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How to Translate Time; the Temporal Aspects of Rodent and Human Pathobiological Processes in Traumatic Brain Injury

Running title: timing of TBI pathologies in human vs. rodent

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ABSTRACT

Traumatic brain injury (TBI) triggers multiple pathobiological responses with differing onsets, magnitudes, and durations. Identifying the therapeutic window of individual pathologies is critical for successful pharmacological treatment. Dozens of experimental pharmacotherapies have been successfully tested in rodent models, yet all of them (to date) have failed in clinical trials. The differing timescales of rodent and human biological and pathological processes may have contributed to these failures. We compared rodent versus human timescales of TBI-induced changes in cerebral glucose metabolism, inflammatory processes, axonal integrity, and water homeostasis based on published data. We found that the trajectories of these pathologies run on different timescales in the two species, and it appears that there is no universal “conversion rate” between rodent and human pathophysiological processes. For example, the inflammatory process appears to have an abbreviated timescale in rodents versus humans relative to cerebral glucose metabolism or axonal pathologies. Limitations toward determining conversion rates for various pathobiological processes include the use of differing outcome measures in experimental and clinical TBI studies and the rarity of longitudinal studies. In order to better translate time and to close the translational gap we suggest a) using clinically relevant outcome measures, primarily in vivo imaging and blood-based proteomics, in experimental TBI studies and b) collecting data at multiple post-injury time points with a frequency exceeding the expected information content by two or three times. Combined with a big data approach, we believe these measures will facilitate the translation of promising experimental treatments into clinical use.

Keywords: axon; edema; glucose; inflammation; timeline

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GENERAL INTRODUCTION

The latest Phase 3 clinical trials of progesterone, ProTECT and SyNAPSe, are the newest contribution to the 100% rate of failure in translating experimentally efficacious pharmacotherapies for traumatic brain injury (TBI) into clinical use.[1, 2] Factors that have potentially contributed to this failure, including heterogeneity of the patient population, varying treatment paradigms, dosing, and poorly defined outcome measures, have been widely discussed elsewhere,[3, 4] with broader discussions about the challenges facing successful translation of experimental TBI pharmacotherapies into clinical use.[4-7] Rodent models of human diseases are the backbone of modern biomedical research. However, a limited understanding of inter-species differences, including the differing timescales of rodent and human pathological processes, can significantly hinder the translation of diseases with dynamically changing molecular pathologies like TBI. The limitations of modeling complex CNS disorders in animals, especially in rodents, have been extensively discussed.[8-10] High-fidelity modeling of TBI in rodents is particularly challenging due to the complexities of physical-biological coupling, i.e., how physical forces impacting the head translate first into biomechanical forces then biological responses.[11] The differences between human and rodent heads and brains in terms of size, gross anatomies, and cytoarchitectures have also been discussed.[12, 13] Importantly, there is substantial variation in the temporal pattern of normal biological processes, such as gestation, sexual maturation, and lifespan.[14] Analysis of the available literature has shown that a “rat hour” or “rat day” is not equivalent to a “human hour” or “human day” (and vice versa), but there seems to be no generally applicable conversion factor. Our limited understanding of the temporal differences in rodents’ and humans’ pathobiological response to injury can be attributed to two main factors: the use of different outcome measures in experimental versus clinical TBI studies, and the limited number of longitudinal studies with sufficient sampling frequency. The dominance of post-mortem outcome measures (e.g., histology) in experimental TBI research, at the expense of more clinically relevant measures (e.g., neurobehavior and in vivo imaging), significantly impedes the translation of experimental findings.

