (1998)
Vestibular nuclei	20 weeks GA	Sarnat et al. (1998)
Arcuate nucleus	22 weeks GA	Sarnat et al. (1998)
Inferior olive	Negative throughout fetal period	Sarnat et al. (1998)
Thalamic nuclei	14–20 weeks GA	Sarnat et al. (1998)
Caudate nucleus	22 weeks GA	Sarnat et al. (1998)

Gilmore et al. (2007), Sarnat et al. (1998).

Table 2
A summary table of the reported gestational age (GA) of the fetus and the postpartum age of the neonate and child at which groups of myelinated nerve fibers may be first visualized in the living, developing fetus, infant or child by means of MRI. The relevant literature reference is listed by the first author and the year of publication below Table 2.

Lobe of the brain	Percentage increase in cortical gray matter volume from 4 yoa to peak (males)	Age at which peak cortical volume is achieved (males)	Percentage of peak volume present at 22 years of age	Percentage increase in cortical gray matter volume from 4 yoa to peak (females)	Age at which peak cortical volume is achieved (females)	Percentage of peak volume present at 22 years of age
Frontal	+5.8%	12.1 years of age	92.8%	+3.4%	11.0 years of age	95.6%
Temporal	+10.2%	16.5 years of age	98.4%	+4.8%	16.7 years of age	97.2%
Parietal	+5.7%	11.8 years of age	92.9%	+4.4%	10.2 years of age	91.2%
Occipital	Linear increase throughout	22 years of age		Linear increase to peak value	17 years of age	96.9%
White matter of whole brain		Age at which peak volume is achieved			Age at which peak volume is achieved	
		25.30 years			20.00 years	

Giedd et al. (1999), Gilmore et al. (2007), Blakemore (2009), Giza (2006), Knickmeyer et al. (2008), Shaw et al. (2008), Groeschel et al. (2010), Tamnes et al. (2010).

or more decades of early life (accessory Table 2). Central white matter increases in a dynamic, linear manner that begins *in utero* and continues in a coordinated, predetermined, manner until about 30 years of age (supplementary Table 2). The volume changes of white matter differ from those of gray matter in that total hemispheric white matter increases by 11% in the first year, by 19% in the second year (Knickmeyer et al., 2008; Hagmann et al., 2010) and by 61.6% between 5 and 30 years (supplementary Table 2). The maximum volume of white matter is not achieved until 20.00 years in females and 25.30 years in males (Groeschel et al., 2010). Changes in fractional anisotropy (FA) of different developing brain pathways may be visualized using magnetic resonance imaging (MRI) and a schema for development of projection, callosal and association fibers is provided in Fig. 1. Major changes in the chemical composition of parts of the fetal, neonate and infant brain occur during development and maturation. These changes are first a fall of water content within the brain; second a change in the ratio of water to macromolecules reflecting the generation of precursors of myelin and its synthesis and; third the process of myelination. These manifest as both different FA values and apparent diffusion coefficients (ADC) at different stages of maturation (Hagmann et al., 2010). At birth and throughout the first 4–6 months *post partum*, the intensities of gray and white matter are opposite to those seen in an adult where white matter has a lower signal intensity than gray matter in T1-weighted images and a greater signal intensity in gray matter in T2-weighted images (Fig. 2). With development and maturation there is a progressive increase in white matter signal intensity visualized using T1-weighted imaging (Fig. 2) and a logarithmic decrease in ADC which is a more sensitive estimator of maturation of white matter (Hagmann et al., 2010). A detailed analysis of development of the corpus callosum (CC) (Ding et al., 2008) illustrates this very well and provides good evidence that reorganization and maturation of the CC extends over the first 12 years of life. Recent work by Asato et al. (2010) has distinguished between white matter tracts that are functionally mature by the onset of adolescence and those that are not. Examples of the former are the following association tracts: the inferior fronto-occipital fasciculus (IFOF), inferior longitudinal fasciculus (ILF), the superior longitudinal fasciculus (SLF), and uncinate fasciculus (UF) (Fig. 1). Among projection tracts are the corticospinal tract, internal capsule and contiguous corona radiata, and the anterior thalamic radiations (ATR). The only commissural tract that is mature by adolescence is the body of the corpus callosum (CC) (Asato et al., 2010). However, and importantly for consideration of the consequences of TBI, the following tracts do not reach maturity until late adolescence: frontal and temporal parts of the UF, a frontal region of the SLF, frontal portions of the ATR, the genu of the IC, a frontal-parietal region of the corona radiata, and the splenium of the CC (Asato et al., 2010). Fig. 2 reproduces examples of T1 and T2 MRI images at a range of stages of development of cerebral gray and white matter and illustrate the differential maturation and myelination of different tracts within the developing brain. Further information may be obtained from supplementary Tables 1 and 2 published on-line

In outline neuron numbers within cortical gray matter have matured by the end of the neonatal phase. Maturation and development of different parts of central white matter, however, continues throughout childhood and adolescence. However, some evidence suggests that insult to the developing brain, especially pre-partum and at birth correlate with cognitive impairment and abnormalities in white and gray matter structure and function (Ball et al., 2011). Preterm birth has a high correlation with a reduced volume of the thalamus, hippocampus, orbitofrontal lobe, posterior cingulate cortex and centrum semiovale (Ball et al., 2011). One conclusion might be that insult and/or injury before partum and around partum compromises subsequent normal development of the post-natal brain, possibly to a greater degree than insult at slightly older ages.

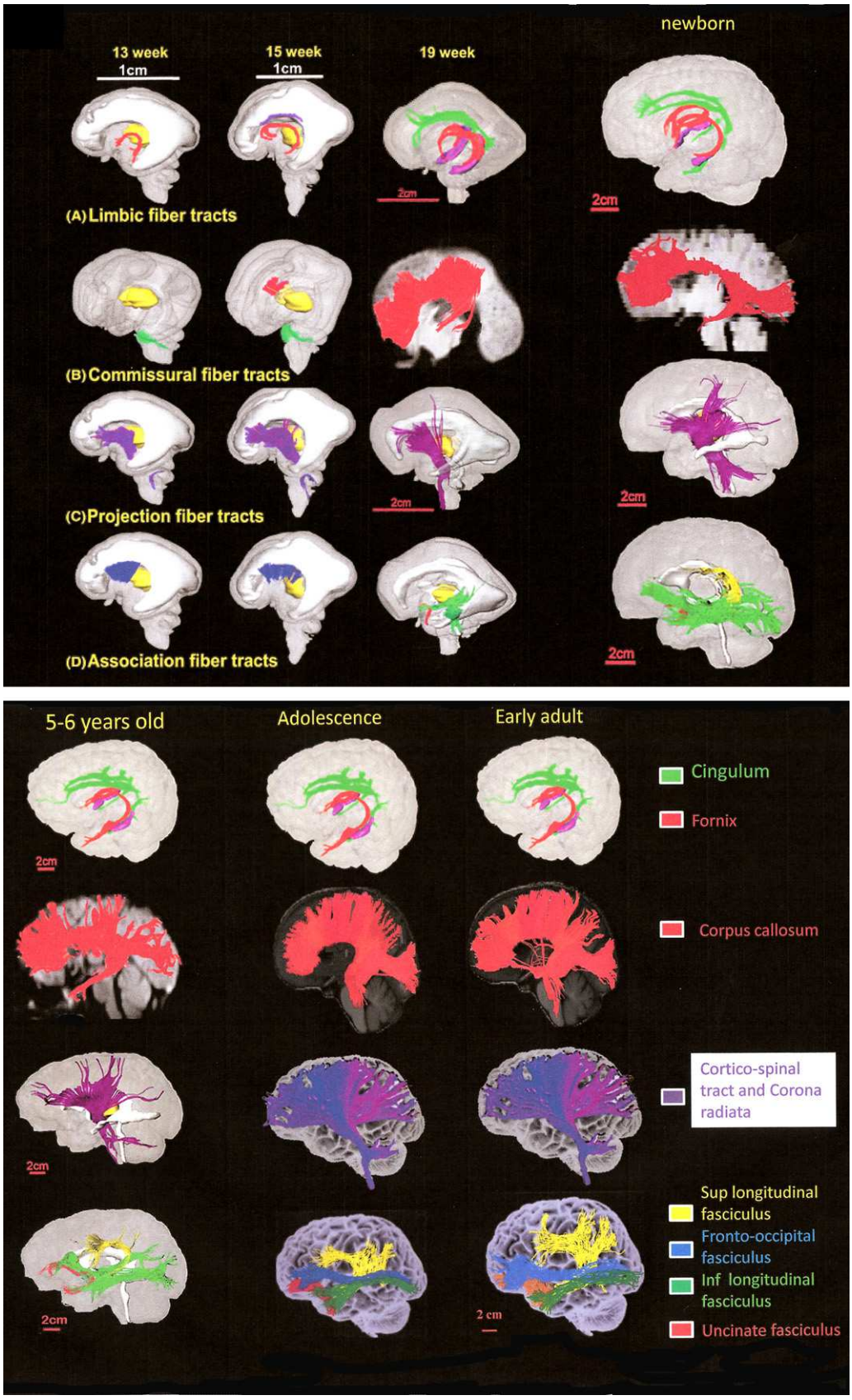

Fig. 1. A schematic to represent the differential development of (A) Limbic fiber tracts (top row), (B) Commissural Fiber Tracts (second row), (C) Projection Fiber Tracts (third row) and (D) Association Fiber Tracts between 13 weeks gestational age and Early Adulthood in successive vertical columns from left to right. = cingulum, = fornix, = corpus callosum, = corona radiata including corticospinal tract, = superior longitudinal fasciculus, = inferior longitudinal fasciculus, = fronto-occipital fasciculus, = uncinate fasciculus.

