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Clinical characteristics and pathophysiological mechanisms of focal and diffuse traumatic brain injury

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Abstract

Traumatic brain injury (TBI) is a frequent and clinically highly heterogeneous neurological disorder with large socioeconomic consequences. TBI severity classification, based on the hospital admission Glasgow Coma Scale (GCS) score, ranges from mild (GCS 13–15) and moderate (GCS 9–12) to severe (GCS $\leq$ 8). The GCS reflects the risk of dying from TBI, which is low after mild (~1%), intermediate after moderate (up to 15%) and high (up to 40%) after severe TBI. Intracranial damage can be focal, such as epidural and subdural haematomas and parenchymal contusions, or diffuse, for example traumatic axonal injury and diffuse cerebral oedema, although this distinction is somewhat arbitrary. Study of the cellular and molecular post-traumatic processes is essential for the understanding of TBI pathophysiology but even more to find therapeutic targets for the development of neuroprotective drugs to be eventually used in human beings. To date, studies *in vitro* and *in vivo*, mainly in animals but also in human beings, are unravelling the pathological TBI mechanisms at high pace. Nevertheless, TBI pathophysiology is all but completely elucidated. Neuroprotective treatment studies in human beings have been disappointing thus far and have not resulted in commonly accepted drugs. This review presents an overview on the clinical aspects and the pathophysiology of focal and diffuse TBI, and it highlights several acknowledged important events that occur on molecular and cellular level after TBI.

Keywords: focal traumatic brain injury ● diffuse traumatic brain injury ● pathophysiology ● traumatic axonal injury ● excitotoxicity ● mitochondria ● axonal disconnection

Introduction

With an incidence of 235–556/100,000, traumatic brain injury (TBI) is among the most frequent neurological disorders worldwide [1, 2]. Traffic accidents, falls and assaults are the main causes of TBI [3].

In severe TBI case fatality rates mount up to 40% and in survivors the disability rate is as high as 55–77% [3–5], leading to reduced quality of life and vast socioeconomic costs. Given the high incidence and impact of TBI on society, interest is directed towards the development of neuroprotective therapies. Unfortunately, results of approximately 30 randomized controlled clinical trials in human beings have been disappointing [6, 7], underlining the complex and heterogeneous pathogenesis of TBI.

In vitro and *in vivo* research in different cell lines and in animal and human beings, has increased knowledge on the pathophysiological processes arising from TBI. TBI is a dynamic process resulting in alterations in function and structure of virtually all

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Table 1 TBI classification, mortality, CT-abnormalities and neurosurgical interventions

TBI category	*N*	Mortality	CT-abnormalities	Neurosurgical intervention*	Refs.
Mild (GCS 13–15)[†,‡]					
GCS = 15	24249	0.1%	7.8%	0.9%[#]	[132]
GCS = 13–15	3181	0.7%	7.6%	0.5%	[133]
GCS = 13–14	1483	5.8%	-	-	[134]
Moderate (GCS 9–12)[†]					
GCS = 9–12	309	3.9%	64.7%	16.5%	[135]
GCS = 9–12	1422	12.7%	-	-	[134]
GCS = 9–12, no other recordings of GCS < 9 at acute phase*	128	9%	-	-	[3]
Severe (GCS ≤ 8)[†]					
GCS ≤ 8; admission ≤ 8 hrs	1914	42.2%	-	-	[134]
GCS = 8 or deterioration < 48 hrs	746	32.5%	92.8%	36.3%	[136]
GCS ≤ 8; death < 24 hrs or obey commands within 24 hrs excluded	304	40%	98%	21%	[4]
GSC ≤ 8 at admission or at least one GCS at acute phase ≤ 8 and none > 8	583	40%	-	37%	[3]

*Variability exists between studies in what is defined as neurosurgical intervention and may include one or more of the following: craniotomy, elevation of skull fracture, intracranial pressure monitoring and ventricular drainage.

[†]There is variability between definitions depending on the moment on which the GCS should be obtained (*e.g.* at accident scene, at emergency department, after resuscitation, after 24 hrs).

[‡]Variability exists in (duration of) additional criteria that should be present upon diagnosing MTBI (*i.e.* loss of consciousness, PTA).

[#]In addition to neurosurgical intervention this number also includes medical treatment for brain oedema and transfer to more intensive care.

elements of the brain that may continue up to years after the injury is sustained, introducing new possible windows for therapeutic intervention.

In this review we aim to provide an overview of the clinical consequences of TBI and current concepts of the pathological processes underlying damage of nerve cells and their axons. Although not all cellular and molecular post-traumatic processes are evaluated exhaustively, this overview can be a starting point for readers with additional interest in TBI pathophysiology.