Furthermore, most experimental and clinical TBI studies use single time points, largely focused on the acute phase of the injury. This, too, is a major limiting factor as both the identity and the intensity of the pathobiological process can substantially change over time.[15]

In this review, we compare the rat and human timescales of (a) cerebral glucose metabolism, (b) inflammation, (c) vascular permeability / water imbalance, and (d) axonal changes following TBI. We have summarized our findings graphically (Fig. 1-4) based on available literature. We selected data based on identical and/or as comparable as possible methodologies used both in experimental and in clinical TBI studies. As discussed below, the differences in outcome measures used in clinical vs. experimental studies majorly affect translatability between the two fields. The quantity of literature data regarding the timelines of these four TBI-related pathobiological processes varies substantially, as reflected in the length and details of the relevant discussions. Our comparison is limited to studies with identical or comparable outcome measures in experimental and clinical TBI. While published literature on stroke, spinal cord injury, and peripheral nerve injury may provide information that is currently unavailable in the TBI field, the primary injury processes and related pathomechanisms are quite different; thus, the information is likely nonapplicable for TBI.

CEREBRAL GLUCOSE METABOLISM

Cerebral metabolism of glucose shows dynamic changes during development and after TBI. Adult cerebral metabolic rates of glucose (CMRglc) are achieved in the rat between 35-45 days after birth (75 µmol/100g/min),[16] and in humans by 16-18 years of age (26-27 µmol/100g/min).[17] These changes in the developmental profile directly influence changes in brain metabolism after TBI. Generally, after TBI there is a transient increase in CMRglc followed by a prolonged decrease in glucose uptake. It is important to note that CMRglc by positron emission tomography (PET) or 2-deoxyglucose autoradiography really demonstrate the uptake of glucose into brain cells up to hexokinase. It does not actually reflect glycolytic processing of glucose. However, the dynamic changes in glucose uptake demarcate 2 important phases. *In energy state 1 ("hyperglycolysis")*, there is an immediate

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increase in glucose uptake that lasts 3-6 h in animals and 7-10 d in humans. Increases in glutamate and ionic disequilibrium are also detected during this phase. The lactate pyruvate ratio (LPR) reflects the balance between glycolytic contribution to the mitochondria and the anaerobic processing of pyruvate to lactate. The interstitial pyruvate and lactate are thought to be in equilibrium with the cytosolic compartment.[18] Increases in LPR have been reported 1-10 d post TBI in numerous clinical studies and in experimental TBI. [19-20] Elevated LPR has been clinically documented after TBI in the absence of ischemia,[19] shown to reflect the degree of cerebral damage,[21] and is associated with poor outcome.[22] The increase in LPR under these conditions may reflect changes in the glycolytic glucose processing. *In energy state 2 (metabolic depression)*, there is a prolonged decrease in glucose metabolism, which is a hallmark response to brain injury.[23,24] During this second phase, there is a 9-12% increase in glucose shunting towards the pentose phosphate pathway.[25] Glycolytic processing of glucose requires a constant supply of nicotinamide adenine dinucleotide (NAD^+), which is decreased in the cytosol, thereby decreasing the carbon supply to the mitochondria.[26-27] A consequence of these acute changes in glucose processing results in a decrease in ATP after TBI.[28,29] Increased shunting of glucose towards the pentose phosphate pathway and/or impairments in glycolysis can decrease the production of pyruvate. Collectively these events disrupt the brain's energy production by decreasing carbon entry into mitochondria and creating a state of cerebral metabolic crisis that is thought to be reflected by the LPR. Ongoing cerebral metabolic dysfunction can lead to mitochondrial damage, energy failure, and neuronal loss after TBI. This dynamic TBI-induced metabolic pattern has been observed in various types of experimental TBI models, injury severities, age groups, and in human TBI. Timelines of CMRglc changes in animal TBI models and in human TBI will allow us to compare the species recovery profiles. Relevant findings are illustrated on Fig. 1.