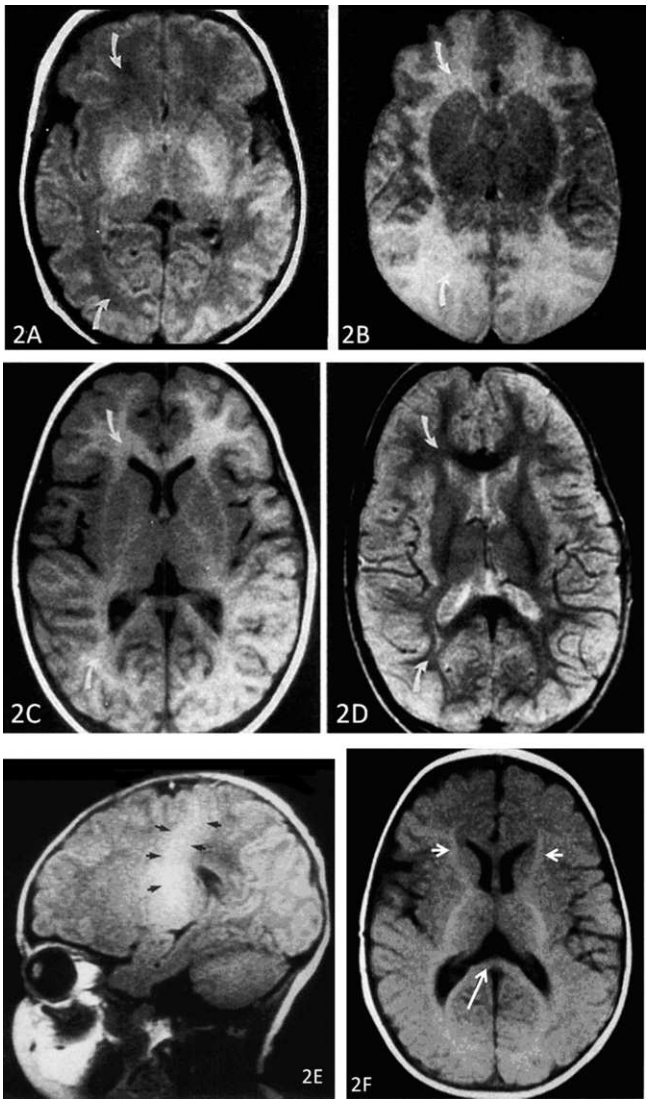

Fig. 2. Axial T1-weighted (A and C) and T2-weighted (B and D) images obtained at the level of the basal ganglia. The white matter (curved arrows) of a neonate (A and B) has a low signal intensity compared to gray matter in a T1 weighted image (A) but a higher signal intensity in a T2-weighted image (B). The reverse appearance occurs in a 14-month-old infant (C and D), showing progress toward an 'early adult' stage. In T2-weighted images, high-signal intensity unmyelinated white matter progressively changes to a signal intensity lower in myelinated white matter than that in gray matter (after Ballesteros et al., 1993). (E) Parasagittal T1-weighted image of a 1-month-old infant showing hyperintensity in the central white matter adjacent to the Rolandic fissure as myelination extends upward from the posterior limb of the internal capsule (arrows) (after Ballesteros et al., 1993). (F) Axial T1 weighted image of a 5-month-old infant. The splenium of the corpus callosum is myelinated (long arrow). Myelinated nerve fibers extend more rostrally in the anterior limb of the internal capsule (short arrows).

However, quantitative analyses in terms of changes in the number of neurons is lacking from the current literature. In parallel, there is increasing evidence in support of the idea that efficiency of long distance connections between functional hubs within brain networks, referred to as "structural connectivity" and reflected by shorter reaction times and improved integration of cognitive control, continues throughout adolescence (Asato et al., 2010; Hagmann et al., 2010). It is suggested that nerve fibers forming these long association pathways may be more susceptible to TBI during adolescence as a result of their relative immaturity. These ideas will be discussed in the following parts of this article.

2. Pathology that may be viewed by CT and/or MRI

There has been rapid development of imaging technologies over the last decade. These imaging techniques may be used either upon first arrival of a patient in the Emergency Room, for example Computerized Tomography (CT) where an image is achieved by means of examination using X-rays, or at hours or days after admission using Magnetic Resonance Imaging (MRI). However, the routine resolution achieved by MRI at typical 1.5 T strength is of the order of a cubic millimeter. Individual nerve fibers, but a few micrometers in diameter, therefore cannot be resolved. Resolution may be improved through increased field strength and many research facilities use 3.0 T, 4.0 T or 8.0 T machines. At increased tesla levels concerns about the weight of the machine, consumption of cryogen, fringe fields and magnetohydronamic effects may be important. Pathophysiology following TBI reflects interactions or responses between many different types of cell to form a lesion large enough to be visualized by MRI. As a result, description of types of pathology frequently occurring in neonates, children and adolescents which may be visualized directly using CT and MRI will be summarized. Then consideration of mechanisms for development of pathology at the cellular level will be reviewed.

2.1. Scenarios and gross pathology of injury

In very young children the cranium is relatively elastic and brain injury is rare unless a hematoma develops to form a space occupying lesion. Indeed in a normal delivery it may be relatively common, in about a quarter of normal, unassisted deliveries, infratentorial hemorrhage may occur if the child is delivered at a relatively young gestational age (Looney et al., 2007). Traumatic brain injury (TBI) refers to a traumatically induced physiologic disruption of brain function. That physiologic disruption may occur as a sequal to the development of intracranial bleeding that may compress the brain, cerebral contusions following impact of the brain with the internal surface of the skull in rapid deceleration of the head, or shearing injuries within the (developing) cerebral white matter as a result of rapid rotational acceleration and/or deceleration. Movement of the head often occurs in a linear plane during a fall over a short (less than 4 ft or 1.2 m) distance, say off a chair or bed, and the head hits an object such as a table or chair seat edge or corner during the fall. In falls over a greater vertical distance, usually referred to as long falls (Duhaime et al., 1987, 1992; Case, 2008a,b) focal impact forces result in fracture of the skull, focal contusions and subarachnoid hemorrhage. When blood vessels are damaged, blood accumulating from these may collect between the skull and the brain to form an extradural or intradural hematoma or a contusion on the surface of the brain and it is notable that Duhaime et al. (1992) report a high incidence (more than 70%) in very young children involved in motor vehicle accidents (MVAs). Intracranial hemorrhage, usually subdural hemorrhage, is common in young children in Abusive Head Trauma (AHT) (Christian and Block, 2009) or non-accidental head injury (NAHI) occurring in 54% of patients reported in (Duhaime et al., 1992); 90–95% of cases reported by Case (2008a); 85% of cases reported by Hafiz and Saffari (2011) (Fig. 3B).

A greater severity of injury is obtained if there is an angular or rotational component of the deceleration of the head (Duhaime et al., 1992). Angular deceleration applies much greater deformation to the brain, as might occur, for example, in falling down stairs or being a passenger in a MVA. During angular deceleration shear strain in central white matter results in axonal shear injury and damage to capillaries. The recent appreciation that axonal disruption after TBI is not a single event but represents an initial loss of tissue over several weeks followed by a long term low level loss of gray and white matter (Maxwell et al., 2010; Sulaiman et al., 2011)

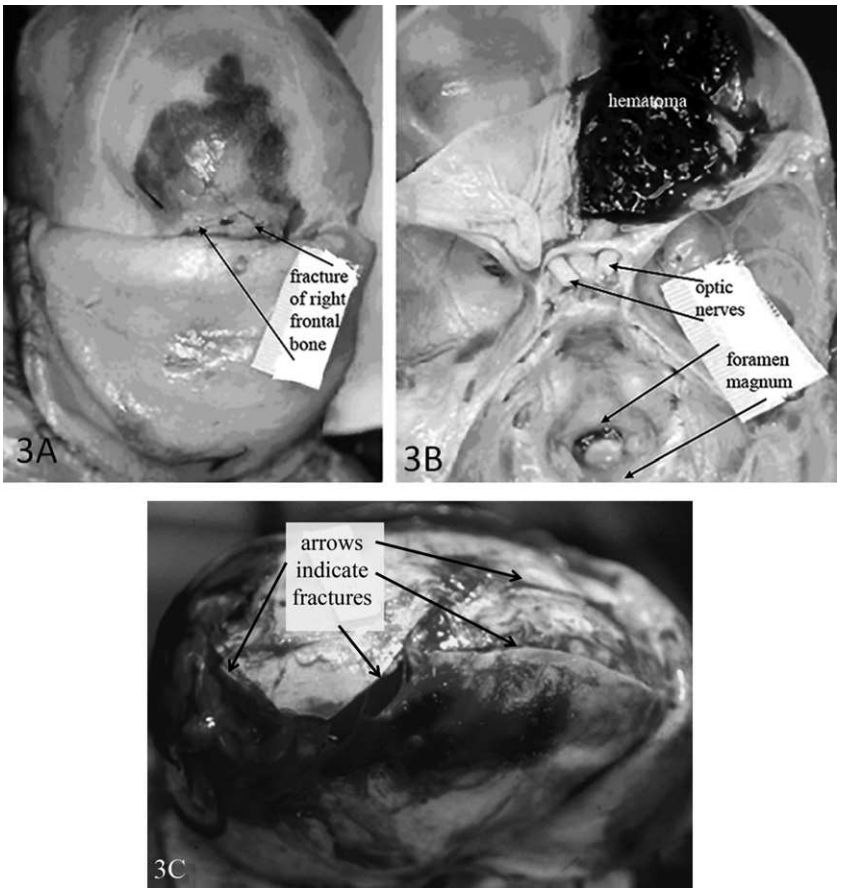

Fig. 3. A and B, two photographs from a four month old patient who fell from a bed toward the floor hitting his head on the corner of a table during the fall. In 3A fractures of the right frontal bone are indicated. In 3B is seen an epidural hemorrhage overlying the floor of the right anterior cranial fossa. Part 3C illustrates widespread fractures of the skull generated during a crush injury to the head of a 21-month toddler in whom the head was crushed by a wheel of a motor vehicle.
All images courtesy of Case M.E.

indicates the complexity of pathology in patients who survive TBI for years or decades.