Classifying TBI

Clinical injury severity

For almost four decades the Glasgow Coma Scale (GCS) score, which measures level of consciousness at the trauma scene or at emergency department admission, has been the primary clinical variable to grade initial brain injury severity in mild (GCS 13–15), moderate (GCS

9–12) or severe (GCS ≤ 8) [8]. In terms of survival the GCS score, especially the GCS motor score, remains one of the strongest predictors [9] (Table 1). However, from the GCS the underlying cerebral pathology cannot be inferred and different structural abnormalities may result in a similar clinical picture (Table 2). Therefore, at present more attention is paid to the pathological features of injury such as the moment of onset (primary or secondary) and distribution of structural damage (focal or diffuse) [10–12] (Fig. 1).

Primary injury consists of the initial damage directly resulting from the mechanical forces affecting the cerebral tissues. Secondary injury refers to the cascade of cellular and molecular processes initiated by the primary injury. In addition, secondary injury consists of the cerebral damage due to hypoglycaemia, hypotensive or hypoxic events, and raised intracranial pressure resulting in cerebral ischemia.

Focal injury or diffuse injury

Focal brain damage is produced by collision forces acting on the skull and resulting in compression of the tissue underneath the

Table 2 How focal and diffuse traumatic brain injuries can lead to similar clinical pictures

	Focal injury	**Diffuse injury**	**Refs.**
Decreased consciousness, coma	Lesions with localized mass effect to diencephalon or brainstem	DAI to diencephalon or brainstem	[137, 138]
	Primary or secondary brain stem lesions	Diffuse oedema with compression of mesencephalic or diencephalic structures	
		Temporary functional inactivation (*i.e.* hypometabolism, excitotoxic effects)	
PTA	Focal medial temporal lobe lesions	DAI affecting the memory network	[139]
	Focal compression of the medial temporal lobe	Temporary functional inactivation (*i.e.* hypometabolism, excitotoxic effects)	
Dysexecutive syndrome / Memory dysfunctions	Frontal and temporal lobe contusions (lobes are vulnerable to mechanical deformation due to their location within the skull)	Axonal injury to fibre bundles such as the uncinate fasciculus and corona radiate	[28, 140]
		Widespread Wallerian degeneration resulting from loss of trophic input (after focal or diffuse injury)	
Motor weakness	Focal lesions with mass affect comprising the motor pathways	DAI affecting the corticospinal tract	[31]
	Basal ganglia haemorrhage	Hypoxic-ischemic injury	

cranium at the site of impact (coup) or of tissue oppositely to the impact (contre-coup) [13]. The location and severity of impact to the skull ultimately determine the cerebral pathology and neurological deficits. Focal injury constitutes subdural and epidural haematomas, intraparenchymal haematomas and (haemorrhagic) contusions (Fig. 1). Traumatic subarachnoid haemorrhage can be a result of focal damage but is also often seen in more diffuse vascular injury.

Diffuse brain injury entails widely distributed damage to axons, diffuse vascular injury, hypoxic-ischemic injury and brain swelling (oedema). The main injury mechanism responsible for diffuse injury is rapid acceleration–deceleration of the head, as seen, for example in high-speed motor-vehicle accidents [14, 15]. Brain structures are heterogeneous both in terms of degree of fixation to other parts of the brain and skull(base) and in terms of tissue consistency. As a result, during movement of the head, certain segments of the brain move at a slower rate than others, causing shear, tensile and compressive forces within the brain tissue [16].

Axonal injury is the most common consequence of diffuse TBI first described in 1956 by the pathologist Strich as a devastating clinicopathological syndrome with extensive damage to the white matter [17]. Later the term 'diffuse axonal injury' (DAI), was suggested by Adams and colleagues, referring to prolonged coma (more than 6 hrs) and widespread injuries to white matter regions [18] that can be pathoanatomically graded into three stages of increasing severity based on the depth of the lesions with grade 1 representing a pattern of lesions confined to the lobar white matter at the grey-and-white matter interface, grade 2 revealing addi-

tional lesions to the corpus callosum and grade 3 further depicting lesions to the rostral lateral–dorsal brainstem [18, 19].

During normal head movement, strain deformation manifested among axons is not harmful: due to their viscoelastic nature axons return to their normal shape and structure [20]. However, under more extreme circumstances the threshold of maximum elasticity is exceeded, resulting in changes in the axonal integrity [21]. Both the degree of the force applied to the axon and the length of time over which the force is applied influence the magnitude of axonal damage [22, 23].

An essential factor in the development of shear strain is the direction of the head movement: lateral head movement (Fig. 3A) is associated with more severe diffuse damage than sagittal head movement [14]. Furthermore, recent studies indicate that head contact has an important additive effect on the development of shear strain levels [24, 25]: findings that raise interest in the protective potential of lateral side airbags [26].