Experimental studies

Experimental models of TBI have been used with ^{14}C or ^{18}F labeled 2-deoxyglucose autoradiography/microPET to determine the magnitude and time course of metabolic changes after injury. Mild fluid percussion injuries have shown immediate increase in glucose uptake and by 6 h glucose metabolic depression was detected, reaching their nadir

at 1 d and recovery by 7-10 d post injury in both the cortex and hippocampus.[23] A similar pattern of CMRglc changes were also observed with ^{18}F-deoxyglucose PET (FDG-PET) in adult mild fluid percussion injured rats with metabolic recovery by 16 d post injury.[30] Experimental data have shown that the magnitude and duration of CMRglc depression is dependent on injury severity. The temporal pattern appears to be similar in different models varying from fluid percussion to controlled cortical impact (CCI). While mild fluid percussion resulted in 20-30% decrease in CMRglc, more severe CCI injury models produced 50-70% CMRglc depression, which continued to remain depressed at 10 d post injury.[31,16] Severe fluid percussion injuries have also been shown to induce chronic hypometabolism lasting 3-6 months after injury.[32] Experimental studies have also shown that the magnitude and duration of CMRglc depression is affected by age. Postnatal day (PND) 17 and adolescent rats showed faster metabolic recoveries than adult rats following fluid percussion injury and CCI injuries especially in subcortical structures.[33,16]

Clinical observations

Traumatic brain injury induced changes in CMRglc have also been analyzed in humans using ^{18}FDG-PET. Mild TBI has been examined with FDG-PET by several groups, but many of these were at chronic time points, the result of motor vehicle accidents, and did not report actual CMRglc making it difficult to directly compare to experimental data.[34-35] These studies do report CMRglc depression in regions associated with specific cognitive functional tests. A comprehensive review of these studies has been compiled by Byrnes.[30] Moderate to severe TBIs have also been studied with PET, but as with mild injury studies, there is often a variety of ages, time points, averaging of cases, as well as an absence of time course profiles and reporting of metabolic rates.[30] One study did show recovery of global cortical CMRglc depression following mild/moderate (GCS 9-15) in adult human TBI patients by 3 months but 6-9 months after severe injury.[24, 36] Chronic CMRglc depression have also been reported after blast injuries, among comatose/vegetative severe TBI patients, and those with significant atrophy. [36-38]

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In animal studies, the nadir is reached within 24 h after impact, whereas the greatest CMRglc depression is attained within the first week in humans. CMRglc recovery after mild fluid percussion injuries is 10-14 d in adults and 21-30 d after CCI injury. These data suggest that the TBI-induced CMRglc depression and the recovery are ~15 times faster in rodents than in humans. The differences between the rat and human timelines should be considered when trying to determine the estimated time for human CMRglc recovery as well as the therapeutic window for clinical trials.

Significance of identifying the temporal pattern of CMRglc depression

From a metabolic point of view, the time of altered (depressed) CMRglc corresponds to the period of increased cerebral vulnerability. During this window of heightened cerebral vulnerability, repeated injury leads to substantially more severe and longer lasting symptoms in both experimental and clinical TBI. Accordingly, identifying the exact temporal relationship between rat and human CMRglc changes is important for civilian as well as military TBI, especially for mild TBI / concussion.

INFLAMMATION

Inflammation involving microglia and astroglia as well as chemokines and cytokines is a key pathological response to TBI. Importantly, neuroinflammation appears to be the link between the acute response to injury and the development of chronic pathological processes that can lead to late-onset chronic neurodegenerative processes such as chronic traumatic encephalopathy (CTE). Similar to its role in other diseases, inflammation is a double-edged sword. In addition to its mostly recognized detrimental effects, inflammation plays a critical role in limiting the extent of cellular damage and facilitating recovery and repair. The ability to identify the point when the beneficial phase of the inflammatory response turns detrimental, is important for timing potential treatments. Equally important is the identification of the molecular and cellular components related to the various phases of the neuroinflammatory process. How closely the temporal profile of neuroinflammation observed in experimental TBI matches the process detected in TBI patients is currently poorly understood. Excellent reviews detailing the chemo- and cytokine responses to TBI in preclinical as well as in clinical TBI studies are available;[39, 40]

however, they also illustrate the lack of temporal resolution in most of these studies. Outcomes between experimental and clinical TBI are likely only partly overlapping, thus knowing how the timescale found under experimental conditions correlate with the clinical process is important to establish the timing of the therapeutic intervention. Importantly, the neuroinflammatory process can be targeted with readily available anti-inflammatory drugs, if we know the therapeutic window and the molecular targets. However, no pharmacotherapies that were proven successful in rodent models of sepsis or trauma have been similarly efficacious in clinical trials. One of the potential factors that may have contributed to these failures is that the therapeutic window was missed due to the different timelines between the rodent and human inflammatory processes. Relevant findings are illustrated on Fig. 2.