In infants, crush injury to the head is particularly pertinent to accidental pediatric neurotrauma. Crushing head injuries are relatively common in the pediatric age group forming 8.7% of all deaths from accidents (Case, 2008a). Injuries to the brain consist of contusions and lacerations caused by fractured bone striking and penetrating the substance of the brain (Fig. 3C). Consciousness may not be lost initially as the head is being crushed, but unconsciousness may occur following the development of brain swelling and complications as a result of raised intracranial pressure.

After the cranial sutures are joined, the sutures offer much greater protection to impact. Mechanical force may then be readily transferred through the skull to affect the brain and adnexae, with correlated injury to related blood vessels, in particular to the middle meningeal artery which becomes more closely related to the cranial bones in the region of the pterion during childhood. The risk for epidural hemorrhage (EDH) therefore increases with age particularly (Fig. 4B) if there is impact to the side of the head. In a report by Gerlach et al. (2009) in 38 patients within Germany, 90% of EDH cases were related to fractures of the skull. With surgical evacuation soon after admission, the outcome was excellent in 90% and good in 10% with survival by all patients. The most frequent cause of accidental TBI in children is falls (52%) usually from more than their own height, with 38% as a result of involvement in a MVA, of which in 36% of the total the patient was a pedestrian (Gerlach et al., 2009).

Case (2008a) reported subdural hemorrhage in only 15% of accidental TBI cases. However, Duhaime et al. (1992) report a high incidence (42%) in MVAs. In contrast, subdural hemorrhage has been reported in 90–95% of fatal inflicted TBI (AHT) in the USA (Case, 2007, 2008b), 85% of AHT cases in Malaysia (Hafiz and Saffari, 2011), and there is an annual incidence of 24.1/100,000 in the UK for children less than one year old, and 12.54/100,000 for children less than 2 years of age (Hobbs et al., 2005).

Subarachnoid and subdural hemorrhages can both result from disruptions of bridging veins, as these veins pass from the cortical surface through the pia-arachnoid membrane of the meninges to the superior sagittal sinus. Subarachnoid hemorrhage occurs in about the same number of cases as subdural hemorrhage in childhood inflicted TBI (AHT) (Case, 2008b). MRI scans frequently provided evidence of repeated injury or abuse as seen by differences in the density of hematomas that reflect the differential longevities of their resolution. There is likely also to be evidence of fractures or partially healed fractures to parts of the rest of the skeleton.

When learning to walk and during the first 4 or 5 years of life children will inevitably fall but not usually through a distance greater than their own height; with exceptions, like falling down stairs, from a swing or roundabout, out of a tree or out of a window. For clinical distinction falls may be classified as either short (through a distance of less than 6 ft or 1.8 m) or long falls from greater heights (Case, 2008a,b). Short falls are almost consistently associated with contact injuries to the body and or scalp when 95% of patients do not experience loss of consciousness or brain injury. Nonetheless, between 1 and 3% of patients may suffer a linear

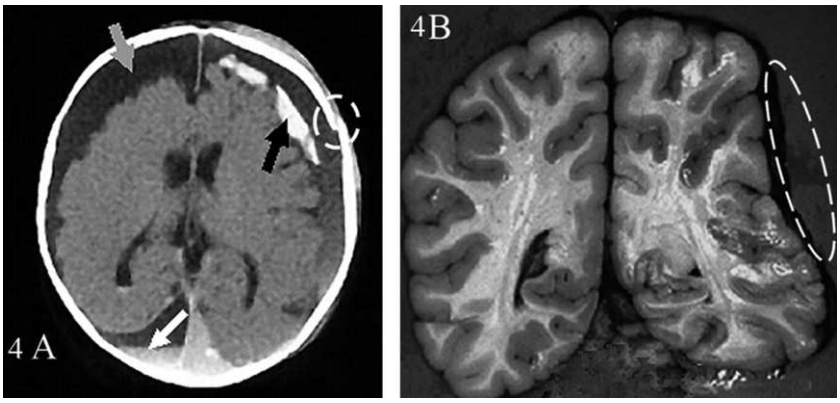

Fig. 4. (A) An axial CT scan of the brain of a child of 2 years of age with repeated abuse and trauma to the head. A hyperdense chronic hemorrhage is visible on the child's left – see black arrow to the right of image. On the Child's right a chronic subdural hemorrhage or hygroma compresses the right cerebral hemisphere (grey arrow) and an acute hemorrhage is present in the occipital region (white arrow). The circular white dotted line indicates a skull fracture. Note the abnormal size and shape of the ventricles. (Courtesy and modified after Vezina, 2009). (http://emedicine.medscape.com). (B) A coronal temporoparietal lobar brain slice from a 2 years old child. The child's head was struck by a heavy object that resulted in fracture of the right parietal bone. Bleeding from damaged branches of the right middle meningeal artery resulted in an 80 ml hematoma, indicated by the dotted oval, that severely compressed the cerebral hemispheres with almost complete occlusion of the right lateral ventricle. Note the flattened profile of the gyri and the reduced width of the sulci in the right, compressed cerebral hemisphere.
(Courtesy and modified after Case (2008a)).

fracture to the skull in short falls (Case, 2008a,b), and of those patients less than 1% may suffer either an epidural or subdural hematoma.

Alternatively, when the fall is from a greater height the biomechanics and risk of TBI and or acceleration/deceleration injury are much increased where the severity of injury is related to the height from which the fall began. Children may have multiple complex fractures of the calvaria and base of the skull and associated subdural and subarachnoid hemorrhages (Reiber, 1993). Injury to white matter of the brain may occur if angular or rotational movement of the head occurs during impact.

As children increase in age falls may occur from bicycles, skateboards, horses, out of trees or out of windows. Risk of injury to central white matter is increased if an angular or rotational movement of the head occurs during an injury and one of the most frequent scenarios for TBI in children and adolescents within Europe is involvement as a pedestrian in a motor vehicle accident (Parslow et al., 2005; Törő et al., 2011). Once children achieve adolescence, injuries as a result of contact sports may occur with concussion or longer periods of loss of consciousness. Traumatic axonal injury occurs in concussion through to moderate or severe TBI and the severity of the injury is thought to relate to the numbers of nerve fibers injured (Bigler and Maxwell, 2011). There is also associated injury to the microvasculature of the brain especially at the junction between cortical gray matter and the underlying white matter of the cerebral hemispheres (Adams et al., 1989). Despite the fact that such petechial hemorrhages are of microscopic proportions the differential magnetic properties of blood cells and neurons due to the presence of iron within erythrocytes allows detection of these micro-hemorrhages using Susceptibility Weighted Imaging (SWI) (Tong et al., 2008; Robinson and Bhuta, 2011) and increasingly the presence of petechial microhemorrhages, frequently termed microbleeds (Fig. 5), are interpreted as markers for axonal shear injuries (Kinnunen et al., 2011). Lastly, in late adolescence and as young people obtain a driving license, injuries as a result of motor vehicle accidents become more prevalent. In these cases the pathology of the injured brain is comparable to that in the adult.

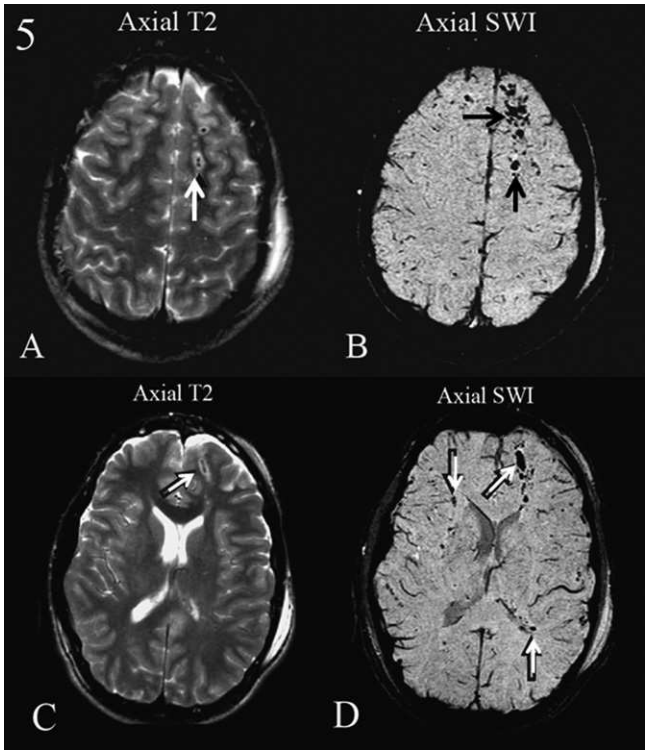

Fig. 5. MRI and Susceptibility-image-weighting (SWI) of diffuse axonal injury. The patient illustrated was a 17 year old youth who experienced severe TBI, initial GCS score = 5, when crashing a motorcycle. Images A and B obtained at the level of the centrum semiovale, and C and D of the lateral ventricles. The SWI images show numerous petechial or shearing hemorrhages that are difficult to perceive in the axial T2 images but well resolved in the axial SWI images. Some examples of focal hemorrhages are indicated by the arrows.
(Courtesy of Tong et al., 2008, American Society of Neuroradiology).