Clinical characteristics

Coma, confusion and subacute impairments

As a consequence of the variety of TBI pathologies, clinical features may be equally heterogeneous in terms of mortality, the time
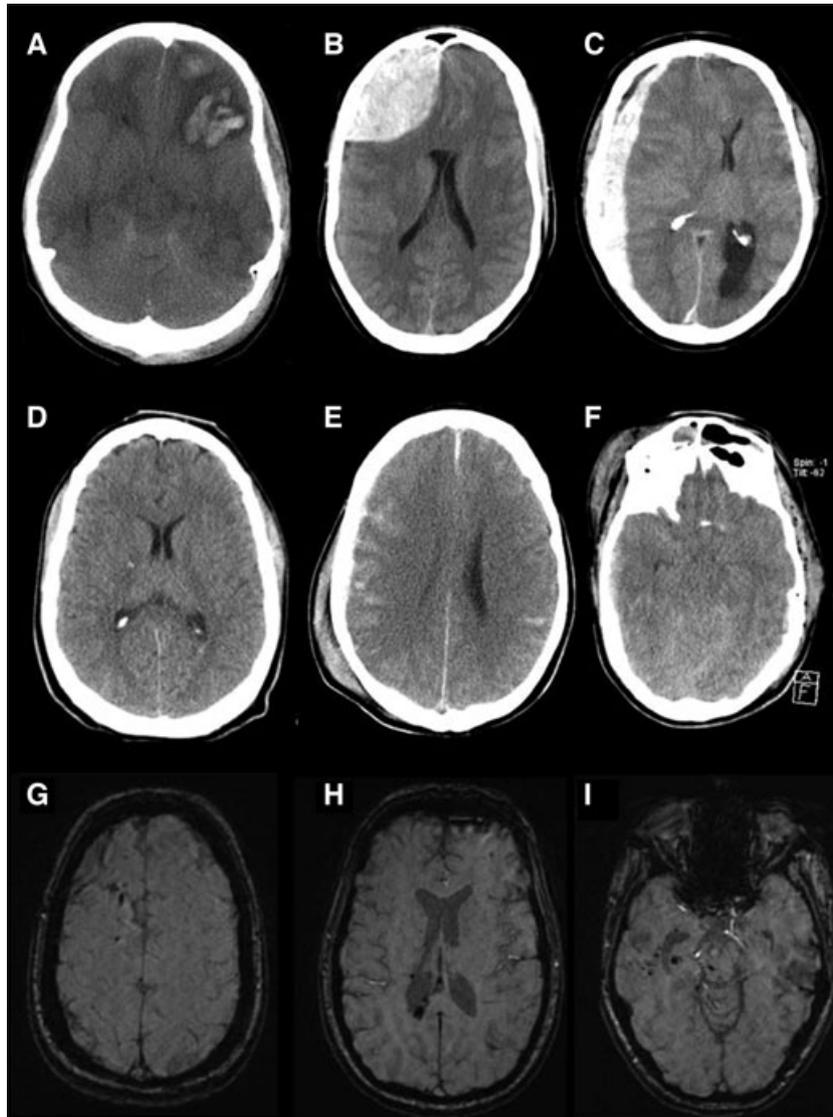
Fig. 1 Focal and diffuse TBI. Examples of focal and diffuse TBI on CT (rows 1 and 2) and MRI (row 3). Focal injury: (**A**) Left frontal contusion with midline shift to the right and compression of the lateral ventricles. (**B**) Right frontal epidural haematoma with midline shift to the left and compression of the anterior part of the lateral ventricle. (**C**) Right frontotemporoparietal subdural haematoma with a midline shift to the left. Diffuse injury: (**D**) Punctate haemorrhage within the right posterior limb of the internal capsule, a sign of DAI; (**E**) Diffuse subarachnoid haemorrhage; (**F**) Diffuse swelling with bilateral compression of the basal cisterns. DAI on MRI: Susceptibility weighted images of one patient revealing punctate haemorrhages (hypo-intense foci) within (**G**) the right frontal hemisphere, (**H**) Splenium of the corpus callosum, and (**I**) mesencephalon, corresponding to grade 3 DAI.

course of recovery and the functions that are affected. In general, recovery after TBI follows a certain pattern of stages [12]. The first stage consists of loss of consciousness or coma, which can vary from seconds up to weeks. Following the comatose phase, severe TBI patients may go through a stage of vegetative state characterized by a day–night cycle, with eye opening, not obeying commands and without speech production. The second phase of the recovery process is characterized by a period of disorientation, memory disorders and behavioural disturbances, also referred to as post-traumatic amnesia (PTA). Again, this period can vary from minutes to months. Both diffuse and focal injuries may result in coma, vegetative state and PTA (Table 2) though the pattern of recovery appears to be less predictable after focal injury than after diffuse TBI. This is emphasized by the finding that after diffuse injury but not after focal injury, duration of unconsciousness

and PTA strongly correlated with functional outcome at 6 and 12 months [27].