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Experimental studies

How closely rodent models of inflammatory diseases mimic the human condition is the focus of intense and contentious debate. Comparing the genomic responses of human conditions with their relevant mouse models of trauma, burn, and endotoxemia has shown that at the whole genome level, there is very little similarity in human versus rodent transcriptional response and signaling pathways involved in the pathological response.[41] However, narrowing down the selection to genes involved in a subset of inflammatory responses showed more similarities in transcriptional response between rodent models and human cases. Importantly, the studies concurred that the temporal pattern of rodent versus human genomic response is very different. An important measure of the temporal pattern of the genomic response to inflammation is the time interval for the expression pattern to normalize. The time interval to return to normal (baseline) pattern of gene expression in the human burn cases averaged ~5 months (1 month to 1 year). In the rodent model, average recovery time was 3 days (1 day up to 7 days) suggesting a ~50-fold faster process in rats. Using a similar definition (return to normal pattern of gene expression) the recovery rate was found to be ~30-fold faster in the rodent model of trauma than in humans. Interestingly, the recovery rate was similar for endotoxemia, around 1 day in both humans and rodents. However, the genomic responses themselves to trauma, burn, and endotoxemia were substantially different in the two species. [41]

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Longitudinal studies tracing microglial response to injury by PET imaging enables comparison of the neuroinflammatory timelines between human cases and rodent models of TBI. The most comprehensive experimental longitudinal study by Wang et al. (2014) used a combination of PET imaging with [^{18}F]-714,[42] a specific ligand to the 18 kD translocator protein (TSPO) for activated microglia, ex vivo autoradiography, and immunohistochemical verification of the PET and autoradiography data. An important caveat is that the various PET ligands also bind to TSPO in (activated) astrocytes, and thus TSPO should be considered a marker of activated astro- and microglia. [43, 44] The study showed that the neuroinflammatory response, as detected by all three methods, peaked at around day 6 after controlled cortical impact (CCI) injury. TSPO expression declined by day 10 and returned to the levels measured in the sham treated group by day 28. This timeline was confirmed by in vitro autoradiography and also by immunohistochemical staining of activated microglia. These findings are in agreement with previous PET studies using different ligands ([^{11}C]PK11195 and [^{18}F]FE-DAA1106) for activated microglia. [45] These studies also showed maximum neuroinflammatory (microglia) response around day 6-10 post-injury that returned to normal levels by around 30 days post impact. It should be noted that activated astroglia as well as peripheral macrophages also bind TSPO, thus the protein is not entirely microglia specific. However, other cell types are also involved in mediating the inflammatory response after TBI.

Clinical observations

There are significant challenges in comparing the temporal sequence of inflammatory events in rodent models vs. human TBI. A key consideration in human neuroinflammation research is defining 'inflammation' in the first instance. If 'inflammation' is considered as the endogenous response to injury, it has to cover a wide spectrum of both humoral and cellular events that span both innate and adaptive immunity.[46] Access to a number of biological compartments (plasma, CSF, brain extracellular fluid) has allowed researchers to measure a range of inflammatory biomarkers following TBI but each of these biocompartments has its limitations with respect to interpretation. For example, plasma sampling is confounded by extracranial trauma; CSF sampling is usually intermittent and the CSF compartment may reflect a sump