3. Cellular pathology and pathophysiology

3.1. Cell types in the CNS

The cell types occurring within the developing CNS are neuronal and glial cell precursors and their progeny, pyramidal and non-pyramidal neurons, astrocytes, oligodendrocytes, the endothelium of developing blood vessels and derivatives of the monocyte-macrophage cell line that form a range of cells collectively termed the microglia. This review suggests that three factors may

influence outcome following injury and/or trauma to the developing and young CNS: (1) the maturity or stage of development of populations of neurons and glia at foci of injury, (2) the stage of development of the white matter of the brain that probably reflects the level of integrity and myelination of association, callosal and projection pathways at the time of injury and (3) the effect of that insult upon succeeding development and maturation of the integrative networks of the brain.

The succeeding sections of this review summarize current knowledge about cellular responses within a brain during the so-termed "acute phase" of TBI which extends between the moment of injury and 24 h thereafter. Obtaining human material within minutes or a few hours after injury is almost impossible because the patient may not appear in the emergency room until at least 1 or 2 h after suffering an insult to the CNS. Of necessity, the acute or early morpho-pathophysiology has been obtained using a number of animal models the great majority of which have been developed using laboratory rodents. This is especially true for material that has been examined at the ultrastructural level using transmission electron microscopy. Recent development of sophisticated MRI has, however, allowed direct visualization of pathology in a living patient and has the potential to provide valuable information about the time-course and spatial extent of pathologic responses over months or years following TBI. This has been a major advance in assessing human patients following TBI although it is still true that resolution at the microscopic scale is difficult to achieve.

3.2. Membrane damage – myelinated or unmyelinated axons

Implicit in the concept of TBI is that mechanical injury occurs to axons coursing through central white matter of the cerebral hemispheres, cerebellum and brain stem under conditions of rapid rotational acceleration or deceleration of the head, for example as occurs in a long fall or motor vehicle accident (MVA). For neonates, however, space occupying lesions like intracranial hemorrhages may be common (Hobbs et al., 2005; Case, 2007, 2008b; Hafiz and Saffari, 2011). In these situations either the axon may be sheared or the brain may be compressed inside the cranial cavity. An important corollary of the latter is that hypoxia and/or ischemia may play a major role in the ensuing pathology. A range of membrane pumps normally function to maintain the differential concentration of ions across cell membranes to both maintain the transmembrane electrical gradient essential for the normal activity of neurons and the differential distribution of ions between the cytoplasm of cells and the brain extracellular or interstitial fluid (Graphical Abstract). It is noteworthy that the injured brain undergoes swelling (Graham et al., 1989; Gorrie et al., 2001) as a result of increasingly widespread loss of ionic homeostasis and dysfunction of cell membrane activity (Graphical Abstract) that results in either or both cytotoxic and vasogenic edema. Spreading depression may occur and is mediated via propagation of astrocyte Ca^{2+} waves over distances of several hundred micrometers from an initial injury locus (Hamilton et al., 2008; Verderio and Matteoli, 2001). A DTI study of acute mild TBI in adolescents having a GCS of 15 and no abnormality indicated with CT but in which patients showed cognitive, affective and somatic post concussion symptoms with change in emotional responses (Wilde et al., 2008), reported an increased fractional anisotropy (FA) and reduced diffusion coefficient (DC) within the corpus callosum between 24 h and 6 days of injury. Wilde et al. (2008) suggest that the increased FA and reduced DC indicate cytotoxic edema and inflammation due to loss of ionic homeostasis and abnormal plasma membrane function. The recently reported maintenance of abnormal intracellular Ca^{2+} in vitro (Staal et al., 2010) over at least 24 h after experimental stretch injury and the suggestion that glia also respond to axonal injury (Fitzgerald et al., 2010) by allowing propagation of Ca^{2+} signals provides experimental support for this hypothesis. This allows generation of a hypothesis that complex interaction between neurons and astrocytes may also serve to exacerbate the number of injured nerve fibers through metabolic compromise or oxidative stress.

A direct correlation between membrane strain and changes in membrane permeability in experimental animals or patients has still not been reported (Laplaca and Prado, 2010). With specific regard to trauma a small number of publications have provided cytochemical/immunolabeling evidence in support of the idea that damage to the cell membrane is associated with influx of free calcium into the cytoplasm or axoplasm with a subsequent activation of neutral calcium activated proteases or calpains (Maxwell et al., 1995; Saatman et al., 2003; Jette et al., 2006). The initial mitochondrial response is to try to ameliorate such influx but it is soon swamped by the uncontrolled influx of calcium and release of calcium from axoplasmic reticulum (Stys, 2005; Maxwell et al., 1995; Saatman et al., 2003). This leads to opening of the mitochondrial transition pore, mitochondrial depolarization and release of cytochrome c into the axoplasm which allows activation of neutral caspases in both traumatic (Büki et al., 1999, 2000; Barrientos et al., 2011) and ischemic injury (Jette et al., 2006). Post-injury influx of free calcium and sodium has been reported in vitro (Wolf et al., 2001) when intra-axonal accumulation of calcium extends over tens of minutes, and Jette et al. (2006) reported a similar time frame during anoxia with an approximate 2.5-fold increase from baseline over 30 min. But, information about immature or unmyelinated nerve fibers such as are present during the fetal and neonatal development of the human CNS and their responses to mechanical injury is largely lacking from the current pathological literature. Experimentally, Reeves et al. (2005) reported responses after injury in unmyelinated nerve fibers of the corpus callosum in rat and suggested a more rapid response to injury associated with a rapid breakdown of the axolemma and cytoskeleton than occurs in myelinated fibers after TBI. In terms of neuronal function and activity, Reeves et al. (2005) reported complete loss of the slower compound action potential (CAP) in unmyelinated nerve fibers at 3 h after injury and the CAP remained significantly lower than in controls out to 7 days following TBI. This is in contrast to the near total recovery of CAP in myelinated fibers at 7 days (Reeves et al., 2005; Tomei et al., 1990).

Unmyelinated, injured nerve fibers form axonal swellings consisting of accumulations of tubulo-vesicular intra-axoplasmic membranous profiles and damaged, swollen mitochondria within an hour of injury followed, at 3 h, by disruption of the axolemma and cytoskeleton (Reeves et al., 2005). At one day after injury unmyelinated axons may be fragmented with remnants consisting of membrane and cytoskeletal debris scattered among and between other intact or swollen, disorganized and shrunken fibers containing remnants of axoplasm of an increased electron density. Reeves et al. (2005) conclude that their electrophysiological and morphological data show that subgroups of unmyelinated, small myelinated or larger myelinated nerve fibers have a differential vulnerability both to injury and the potential for repair and recovery. This extended earlier work upon myelinated axons (Jafari et al., 1997, 1998). There is a growing consensus that degeneration or loss of unmyelinated nerve fibers occurs more rapidly than myelinated nerve fibers. A recent publication has confirmed and extended these findings in an elegant *in vitro* study (Staal and Vickers, 2011) in which axon bundles outgrowing from cultures of embryonic rat neocortex obtained from 18 days GA fetuses and co-cultured either with oligodendrocyte precursor (OPC) or without OPC cells provided in the former case myelinated nerve bundles and, in the latter, unmyelinated nerve bundles. Both types of nerve fiber were exposed to an acute 3–6% strain to provide moderate axonal injury. Injury to bundles of unmyelinated axons resulted in