The third phase of recovery is characterized by a diversity of cognitive, behavioural, mood and sensorimotor disturbances. The frontal and temporal lobes in general are the most frequently affected brain regions. Sudden movement against the skull base renders the frontal-temporal regions especially vulnerable to cortical contusions and therefore not surprisingly impairments in attention, concentration, memory and executive functioning are the most frequent deficits after TBI [28]. However, also after diffuse (axonal) injury, impairments in memory and executive functioning are common [29, 30]. Compared to cognitive or behaviour problems, persisting motor weakness after TBI has a relatively low incidence [31]. In a study by Katz *et al.*, the time course of recovery of arm function in patients with diffuse TBI was

Fig. 2 Simplified pathophysiological molecular and cellular processes after focal TBI. The figure accompanies the transcript given in the main text. In short, increases in extracellular glutamate result in a supraphysiological Ca^{2+} influx that, subsequently, initiates several in parallel operating intracellular cascades (four are shown). Left upper corner: Increased activity of the calcium-dependent enzymes nNOS and eNOS enhances nitric oxide production leading to lipid peroxidation and eventually cellular necrosis. Left middle part: Calpain (a cystein protease) activity is also augmented due to an intracellular calcium increase, ultimately resulting in cellular necrosis pathways. Lysosomal membrane rupture and cathepsin release play an important role in this process. Right upper corner: Increases in intracellular calcium causing mitochondrial calcium overload result in increased mitochondrial membrane permeability. As a result ROS causing oxidative stress and the protein cytochrome-c are released into the cytoplasm. Cytochrome-c binds to the apoptosis activating protein-1 (apaf-1) activating the apoptotis inducing caspase pathway. Glu: Glutamate; $[Glu]_e$: extracellular glutamate concentration; NMDA: N-methyl-D-aspartate aspartic acid; E.R.: endoplasmatic reticulum; $[Ca^{2+}]_i$: intracellular Ca^{2+} concentration; nNOS: neuronal NOS; eNOS: endothelial NOS; MPT: membrane permeability transition; ATP: adenosine triphosphate; ADP: adenosine diphosphate; ROS: reactive oxygen species; apaf-1: apoptosis activating protein-1; $\cdot O_2$: oxygen radical.
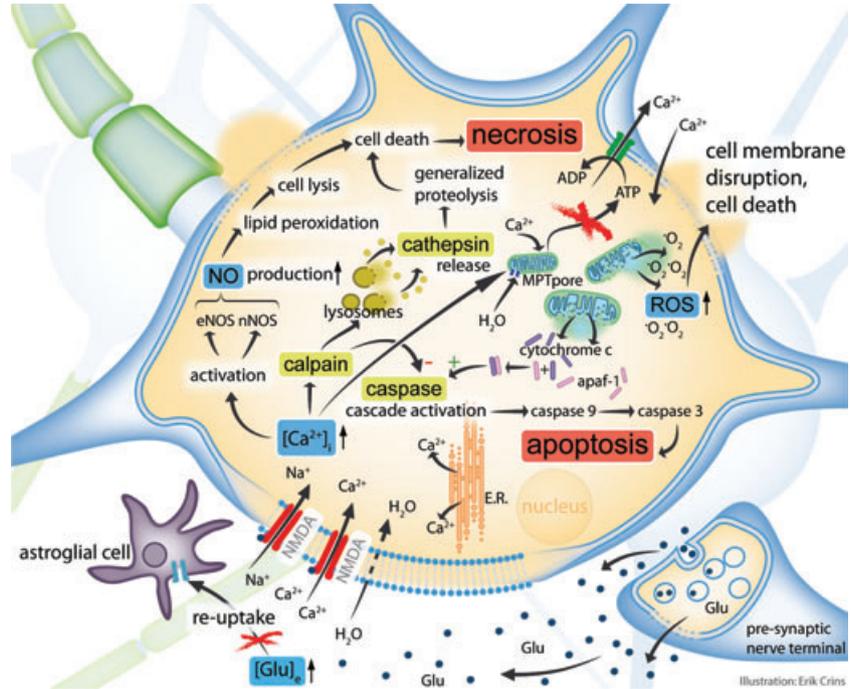
Imaging TBI

slower compared to the time course seen in patients with focal injuries [32]. This difference in recovery time between injury mechanisms however was not present when recovery of ambulation was considered [33].

Visualization of the full extent of damage after TBI is complex. In the acute phase work-up, the fast and easy to obtain CT is favoured due to its high sensitivity for (focal) injuries that may require intervention (Fig. 1). In the subacute and chronic phase of TBI, MRI techniques like T2-weighted imaging or fluid attenuated inversion recovery are preferred, because of their superior detection of lesions such as non-haemorrhagic contusions and oedema [34, 35].