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for clearance of inflammatory substances;[47,48] and microdialysate sampling from the brain extracellular space is limited by the proportion of a soluble mediator that crosses into the microdialysis catheter which varies with molecular weight, charge and methodological variations in microdialysis technique.[49] The absolute concentrations of a given mediator are therefore difficult to relate directly to equivalent rodent studies when extrapolating data on the range of concentrations that may inflict neuronal injury. Human TBI research is necessarily opportunistic and very early events that occur in the clinical resuscitative phase are beyond the reach of clinical studies that require sampling of biological fluids. However, an early post-mortem study carried out on patients succumbing to traumatic brain injuries at various intervals has empirically demonstrated that at the mRNA and protein level, early pro-inflammatory drivers of innate inflammation, including IL1β and TNF, are significantly increased in the human brain within 24 h after TBI.[50] Following this early period, once patients are admitted to specialist neurointensive care, invasive monitoring allows a more detailed assessment of inflammatory biomarkers. Interestingly, serendipitous monitoring within the first 24 h picks up this early pro-inflammatory signal while subsequent monitoring time points do not.[47] Beyond this time frame, a strong IL6 signal has been detected within the first 24-72 h in both microdialysate and CSF.[47,51,52,53] IL6 is an interesting cytokine as it may have both damaging and protective properties depending on the context in which it is produced.[54] In absolute terms, IL6 is produced at concentrations several orders of magnitude higher than IL1β and TNF,[47] which may introduce a selection bias into the literature as it is easier to detect analytically.

Alongside these key cytokines, several chemokines are detected and likely reflect recruitment or activation of inflammatory cells. GRO-alpha and IL8 are produced in the first 24-48 h and may reflect neutrophil recruitment. Beyond this, chemokines related to microglial activation, recruitment, activation, and expansion are found in an overlapping time frame including monocyte chemoattractant protein 1 (MCP1/CCL2), regulated upon activation normal T cell expressed and secreted (RANTES/CCL5), and granulocyte colony stimulating factor (GCSF). When comparing studies, it is important to interpret the data in relation to time of injury rather than the time that monitoring or sampling commences. A

late IL10 response at around day 4-7 may subsequently reflect a shift in microglial activation towards a reparatory or regulatory phenotype.[47,55]

Several authors have considered the role of these inflammatory biomarkers in TBI pathophysiology and correlated concentrations with specific sequelae rather than considering the temporal sequence per se.[56,57] Several studies relate this acute response to chronic sequelae,[58, 59] and outcomes at 6 months following TBI,[60, 61] suggesting that this acute response plays a mechanistic role in inflicting injury.

In the chronic phase, there is evidence that the plasma cytokines (IL1β, IL6, IL8, and IL10) are raised for over 3 months following injury and this may relate to clinical outcomes, albeit demonstrated in small groups of patients.[61] While no comprehensive study similar to Wang et al. (2014) is available for human TBI patients, [42] published works indicate that the inflammatory response, as defined by the presence of activated microglia, persists for many years after the injury. Using the ligand [^{11}C]PK11195, Ramlackhansingh et al (2011) have found activated microglia persisting in patients up to 17 years after TBI.[62] Interestingly, the patients showed persistent activation of microglia distant from the lesion site, raising the possibility that this represents diaschisis or a long-lasting reorganization that underlies plasticity and recovery, distinct from the original injury. These findings are consistent with data obtained from post-mortem studies showing long-term presence of activated microglia ~16 years after TBI as well as studies performed in non-human primates showing substantial neuroinflammation one year after injury (the latest time point studied) indicating that TBI induced neuroinflammatory response can be 4 to 30 times faster in rats than in humans (Summarized in Fig 2). An additional and often underappreciated aspect of inflammation is the adaptive immune response.[63] There is a paucity of clinical studies in this context but information about the additional phase of autoimmunization over a 10 to 14-day typical study period is missing.