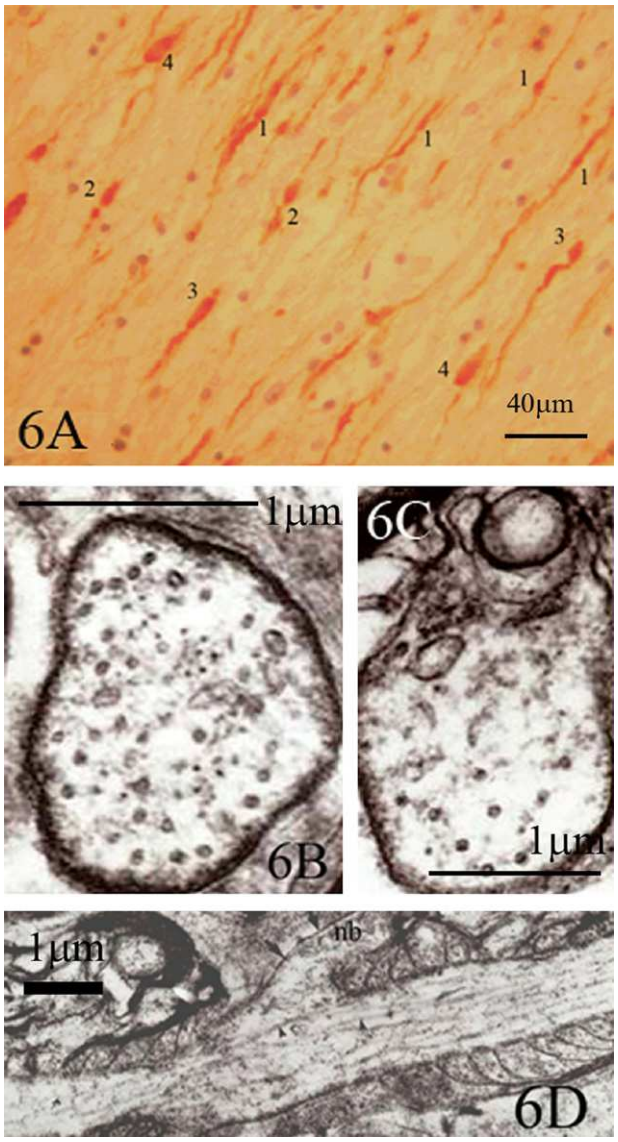

Fig. 6. (A) A medium power field of deep white matter labeled for β-APP which highlights changes in axonal profiles in a patient at 24 h survival after TBI. The number adjacent to axonal profiles indicate different stages of the axonal reaction. 1 = the axons are elongate in profile but there is variation in axonal caliber and suggestion of formation of axonal swellings. 2 = Axons showing larger caliber swellings within which is a central, focal constriction. These axons are undergoing axonal disconnection or secondary axotomy to give rise to separate fragments. 3 and 4 represent terminal bulbs of either proximal or distal axonal fragments at the end of which are axonal bulbs. Wang et al. (2011) have recently described the ultrastructure of proximal and distal degeneration bulbs. Using their criteria it is suggested that axons labeled 3 are proximal bulbs and those labeled 4 are distal degeneration bulbs. (B) A transverse thin section electron micrograph of a node of Ranvier from a normal animal. The peripheral axolemma appears dark because of the presence of the dense undercoating characteristic of the nodal axolemma. The dense undercoating is formed by a protein complex containing spectrin and is thought to stabilize the large number of Na^+ channels found in the nodal axolemma. Within the axoplasm are a number of small circular profiles and a number of slightly smaller dark dots. The former are transverse sections of longitudinally orientated microtubules, the latter of neurofilaments which together form major parts of the axonal cytoskeleton. A few oval or irregular profiles represent sections through parts of the axoplasmic reticulum. (C) In this transverse section from an injured node of Ranvier after stretch injury, the axolemma and dense undercoating are visible in the lower part of this image. The axolemma extends upwards forming an irregular profile, lacks the dense undercoating and is related to a group of membranous profiles. The upper region of this axon forms a "nodal bleb". In the lower region a few microtubules are present together with an even lower number of neurofilaments which are more widely separated from their neighbors than in the normal node, 6B. Loss of at least part of the normal complement of the axonal cytoskeleton will compromise axonal transport along this injured fiber. (D) A longitudinal thin section of an injured node of Ranvier at 15 min after stretch injury. A nodal bleb (nb) extends away from the nodal cylinder towards the upper right and contains several membrane limited profiles. The axolemma limiting the bleb lacks the dense undercoating of the remaining nodal membrane with which it is contiguous. Intact paranodal oligodendrocyte loops occur on either side of the node. There is suggestion of a reduced content of cytoskeletal elements beneath the stalk of the nodal bleb.

misalignment of intrinsic neurofilaments by 24 h, the formation of axonal swellings by 48 h and secondary axotomy which occurred maximally by 72 h after injury. When myelinated axon bundles were injured, however, only minimal cytoskeletal damage occurred at 48 h and significantly fewer axons progressed to secondary axotomy (Staal and Vickers, 2011). This novel information may allow the suggestion that myelination of axons provides not only for the occurrence of salutatory conduction but also reduces the vulnerability of myelinated axons to acute, mechanical strain. However, as pointed out by Staal and Vickers (2011), myelination also influences the development and content of neurofilaments within a myelinated axon and it is not beyond possibility that the higher content of neurofilaments, together with the increased spacing between those neurofilaments resulting from greater phosphorylation of neurofilament sidearms (Kumar et al., 2002), may also increase mechanical resistance in these axons. Nonetheless this adds support to the hypothesis that pre-myelinated axons in the early developing human brain may be more susceptible to mechanical injury than myelinated axons within developing central tracts. In conclusion, the current lack of information about responses by human fetal immature or developing nerve fibers does not allow generation of definitive conclusions although it is acknowledged that outcome for neonates after TBI is usually poorer, occurs more rapidly with a slowing of the rate of growth of the brain and head; that a reduced motor, visual and cognitive development results; and a reduced capacity in lexical and discourse components of linguistic development, working memory and inhibitory control results (Bonnier et al., 2007; Ewing-Cobbs et al., 2004, 2008; Max et al., 2004).

3.3. Neuronal responses

Following death after TBI, damaged or reactive axons may be visualized if the patient survives for more than several hours before death. This time period is illustrative of the fact that for the most widely used immunocytochemical marker of axonal injury, antibodies against beta-amyloid precursor protein (β-APP), the antigen is not present in intact or normal axons at concentrations great enough to allow immunocytochemical labeling. Following trauma, continued axonal transport on either side of a locus of axonal injury allows sufficient increase in the intra-axonal concentration of β-APP to the level at which immunocytochemical labeling allows resolution of foci of increased axonal caliber that are termed "axonal swellings" (Maxwell et al., 1997) (Fig. 6A). The β-APP accumulates therein due to the presence of loci where there is loss of axonal transport. Beta-amyloid precursor protein is transported within membrane limited axoplasmic vesicles via fast-axonal-transport (FAT) in association with the microtubule component of the axonal cytoskeleton. Ultrastructural experimental studies within minutes of injury have reported axonal foci in which microtubules are absent (Fig. 6B–D) and these frequently occur at nodes of Ranvier at which nodal blebs develop (Maxwell et al., 1991) (Fig. 6D). Ultrastructure also suggests loss of the characteristic nodal dense undercoating (Fig. 6C) that maintains localization of sodium channels and other transmembrane proteins. Saatman et al. (2003) reported transient, focal aggregates of spectrin breakdown product within 15 min of stretch-injury. Later, von Reyn et al. (2009) reported calpain mediated proteolysis of voltage-gated sodium channel α-subunits after in vitro stretch-injury to cortical neurons where proteolysis of sodium channels occurred prior

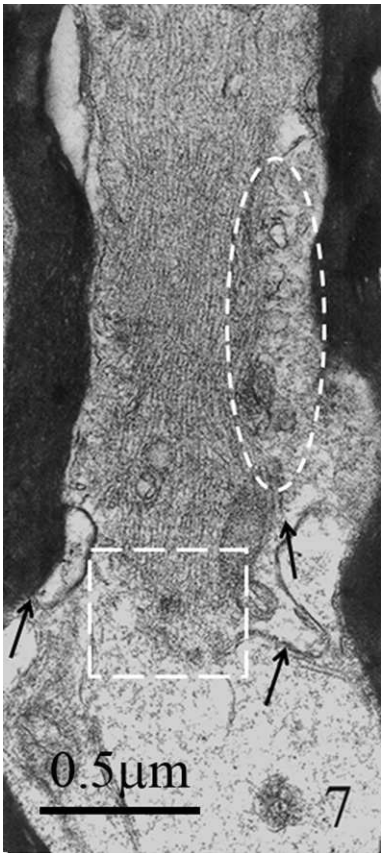

Fig. 7. This is a field in an injured axon from a stretch-injured optic nerve to illustrate "secondary axotomy" taken from a 4 h survival experimental specimen. Compacted neurofilaments are seen at the top of the image and the axon is still surrounded by the myelin sheath. To the right within the dotted oval occur numerous vesicular membrane profiles suggestive of breakdown of the axolemma. The black arrows indicate several profiles of fragmented axolemma. Within the white outlined box neurofilaments terminate at a lucent region which contains a flocculent precipitate, suggestive of terminal neurofilament proteolysis.

to loss of the axolemma. Ultrastrastructural immunocytochemical evidence of calpain mediated breakdown products has also been reported at an initial subaxolemmal locus which then, secondarily over several hours, extends throughout the axon cylinder (Büki et al., 1999). von Reyn et al. (2009) suggest that proteolysis of sodium channels may contribute to an increased content of cytosolic calcium with subsequent calpain activation. Saatman et al. (2003) also reported a second phase of spectrin breakdown out to 24 h after injury that, it is here suggested, may reflect secondary disruption of axonal neurofilaments. However, focal resolution of spectrin breakdown product has not been reported in optic nerve fibers following anoxia (Jette et al., 2006) when a diffuse distribution of labeling was reported such that initial localization to presumed nodes of Ranvier was not confirmed and provides for some controversy in this area which is still unresolved.

Damaged axons may also be identified experimentally by the compaction of neurofilaments (NF) with a reduced spacing between adjacent NF (Pettus and Povlishock, 1996) and is associated with spectrin breakdown (Susuki and Rasband, 2008). The pathophysiological result is a loss of axonal transport (Jafari et al., 1997, 1998; Stone et al., 2004; Marmarou et al., 2005). Compacted NF also occur immediately adjacent to the point of axonal disconnection or secondary axotomy (Maxwell et al., 2003a) (Fig. 7). Furthermore, Liu et al. (2006) have reported damage to the related myelin sheath using myelin basic protein (MBP) C-terminal fragment-specific immunocytochemistry where separation of myelin lamellae occurs followed by formation of myelin figures. In terms of the mechanism(s) of breakdown all of the neurofilament proteins, APP, αII-spectrin, MBP, tau, tubulin, ankyrin among others have been reported as substrates of calpains (reviewed by Liu et al., 2006; Jette et al., 2006).

In summary, stretch-injury to the axolemma damages sodium channels and results in an uncontrolled influx of sodium and calcium ions. The increased axoplasmic content of calcium ions diffuses rapidly along a considerable length of any injured axon, at least under anoxia (Jette et al., 2006), and allows activation of neutral proteases that breakdown the subaxolemma cytoskeleton to exacerbate damage to the axolemma (Graphical Abstract). Calcium influx also injures closely related mitochondria which cease synthesis of ATP resulting in failure of membrane related transporters and pumps to further exacerbate calcium influx (Graphical Abstract). The focal activation of neutral proteases then acts upon neurofilament proteins to alter their spacing, lead to their proteolysis and loss of the remnants of the cytoskeleton at which point secondary axotomy has occurred (Graphical Abstract). Activated calpains have a wide spectrum of substrates (Saatman et al., 2010) of which mitochondria are one. Calpain damage to mitochondria results in release of cytochrome C into the axoplasm and apoptosis inducing factor that, hypothetically, may feedback to the cell soma to further exacerbate the effects of injury through induction of loss of neurons.