Because of its microscopic nature, DAI is commonly undetected with conventional neuroimaging techniques like CT or MR. Due to simultaneous shear injury affecting neighbouring micro vascular structures, DAI is frequently accompanied by small punctate haemorrhages, visible at the DAI predilection sites. MR imaging techniques that are highly susceptible to blood products, such as T2* gradient echo and susceptibility weighted imaging, are now increasingly used within the clinical setting to enable diagnosis of DAI [36, 37] (Fig. 1).

Diffusion tensor imaging is a relatively new MRI modality that produces *in vivo* information of brain tissue integrity by yielding an image on the basis of the diffusion of water molecules [38]. Because of this property the technique offers great potential in the detection and delineation of (diffuse) traumatic lesions [36].

Focal and diffuse TBI: separate entities?

Though described in this paper as separate entities, it should be noted that focal and diffuse injuries may both arise and interact within a single individual. A recent MRI study in moderate and severe TBI, revealed both focal lesions (contusions or haematomas) and DAI in 50% of the patients [39]. The coexistence of multiple injury types provides a further difficulty and it has been suggested that for effective treatment multi-therapy strategies should be applied.

In this review, pathological processes at the neuronal cell body are described under the header of focal injuries whereas traumatic axonal pathology is placed under diffuse brain injury. Neuronal cell death has indeed mostly been studied in focal contusional or pericontusional regions whereas axonal injury is considered a consequence of diffuse TBI. However, the distinction between focal and diffuse injuries is artificial. Diffuse neuronal cell death remote from or unrelated to focal injuries is commonly reported after TBI [40] whereas in animal models, axonal injury is often induced at specific locations instead of diffusely distributed [41].
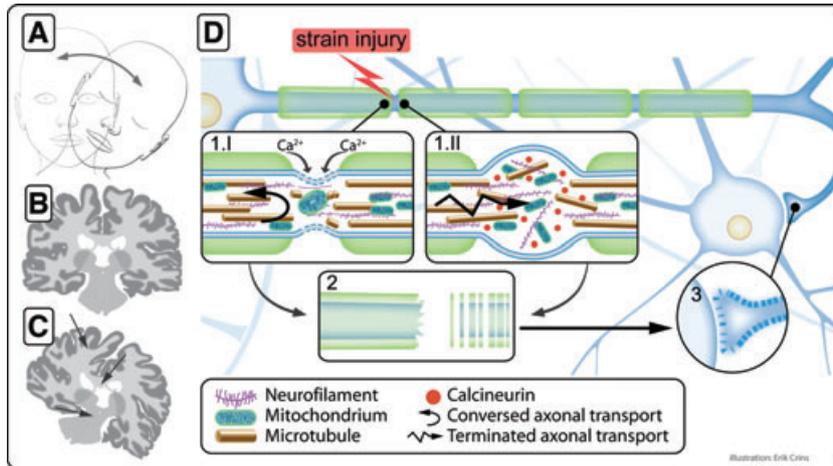
Fig. 3 Mechanical and pathophysiological mechanisms of TAI. Sudden lateral acceleration–deceleration of the head (**A**) is the main injury mechanism causing TAI. (**B**) and (**C**) are schematic representations of the brain during rest and movement, respectively. Deformation of the brain during sudden head movement causes shear, tensile and compressive strains within the brain tissue. Predilection sites of axonal injury are the grey and white matter interface, the corpus callosum and the brain stem (**C**). Sufficiently high strains, as occur, for example in motor-vehicle accidents, cause a cascade of pathological changes within the axon that may finally lead to axonal disconnection (**D**). It is suggested that two different processes of axonal injury exist [110]. The first is characterized by altered focal axolemma permeability (**D**1.I) whereby ionic homeostasis leads to local Ca^{2+} influx and mitochondrial swelling. Both local calcium dysregulation and release of cytochrome-c from damaged mitochondria result in activation of cysteine proteases and breakdown of essential axonal cytoskeleton products including loss of microtubules, neurofilament side-arm cleavage and neurofilament compaction impending normal axonal transport. In contrast to previous suggestions there is no termination of axonal transport or axonal swelling. Rather, it is suggested that there is a conversion of anterograde into retrograde axonal transport that prevents the axon from swelling. The second type of axonal injury (**D**1.II) is characterized by a combination of local axonal swelling and altered axonal transport but no overtly altered axolemma permeability. It is suggested that with this injury type there may be subtle alterations of membrane permeability triggering the activation of calcineurin. Calcineurin in turn alters the microtubular network, causing a disruption in axonal transport, with accumulation of organelles and swelling. After axonal disconnection, which may occur after both injury types, the axon undergoes a process of Wallerian degeneration (**D**2) consisting of a breakdown of the myelin sheath and the axon cylinder. The target site has now lost its input from the disconnected axon (**D**3) and may undergo synaptic reorganization, for example through axonal sprouting of neighbouring intact fibres. This process of synaptic reorganization may by adaptive or maladaptive.

Pathophysiological mechanisms of focal injury

The essentials: glutamate and Ca^{2+}

A key feature of focal TBI is impact to the head and the sequential energy transfer to the cerebral tissues, causing depolarization of nerve cells which results in uncontrolled excessive release of excitatory neurotransmitters leading to a cascade of pathological events called excitotoxicity. The main excitatory neurotransmitter in brain injury pathophysiology is glutamate and extracellular concentrations become significantly increased after injury. In human beings up to 50-fold increased glutamate levels have been found, especially in focal parenchymal contusions [42, 43]. The potency of glutamate as neurotoxin has been appreciated since several decades, and *in vitro* studies have suggested a dose–response relationship [44]. Human microdialysis studies demonstrate that raised extracellular glutamate levels are associated with worse outcome [42, 45, 46]. Glutamate is released by pre-synaptic vesicles after depolarization but also leaks through damaged cell membranes (Fig. 2). In addition, the normal glutamate re-uptake by astrocytes, *via* an ATP-dependent sodium-cotransport system, decreases or is abolished due to destruction and energy depletion of neighbouring astrocytes.

Excessive extracellular glutamate, initiates a massive influx of Ca^{2+} and Na^+ influx into neurons and glial cells [47]. Glutamate binds the *N*-methyl-D-aspartate aspartic acid and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors resulting in an over activation of the ion channels responsible for Na^+ and Ca^{2+} influxes [48]. More Ca^{2+} is sequentially released from intracellular stores like the endoplasmic reticulum further raising intracellular Ca^{2+} levels causing depolarization of the neuronal membrane activating voltage-dependent Ca^{2+} channels (VGCC) which also results in an additional increase of Ca^{2+} influx [47]. Experimental blocking of the VGCC's improves behavioural outcome in rats after TBI suggesting a neuroprotective therapeutic target [49]. Passive water movement as a consequence of the Na^+/Ca^{2+} influx produces sequential neuronal swelling. On the one hand, high cytosolic Ca^{2+} disrupts protein phosphorylation, microtubule construction and protease formation causing los of neuronal function. On the other hand, calcium-dependent enzymes are activated, especially calpain-1 and -2 that in turn results in protein and enzyme destruction (see below) [50].

Furthermore, nitric oxide generation by nitric oxide synthase (NOS) is partially calcium dependent (isoforms: neuronal NOS [nNOS] and endothelial NOS [eNOS]) and consequently nitric oxide production becomes increased [51, 52]. Besides a function as signalling molecule nitric oxide is a free radical with detrimental effects when it reacts with oxygen radicals to form peroxynitrite that will result in lipid peroxidation, cell membrane lysis and DNA

fragmentation [53, 54]. Nitric oxide synthetase inhibitors given early after trauma reduce the neurological deficits in mice after closed head injury [55] and specific blockage of nNOS significantly reduced contusion volume in moderate TBI in rats [56]. In its turn membrane disruption may result in an additive calcium influx caused by an increased non-specific permeability to ions.

Mitochondria

The imperative role of mitochondrial dysfunction in the eventual cascade to cell death renders mitochondria as a potential neuroprotective therapeutic target [57]. To maintain cytoplasmic Ca^{2+} homeostasis the raised intracellular Ca^{2+} concentration results in calcium sequestration within mitochondria [58]. However, mitochondrial Ca^{2+} overload directly impairs oxidative phosphorylation (OXPHOS) processes [59], leading to membrane depolarization with increased mitochondrial permeability by formation and opening of mitochondrial membrane permeability transition (MPT) pores [60–63]. Subsequently, passive water entry into the mitochondrion results in osmotic swelling [64, 65] and eventually loss of mitochondrial function [59, 65]. With mitochondrial dysfunction the energy production of the cell, *i.e.* OXPHOS and ATP production, is compromised [64] as has been demonstrated in human mitochondria after focal TBI [66]. Since energy demands are high shortly after TBI, the decreased ATP levels are extra harmful [67]. In patients with severe TBI, the severity of mitochondrial impairment assessed using high-resolution proton magnetic resonance spectroscopy, correlates with outcome [68].