It is essential to acknowledge that a time frame of at least several hours is required before focal disruption of axonal transport allows adequate accumulation of beta-amyloid to be resolved by light microscopy (Geddes et al., 1997, 2000; Maxwell et al., 1997). It is important to appreciate that labeling for β-APP provides no information about the presence of immediate axonal shearing as occurs in "primary axotomy", but does provide insight into the occurrence of "secondary axotomy". And that a post-traumatic survival period extending to at least several hours is required to determine that any axon has been injured. Careful examination of a field of β-APP labeled axons will also illustrate stages in the process reflecting axonal swelling, focal constriction within axonal swelling, axonal fragmentation or axotomy and formation of proximal and distal axonal bulbs (Fig. 6A). Importantly, however, β-APP labeling is not diagnostic of or specific to traumatic axonal injury since such labeling has also been reported, for example, after ischemia (Waxman et al., 1992), in multiple sclerosis (Mahad et al., 2009); in herpes simplex encephalitis (Mori et al., 2005), in HIV infection (Raja et al., 1997) and a variety of types of brain injury in adults (Stys, 2005; Coleman, 2005) and pediatric subjects (Johnson et al., 2011). Axonal injury after TBI is frequently associated with vascular damage manifested as petechial hemorrhages in both white matter and the cortical ribbon (Gorrie et al., 2001). Gorrie et al. (2001) obtained 32 cases of children that died at or after a MVA. Within the 32 patients, subarachnoid hemorrhage occurred in 68.7%, subdural hemorrhage in 31.3%, and brain swelling in 21.8%. Importantly, however, in younger children a greater proportion (64.3%) of children died at the scene of an accident and their outcomes were more severe in that more than 80% of patients died within one hour of injury. For older children, 27.7% died at the scene of the accident (Gorrie et al., 2001). Gorrie et al. (2001) provide support for the hypothesis that young children are more sensitive to trauma to the brain and that death occurs much more frequently after TBI than in older children and adolescents, a concept that differs from the earlier widely held idea that younger children may recover better. The latter concept arose in the 1970s from earlier work done by Margaret Kennard. This sometimes, erroneously, is termed the "Kennard's Principle" but that does not really reflect the work done by Margaret Kennard and was not an idea that she actually coined (Kennard, 1940; Anderson et al., 2011). Gorrie et al. (2002) extended their analysis through examination of the distribution of β-APP positive reactive axons in the same patients referred to

above. No labeled axons occurred in the brains of patients who died within 1 h of injury and this strengthens the current view that β-APP immunocytochemical labeling highlights a population of axons within which axonal transport continues immediately after injury but at a locus transport becomes compromised over the first few hours after TBI. There is a growing consensus that primary axotomy occurs relatively rarely and only in the most severe cases of human TBI when a patient dies or enters coma immediately after an accident (Maxwell et al., 1997; Smith and Meaney, 2000). It must also be recognized that current histopathological tools used in forensic examinations of patients provide no evidence to allow recognition of primary axotomy. The data provided by Gorrie et al. (2002) supports the hypothesis that with any survival of greater than a few hours hypoxic changes are a major secondary insult in both children under two and aged between 2 and 16 years because swollen axons occurred in more than 85% of the white matter of the injured brain in children that survived for about thirty hours (Gorrie et al., 2002). Gorrie et al. (2002) also provide good data that the number of damaged, immunolabeled axons increases rapidly until 12 h after injury and then continues to increase but at a lesser rate until about 90 h after TBI. A recent experimental study (Sulaiman et al., 2011) has provided quantitative evidence that the number of damaged or injured axons exhibiting ultrastructural morphological evidence for degenerative change in an experimental model continues to climb with increasing survival out to 12 weeks after injury and is closely correlated with an increasing number of degenerating neuronal cell bodies from which injured axons probably originated. It is here suggested that too few authors recognize that both traumatic and hypoxic injury mechanisms result in β-APP labeling of axons when labeling is frequently held as being specific for TAI which it is not (Waxman et al., 1992; Coleman, 2005; Stys, 2005). Rather β-APP labeling identifies axons within which the axolemma has been damaged and there has been injury to mitochondria that result in a compromise of axonal transport which allow for focal accumulation of β-APP to give rise to axonal swellings which may develop in a range of brain insults. It is suggested that this should become more widely recognized, particularly since vascular associated axonal injury (VAI) has been well documented in pediatric and childhood TBI (Geddes et al., 2001a,b). More recently, SWI enables microbleeds or petechial hemorrhages at the cortex-white matter boundary to be resolved directly in living patients (Tong et al., 2008), and, as mentioned earlier, is being increasingly used as a marker of DAI.

3.4. Glial cells within the CNS: Astrocytes

Astrocytes first become demonstrable within the human developing CNS at about 15 weeks GA (Roessman and Gambetti, 1986) using the now universal marker for reactive astrocytes, immunocytochemical labeling for glial fibrillary acidic protein (GFAP). An example is provided (Fig. 8A) at 2 weeks after TBI. It is important to note, however, that GFAP expression is not exclusive to protoplasmic and fibrous astrocytes but also include Muller glia in the retina, Bergmann glia of the cerebellum, tanycytes, pituicytes, cribriosocytes, and GFAP-expressing radial glia (Sofroniew and Vinters, 2010). Astrocytes fulfil many functions within the brain, in pH homeostasis, neurotransmitter re-uptake, communication via gap junctions with neighboring astrocytes to modulate neuronal and vascular function, up-regulation of astrocyte glycolysis via Na^+ waves, guidance in capillary tube formation, maintenance of the blood-brain barrier (BBB) and regulation of the cerebral circulation, perhaps focally when neuronal activity rises, or by elevated Ca^{2+} levels within astrocyte foot processes stimulating arteriolar caliber changes (Koehler et al., 2006; Sofroniew and Vinters, 2010). Astrocytes are also responsive in different pathophysiological states and astrocyte swelling and increased K^+ content following ischemia may disturb intracellular signaling by Ca^{2+} and depress blood flow and glycolytic responses for hours or days (Koehler et al., 2006). However, there is increasing evidence that astrocyte responses to Ca^{2+}, NO, neurotransmitters and a spectrum of other agents varies between different brain regions and physicochemical microenvironments (Nimmerjahn, 2009) which makes it presently impossible to provide a simple overview. The purpose of the above discussion is to bring to the attention of the reader a novel area of investigation that may have wide ranging impact or impacts upon future understanding of the close and complex interactions between neurons, astrocytes and capillaries in both intact and injured brain.

Reactive gliosis occurs as either a focal or a diffuse response with the latter being more frequent (Roessman and Gambetti, 1986). However, astrocyte reactivity to GFAP antibodies has been reported to be relatively short lived with labeling becoming faint in survivals of greater than 3 weeks (Roessman and Gambetti, 1986 since confirmed by Geddes et al., 2000). A diffuse response by astrocytes with the response being more notable in the frontal lobes of the brain has been reported after TBI in a primate model (Maxwell et al., 1992) and in humans (Bigler and Maxwell, 2011). With post-natal increased growth and maturation of the brain, reactive astrocytosis becomes more widespread with a marked difference in cell size between fibrous and protoplasmic astrocytes the former being considerably larger. A diffuse, widespread response by fibrous astrocytes following TBI is widely recognized in mature or near mature brain and spinal cord although tending to be more discrete close to a lesion site (White et al., 2010). When mild or moderate injury to the brain had occurred, Myer et al. (2006) reported that astrocytes provided an overall protective effect against degeneration of neural tissue since when astrocytes were experimentally ablated cortical loss was increased. Any protective influence was, however, lost when more severe TBI occurred.

Bell et al. (2005) suggest that reactive gliosis in the neonatal and infant brain may well be missed under routine staining and that use of GFAP immmunocytochemistry should be routine in diagnostic pathology to reduce the risk of failing to observe astrocytic hyperplasia.

Very recently, Budde et al. (2011) used a controlled cortical impact model of TBI in rat. Two months after injury FA values increased adjacent to the lesion. This has often been interpreted as indicating axonal re-growth or regeneration (Voss et al., 2006; Wilde et al., 2008; Lo et al., 2009). Budde et al. (2011), however, using correlated histological analysis, report that increased FA at the margin of a cortical lesion reflects a highly ordered spatial orientation of GFAP positive astrocytes rather than SMI-32 positive neurite processes so that the perilesional increased FA reflects gliosis rather than neuroregeneration. This novel finding needs to be replicated experimentally in a variety of experimental models or post-mortem human specimens before consensus may be achieved. This illustrates the possible interpretive problems due to use of only a single type of test during examination of injured human brain. Moreover, the functional consequences of mild or moderate diffuse gliosis are not well understood (Sofroniew and Vinters, 2010).