Following the increased membrane permeability oxygen radicals (reactive oxygen species [ROS]), a by-product of the regular OXPHOS process, and the pro-apoptotic protein cytochrome-c, located between the inner and outer membranes of the mitochondria, are released into the cytoplasm [69–71]. The release of ROC into the cytosol leads to oxidative stress and ROS generation is further enhanced after the initial mitochondrial calcium uptake [72]. Within the mitochondrion itself ROS cause lipid and protein damage [65]. Cardiolipin (CL) is one of the mitochondria-specific phospholipids. Peroxidation of CL, a process propagated by cytochrome-c [73, 74], is found after TBI but before peroxidation of other phospholipids and it has been proposed that CL oxidation products play an important role in apoptotic signalling pathways [75, 76]. Additionally, oxidative stress products have been found in cerebrospinal fluid (CSF) of paediatric patients suffering from severe TBI [77].

In experimental TBI in rats the application of cyclosporin-A (CsA), a known inhibitor of the MPT pore, showed lower levels of intramitochondrial Ca^{2+} and decreased ROS production compared to untreated animals, implying a neuroprotective property of CsA [78]. This has resulted in a phase II trial in human beings. Cytochrome-c binds to the apoptosis activating protein-1 (Apaf-1) subsequently activating the caspase cascade: First caspase-9 is activated followed by caspase-3, eventually resulting in apoptotic cell death [70, 79–82]. This is considered the 'intrinsic' caspase activation pathway. Nevertheless, mitochondrial involvement is seen in both apoptosis and necrosis [83] and other parallel extramitochondrial apoptotic pathways are proposed [84]. Moreover, cytochrome-c release and caspase-3 activation have been shown in traumatic axonal injury (TAI) [69].

Caspase and calpain

Both the caspase and the calpain proteins belong to the cysteine protease family and are key regulatory enzymes in the molecular processes of cellular necrosis and apoptosis [85–87]. Caspases play a central role in apoptotic cell death [83]. Next to the 'intrinsic' caspase activation pathway, as described above, there is also an 'extrinsic' pathway mediated through the cell surface death receptors like the tumour necrosis factor α receptor 1 and caspase-8 [87, 88] – in this review not elaborated further.

Calpain activity is calcium-mediated and results ultimately in cellular necrosis, although apoptosis has also been associated with calpain activation [50]. After TBI especially calpain-1 (calpain-μ) and calpain-2 (calpain-m) are activated. Active calpain cleaves several enzymes and structural proteins [89], a process that is also found in TAI. The elevation of spectrin breakdown products, *i.e.* breakdown products resulting from cleavage of the cytoskeletal protein α_{II}-spectrin by calpain [90, 91] and to a lesser extent by caspase [92], has been demonstrated in CSF of rats after experimental TBI [93] and of severe TBI patients [94]. Furthermore, it has been suggested that calpain activation is associated with lysosomal membrane disruption leading to leakage of hydrolytic lysosomal enzymes, like cathepsin, that in turn will cause severe damage to the cytoplasm and eventually cellular necrosis [95–97]. Also, intracellular activation of calpains may lead to the inactivation of the caspase activation pathway [89, 98]. Finally, a calpain inhibitor administered to rats following brain injury has been shown to the attenuate motor and cognitive deficits compared to control animals [99].

Necrosis *versus* apoptosis

Both necrosis and apoptosis simultaneously occur in traumatic injured brain tissues [100, 101]. Whereas brain cell necrosis is energy independent, apoptosis occurs only in the presence of ATP, *i.e.* in the presence of functional mitochondria [82, 102]. Therefore, in tissue with extensive mitochondrial destruction and energy depletion mainly cellular necrosis will be found. In apoptosis, also referred to as programmed cell death, cell membranes do not rupture and no inflammatory response is elicited. An increasing portion of neurons succumbed to cellular necrosis when exposed to higher extracellular concentrations of glutamate [102]. Furthermore, it has been suggested that the occurrence of apoptosis or necrosis is associated with the intracellular Ca^{2+} levels [103, 104]. Relatively low intracellular Ca^{2+} might favour apoptosis and high intracellular Ca^{2+} would promote necrosis.

Pathophysiological mechanisms of diffuse injury

A heterogeneous cascade of changes

As indicated above, axonal injury is the most common consequence of diffuse TBI. Confusion can arise from the variable terminology used to describe this type of injury. The term 'diffuse axonal injury' was originally intended to describe a devastating clinical and pathological syndrome [18] suggesting that DAI may be interpreted as solely occurring at the severe end of the injury spectrum. In the following text we will use the term traumatic axonal injury (TAI), which is better suited to describe the whole spectrum of axonal injury severity in both human beings and animal models [105].

Axonal bulbs, grossly swollen axons detected upon microscopic investigation, are a pathological hallmark of TAI [106]. Initial suggestions that these axonal bulbs are the result of immediate mechanical tearing of the axon, followed by axonal retraction and axoplasmic leakage [17, 107], proved to be unsatisfactory for the larger part of the injured axon population. Rather, trauma evokes a cascade of changes to the axon that may ultimately result in secondary disconnection [108, 109].