Diffuse swelling of the brain is a notable feature of short term responses to brain injury and the occurrence of microhemorrhages within the cortico-medullary boundary is increasingly interpreted as a marker for DAI/TAI (Robinson and Bhuta, 2011). Astrocyte foot processes surround the basement membrane of the majority of capillaries within the brain and assume an electron lucent appearance within a few hours of TBI (Fig. 8B). The cytoplasm of these astrocytes frequently contains groups of glycogen granules (Fig. 8C) that appear within a few hours of injury and later disappear. Astrocytes are also linked to neighboring astrocytes by gap junctions that allow propagation of astrocyte Ca^{2+} waves that may

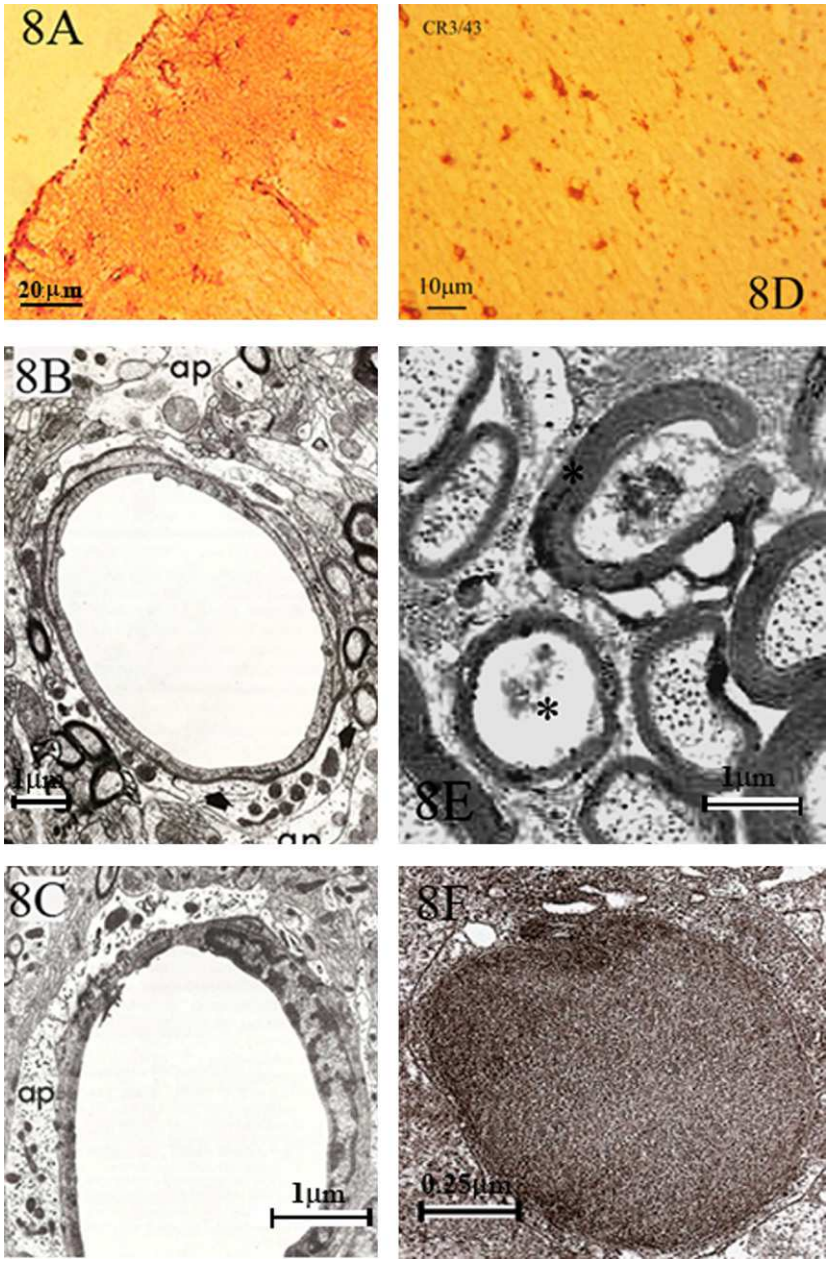

Fig. 8. Part (A) is a photomicrograph of a piece of human frontal cerebral cortex following death at 1 week after TBI, in which the section has been labeled for GFAP, showing numbers of stellate astrocytes in cortical layers 1 and 2. The fibrous astrocyte layer of the glia limitans is highlighted by the label. A few oval or tubular profiles occur and these are surrounded by labeled perivascular astrocytes. (B and C) Transverse thin sections of capillaries within the thalamus at (B) 12 h and (C) 7 days after TBI from an experimental preparation in non-human primate following lateral rotational acceleration of the head. The astrocyte perivascular (ap) foot processes are pale and lucent. Numbers of glycogen granules occur within the cytoplasm of the foot processes being more numerous at 7 days after injury. Note that mitochondria within the foot processes have a normal appearance which indicates that respiratory distress is not present. (D) is a photomicrograph of a section of human subcortical white matter at 6 months after TBI. The section has been labeled with the antibody CR3/43 which identifies reactive microglia that are highlighted in brown (DAB label). Reactive microglia are numerous among myelinated nerve fibers and are suggestive of an ongoing, long term degeneration or loss of a proportion of nerve fibers within the field of the micrograph. (E) is a medium power thin section electron micrograph from a transverse section of stretch-injured optic nerve at 3 months after injury. Two axonal remnants from which the axon has degenerated but a recognizable myelin sheath remains are seen among other normal fibers. In the lower fiber remnant the myelin sheath retains a tubular profile. In the upper fiber the myelin sheath remnant has collapsed and a process from an adjacent astrocyte has penetrated the resultant cavity. This demonstrates that despite axonal loss after stretch-injury the integrity of the myelin sheath is retained for a period thereafter. (F) is a medium power thin section electron micrograph of retina at 3 months after stretch-injury at the stage of nuclear fragmentation prior to dissolution of the nuclear envelope during programmed cell death. This shows condensed nucleoplasm and cytoplasm within which are autophagic vesicles characteristic of the earlier stages of programmed cell death.

spread over several hundred micrometers from an initial stimulus (Verderio and Matteoli, 2001; Hamilton et al., 2008). When pure astrocyte cultures are stretched the mitochondrial membrane potential ($\Delta\psi_m$) and the cellular level of ATP falls, returning to normal over 24 h (Ahmed et al., 2000). In pure neuron cultures $\Delta\psi_m$ falls only after severe stretch injury. However, in mixed astrocyte and neuron cultures the fall in $\Delta\psi_m$ and ATP levels occurs at mild stretch injury by 28% and in severe injury by 38% (Ahmed et al., 2000). This synergistic interaction between astrocytes and neurons thereby exacerbates disruption of mitochondrial function in injured neurons with a resulting deficit in cellular ATP content. Furthermore, release of calcium from the endoplasmic reticulum allows activation of calcium activated neutral proteases within neurons which may disrupt spectrin structure and allow

proteolysis of voltage-gated sodium channels at nodes of Ranvier (von Reyn et al., 2009) to exacerbate injury, loss of ATP production and axonal ionic homeostasis. Within several days of TBI, astrocyte swelling is resolved and reactive astrocytes undergo hypertrophy and a limited amount of proliferation to occupy an increased volume within injured white matter over at least several weeks after TAI (Sulaiman et al., 2011). The cytoplasmic content of the unique intracellular intermediate filaments of astrocytes increases and astrocytes become hypertrophied and form areas of increased fractional anisotropy (Budde et al., 2011). Knowledge about astrocyte function and responses after brain injury, although having increased significantly over the last few years, is still not well understood. It is clear (Koehler et al., 2006) that astrocytes are a very responsive and reactive component of the neurovascular unit. And it has recently been appreciated that the response by astrocytes is much more extensive or widespread throughout the brain than being limited to the locus of injury. But our understanding is limited and it is suggested that investigation of cellular responses after trauma to the brain should not be limited only to analysis of neuronal responses.

3.5. Oligodendrocytes

Development of oligodendrocyte progenitor cells is initiated in the second trimester under the influence of the morphogen Sonic Hedgehog in the ventral region of the neural tube at between 10 and 15 weeks GA. The progenitor cells migrate to dorsal regions of the wall of the telecephalon and differentiate to oligodendrocyte precursor cells, that may be distinguished by immunocytochemical techniques, and are located at the subplate layer immediately below the cortical plate (reviewed in Jakovceski et al., 2009) between 19 and 22 weeks GA. Maturation of oligodendrocytes is marked by expression of two myelin proteins, myelin basic protein (MBP) and proteolipid protein (PLP). MBP positive cells occur within the intermediate zone, the future white matter, at about 18 weeks GA (Jakovceski and Zecevic, 2005) and numerically increase as development progresses. Myelin formation entails proliferation, migration and differentiation of oligodendrocyte precursor cells into myelin forming oligodendrocytes under the influence of cytokines and other mediators, for example, extrinsic myelin gene promoters, electrical activity in axons, and microRNAs although our specific knowledge is still incomplete (Emery, 2010).

Experimental studies in TBI have reported loss of oligodendrocytes from gray and white matter within days of fluid percussion injury in rats (Lotocki et al., 2011). Quantitative data for the cerebral cortex, corpus callosum and external capsule showed a reduction in number of oligodendrocytes by −73% from cortex, −64.2% from the corpus callosum and −86% from the external capsule by three days after injury in rat. At 7 days the loss was comparable −72.9% for cortex, −60% in corpus callosum and −82.5% from the internal capsule (Lotocki et al., 2011). In humans after TBI and with axonal loss in adults, oligodendrocytes undergo programmed cell death during survival (Williams et al., 2001) within degenerating tracts over at least 12 months after TBI. Axonal loss via Wallerian degeneration occurs in stretch- injured axons over 14–84 days (Sulaiman et al., 2011). This is considerably longer than reported after transection and crush injury (Beirowski et al., 2005; Berkelaar et al., 1994) but even so, morphological evidence for axoplasmic disruption out to 3 months (Sulaiman et al., 2011) after TBI suggests that with increasing post-traumatic survival increasing numbers of axons within white matter tracts may be induced to undergo secondary axotomy. The reported evidence of PCD by oligodendrocytes in human cortico-spinal tract out to at least 12 months after TBI (Williams et al., 2001) strengthens the suggestion that axonal loss is a progressive and on-going process with post-traumatic survival. This has not previously been suggested in the literature to date.

3.6. Inflammatory response

There is increasing appreciation that an inflammatory response occurs within the injured brain and there is some evidence that such a response may extend over months or years after trauma. Within a few hours of TBI astrocyte swelling and enlarged perivascular spaces become apparent (Inglese et al., 2005). Most notably, this posttraumatic inflammation is associated with the aggregation of both cyclo-oxygenases (COX-1) and endothelial monocyte-activating polypeptide II (EMPA II) positive microglia and macrophages in the perivascular space of the injured brain (Inglese et al., 2005). These cells reach their highest numbers at 5–7 days after injury and thereafter numbers fall. Inflammation involves the activation of signaling pathways that mobilize inflammatory cells, and stimulate the secretion of multiple inflammatory mediators/biomarkers or cytokines, for example macrophage inflammatory protein-1 alpha MIP-1β and MIP-1α, monocyte chemoattractant protein (MCP-1), pathogen-associated molecular patterns (PAMP's), damage-associated molecular patterns (DAMP's), or receptor for advanced glycation end products (RAGE) to provide some examples. The cellular and tissue response is extremely complex and incompletely understood and this has frustrated attempts to therapeutically modulate trauma-induced inflammation (Namas et al., 2009) such that there is a current dearth of therapeutic options. Blood derived macrophages either enter the injured CNS via the damaged walls of the microvasculature where the integrity of the blood–brain barrier is compromised within several days of injury or arise from intrinsic microglia within oedematous neuropil. Reactive microglia may be identified, for example, using the CR43R antibody and examples of labeled cells may be seen in damaged central white matter both a few days and out to at least several months after injury (Rezaie and Dean, 2002). Evidence obtained from adult patients following TBI suggests that reactive microglia and macrophages may be associated with central white matter tracts (Fig. 8D) and a proportion of nerve fibers may degenerate over months or years after TBI (Williams et al., 2001). However, it is notable that microglia and macrophages have been reported as also undergoing programmed cell death in white matter, for example the corticospinal tract of the human spinal cord up to 12 months after TBI (Wilson et al., 2004). Cagin et al. (2001) reported widespread and continued activation of microglia over more than 12 months after viral infection. Volumetric MRI and PET studies indicated that *in vivo* imaging of activated microglia showed increased signal from loci of initial infection along white matter tracts passing both toward and away from the original focus of infection. The widespread disposition of activated microglia was associated with both atrophy of parts of the nervous system and cognitive deficits. Cagin et al. (2001) indicate that following infection and/or trauma the activity of immune cells or microglia may extend throughout the neural tracts related to an initial locus of damage to widespread sites elsewhere in the CNS. The integrity of oligodendrocytes appears to be closely related to the integrity and functional stage of the axon around which an oligodendrocyte process forms a segment of the myelin sheath. The closely organized disposition of the myelin sheath undergoes a loosening or loss of cohesion early as the axon degenerates (Fig. 8E) (Sulaiman et al., 2011) to the point when vacated myelin tubes of sheaths occur even though the axon has degenerated. A possible interpretation is that once an axon has undergone secondary axotomy, the distal or separated portion is no longer able to maintain the integrity of its related myelin sheath and both eventually degenerate with the loss of the axon remnant preceding loss of myelin and associated oligodendrocytes. A recent study by Venkatesan et al. (2010) in mouse has

reported active engulfment of myelin debris and damaged axons by activated microglia over 28 days after injury to the corpus callosum during myelin phagocytosis. But it is also significant that heterogeneous subpopulations of microglia which secrete different neural effectors, including growth factors, occur at differing times and locations within an injured white matter tract following injury (Venkatesan et al., 2010). There is, however, only limited information currently available for studies in the immature or developing central nervous system.

3.7. Programmed cell death

Programmed cell death (PCD) is an energy consuming process that requires a continued provision of metabolic substrates. Frequently the term apoptosis is used to refer to this pathological state. But apoptosis is, in *senso-stricto*, only applicable to the phase of morphological changes of the cell nucleus after cell death (Elmore, 2007) correlated with the generation of apoptotic bodies, and the more widely applicable term PCD will be used henceforth in this article. Nonetheless, the characteristic nuclear morphology of apoptosis may be readily recognized and noted during neuropathological diagnostic screening whereas the more subtle changes of nuclear morphology preceding apoptosis may be missed unless transmission electron microscopy (TEM) is used (Fig. 8F). Programmed cell death has been widely reported and recognized in a wide variety of human brain injuries or insults and has been documented over months, years or decades after TBI (Hausmann et al., 2004; Dratviman-Storobinsky et al., 2008; Minambres et al., 2008; Maxwell et al., 2010; Sulaiman et al., 2011). However, PCD is also an intrinsic component of development within the very young, growing brain. Normally, the human brain growth spurt begins around mid-gestation (Dobbing and Sands, 1973) and extends until the third year of life (Bittigau et al., 2003). During the growth spurt there is widespread PCD during synaptogenesis and physiological cell death for establishment and maturation of neural circuits toward formation of brain networks as interactions and responses with the external environment develop and mature. The latter is mediated via systematic pruning of local connections and strengthening of long-range connectivity and wiring distance characteristic of adults (Supekar et al., 2009). In the weight drop model of TBI Bittigau et al. (2003) reported an initial, brief excitotoxic, expanding lesion achieving maximal spatial extent within 4 h after trauma in rat pups. There was then a widespread occurrence of PCD within numerous cortical regions, thalamic nuclei, the hippocampal dentate gyrus, subiculum and striatum at 24, 48 h and 5 days after trauma. Greatest numbers of apoptotic cells occurred when 3 day old pups and least when 14 day old pups were injured. Changes in number of neurons provides a partial explanation for the widely reported changes in volume of specific gray matter landmarks within the chronic TBI brain. Loss of neurons must reflect the loss of their respective cell processes, their axon and dendrites, and will alter the parameters in at least a number of neuronal circuits or pathways in the injured, developing brain. These changes in pathways may be manifested at the level of the widely reported changes in behavioral, memory, emotional and cognitive functions in an injured patient (Duhaime et al., 1992; Barlow et al., 2005; Levin et al., 2008) where outcome is worst in children injured at less than 1 year of age (reviewed in Bittigau et al., 2003) with deficits in intellectual and verbal capacity at 10 years survival (Horneman and Emanuelson, 2009).

Although there is still considerable controversy between the "plasticity" and "early vulnerability" camps there is suggested to be a growing consensus, at least in regard to traumatic injury to the young brain, that outcome for the injured young child is worse than in children or adolescents and outcome is more severe in terms of reduced cognitive function for children injured at younger ages (Anderson et al., 2009).

4. Concluding remarks

Several simple but illustrative observations about the relative immaturity or maturity of gray and white matter across childhood and adolescence may be drawn from this review and serve to show that different parts of the CNS may be at differing levels of maturation at the time when a brain injury may happen.

- If brain injury occurs *in utero* during the third trimester or around birth the major proportion of the developing CNS is at an early developmental stage and the outcome after TBI is very poor.
- Mechanical injury to the brain of a neonate is unlikely to occur unless another individual such as an adult is involved or the neonate is involved in a MVA.
- In the very young child parts of the brain are interconnected by immature/unmyelinated nerve fibers. Responses and outcomes after brain injury probably develop more quickly, reflecting the more rapid degeneration of unmyelinated nerve fibers, and may have a far more widespread field of effect due to compromised development of early brain networks, so that with survival the child may be severely handicapped.
- Traumatic or mechanically induced injury to the brain in late childhood and adolescence differs from the above in that the injury scenario is frequently related to sports, interpersonal violence and MVA.
- In late childhood and adolescence groups of immature nerve fibers still occur within the frontal lobes which are at highest risk of injury. The most common outcomes after TBI are deficits in cognitive, planning, emotional, memory and integrative activities.
- The major degenerative pathway for injured nerve fibers is secondary axotomy which involves a pathological cascade of post-traumatic events resulting in axonal fragmentation or disconnection between 12 h and at least several days after injury.
- The key feature that results in an axon entering the pathological cascade is injury to mitochondria within the axon.
- There is a resulting focal loss of axonal transport at the point of injury. Axonal transport is retained elsewhere along the axonal length and allows accumulation of normally present chemicals that may be used as histopathological markers of axonal injury. The most frequently used marker is antibodies against beta-amyloid precursor protein.
- Labeling of an axon with beta-amyloid precursor protein is not specific to TBI but reflects loss of axonal transport that may occur following a variety of insults to the brain.
- Following secondary axotomy, the distal portion of the axon undergoes Wallerian degeneration.
- The proximal fragment of the axon still communicates with its cell body and is thought to either result in the neuron undergoing programmed cell death or the neuron assuming an injured state.
- The duration over which programmed cell death continues within the injured brain has not yet been determined experimentally.
- It is presently thought that post-traumatic continuance of axonal loss results in loss of central white matter that may be most readily appreciated by the increase in size of the lateral ventricles within the cerebral hemispheres.
- Programmed cell death is probably the major pathway for loss of neurons. This cannot be visualized directly by MRI or DTI. But loss of neurons provides an explanation for the recognized loss of volume of the hippocampus, thalamus and parts of the cerebral cortex that has been reported during post-traumatic survival.

- Astrocytes actively respond to TBI, first reflecting focal disruption of the blood-brain barrier, increased release of glutamate and homeostatic disruption. Later, astrocytes undergo hypertrophy.
- Astrocytes spontaneously generate calcium waves which propagate over hundreds of microns from the initial locus of injury. These calcium waves, together with disruption of potassium homeostasis may provide a mechanism for exacerbation of the volume of injured neuropil.
- White matter injury in TBI may often be associated with injury to the brain's microvasculature and the consequent deterioration in supply of glucose and oxygen may exacerbate the primary injury.
- Collectively the above may result in an inflammatory response with increased numbers of macrophages and reactive microglia. These may extend throughout nerve fiber pathways or tracts to other parts of the cerebrum and result in exacerbation of functional loss.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijdevneu.2011.12.008.

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