These changes have long been thought to consist of a single pathological process but the important work of Povlishock and colleagues has now led to the hypothesis that two distinct forms of axonal injury exist [110] (Fig. 3D). The first process is characterized by altered axolemma permeability [111], mitochondrial swelling [69] and cytoskeleton breakdown consisting of a loss of microtubules, neurofilament side-arm cleavage and neurofilament compaction [112–114]. In contrast to what was previously posed, axonal disconnection resulting from this first type of axonal damage is not preceded by a termination of axonal transport and no axonal swelling occurs [115]. Rather, it is suggested that a conversion of anterograde into retrograde axonal transport occurs that prevents the axon from swelling [116] (Fig. 3D).

The second type of axonal injury is characterized by a combination of terminated axonal transport and local axonal swelling but not by overtly altered axolemma permeability [116] or neurofilament compaction [110, 117–120]. What in this latter processes leads to terminated axonal transport is unclear but possibly more subtle alterations of membrane permeability are involved, activating micromolar calpains and triggering the activation of calcineurin [110, 116]. In rats with experimental TAI, administration of a calcineurin antagonist, resulted in an attenuation of axons showing terminated axonal transport [121]. As a result of the activation of calcineurin, the microtubular network may be altered, which in turn disrupts local axonal transport kinetics, eliciting swelling, accumulation of organelles and finally disconnection (Fig. 3D).

Although unmyelinated fibres comprise a great part of the axonal population, current research has focused on the effects of traumatic impact to long myelinated axons [12]. A recent study of compound action potentials in the rat corpus callosum revealed that fine-calibre fibres were more vulnerable to traumatic impact than long myelinated fibres [122, 123]. This suggests that to date, the extent of traumatic axonal damage has probably been underestimated.

After axonal disconnection

After disconnection, the downstream segments of the axons undergo Wallerian degeneration consisting of breakdown of the myelin sheath and the axon cylinder. The time course of this process is highly variable with degeneration initiating as early as 1 to 3 hrs after injury but potentially proceeding up to several months after the impact [113, 124].

In diffuse brain injury, data on the processes following the deafferentation of target sites, now failing to receive input from the detached axon, is sparse. In an experimental model of TAI in a cat, in the dorsal lateral vestibular nucleus a diffuse pattern of deafferentation and nerve terminal loss was followed by a process of terminal recovery. The source of the return of the terminals is unknown though it is suggested that sprouting of adjacent intact nerve fibres leads to the recovery of synaptic input [125]. The quality of synaptic reorganization differs across the spectrum of TBI with maladaptive changes potentially consisting of inapt fibre ingrowth or abnormal alterations of the cytoarchitecture [126]. One factor that appears to be of influence is the presence of multiple injury types within in a single patient. In a series of *in vivo* experiments, interaction between TBI neuroexcitation and diffuse deafferentation was shown to be related to abnormal synaptic reorganization [127–129].

Upstream, axonal disconnection triggers temporary changes within the soma. Interestingly, contrary to what happens in models of primary axotomy produced by transaction of axons, cell-soma dysfunction does not necessarily lead to neuronal cell death. [130]. A possible explanation for this finding is that the process leading to secondary axonal disconnection is slower compared to immediate axotomy allowing the neuronal cell body time to reorganize and survive. However, opposed findings exist indicating that even ultrarapid disconnection of axons (occurring within 30 min. after traumatic impact), do not trigger rapid cell-soma death [124].

Conclusions

Following TBI both focal injury and TAI show multifaceted complex pathophysiological processes that are anything but completely elucidated. Nevertheless, the cellular and molecular processes after TBI are more and more unravelled resulting in an increasing number of possible therapeutic targets [131]. Although several *in vitro* and animal studies have consistently demonstrated beneficial effects of drug interventions (like VGCC blockers, nitric

oxide synthetase inhibitors, cyclosporine-A or calcineurin antagonists) this has not led to effective treatments for human beings. One explanation might be that these studies focused on single events rather than taking the heterogeneous TBI pathology into account. Probably neuroprotective treatment in TBI must target several coexistent pathological mechanisms in single patients.

To enlarge the neuroprotective and therapeutic arsenal, future research on the post-traumatic molecular and cellular mechanisms is essential but also trials in animals and subsequent randomized control trials in human beings are mandatory. A prerequisite for successful TBI research in human beings seems the application of new diagnostic techniques that enable the study TBI pathology at an increasingly detailed level. For example, Diffusion tensor MRI to quantify post-traumatic axonal alterations at different stages post-injury, and CSF microdialysis and MR spectroscopy to follow the metabolic changes of focal injury may be helpful to disentangle the heterogeneity of the specific pathological processes.

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Conflict of interest

The authors confirm that there are no conflicts of interest or financial disclosures for any of the authors.

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