Significance of identifying the temporal pattern of inflammation

The importance of defining and targeting an appropriate therapeutic window has been well-described in the TBI literature. Inflammatory processes are a promising target in that the time frames described above span several days, providing ample opportunity for

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clinical intervention. Nevertheless, there are several caveats that must be considered. A relatively small group of cytokines and chemokines has been measured in the clinical TBI literature,[46] and as these mediators act in complex cascades and show a high degree of collinearity it is impossible to draw conclusions about causality from observational correlative studies. When choosing putative therapeutic targets, an assessment of the biological consequences of administration should provide confirmation that the drug is exerting the desired effect.[64] Furthermore, with the heavy bias in the literature towards assessment of soluble mediators, there is ambiguity over the cellular origin of the measured cytokines and chemokines. Microglia, astrocytes, neutrophils, and neurons are all capable of producing these mediators.[46] Considering the functional heterogeneity of these cells, immunomodulatory pharmacotherapies may require delivery at specific times to target distinct cellular subsets to have a beneficial effect.

From a therapeutic point of view, the onset and extent of the inflammatory response to injury represents a window for anti-inflammatory therapies. Given the abundance and availability of anti-inflammatory therapies that target specific molecules of the inflammatory process, it is critical that the temporal differences between rodent and human inflammatory response are taken into account when trying to identify the therapeutic window for anti-inflammatory molecules in clinical trials.

VASCULAR PERMEABILITY CHANGES AND EDEMA

A frequent consequence of TBI is altered water homeostasis manifested as cerebral edema, particularly in severe TBIs. Cerebral edema is broadly classified as either vasogenic or cytotoxic (cellular) edema. The former occurs due to increased vascular permeability to proteins, resulting in increased water content of the extracellular space, while the latter occurs due to water accumulating in the intracellular space because of abnormal ionic distribution caused by increased ion channel permeability and/or inefficiency of ion pumps. Although the two forms of edema are considered distinct, in reality one form usually leads to the other and the two mechanisms often overlap. Indeed, vasogenic edema following TBI has been found to be permissive for cellular edema.[65] Generally, vasogenic edema is more prominent in the white matter after CNS injury while cytotoxic

edema dominates in the grey matter.[66] The development of edema is initiated by vascular and/or metabolic events, with inflammation contributing to both onset and persistence of cerebral edema. Relevant findings are illustrated on Fig. 3.

Experimental Studies

Following experimental TBI, there is a rapid edema formation within the first hour which diffusion-weighted magnetic resonance (MR) imaging studies have shown as vasogenic edema.[67,68, 69] The presence of a blood brain barrier (BBB) that is more permeable to vascular proteins has confirmed the vasogenic nature of the edema.[70] The initial increased permeability of the BBB to large vascular proteins was, however, limited to the first few hours after TBI with the BBB becoming increasingly less permeable to vascular proteins and normalizing over the next week.[70,71] As the BBB became less permeable, cytotoxic edema became more apparent, signifying a net movement of water from the extracellular to the intracellular compartment. Maximum cytotoxic edema was observed between days 7 and 14 after TBI, resolving thereafter.[68, 69]

Some reports have suggested a second increase in BBB permeability to vascular proteins on day 3,[72] which may lead to a surge in intracranial pressure (ICP). While not universally observed in all models of experimental trauma, this second opening of the BBB is consistent with the reported delayed tight junction breakdown,[73] most likely mediated by activation of matrix metalloproteinases. This is in contrast to the functional transcytotic upregulation of albumin transport, with tight junctions intact, seen in the early phase after TBI,[73,74] and indeed as early as 3 minutes after TBI.[75] Such delayed opening of the BBB suggests the involvement of brain metabolic processes and inflammation that are initiated by the injury in contrast to a largely neural/vascular interaction in the immediate post-injury period.[76]

Clinical observations

Studies of edema and BBB permeability in patients have been difficult given the inherent instability of TBI patients, particularly those that are severely injured, and when imaging studies are potentially complicated by mechanical ventilation. Nonetheless, there have been several studies, albeit most began many hours to days after the primary event.

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Both vasogenic and cytotoxic edema have been reported after TBI using magnetic resonance imaging (MRI) techniques,[77] although a predominance of cytotoxic edema is noted as time progresses with any subsequent tissue loss being replaced by extracellular water.[78] MRI-detectable abnormalities in BBB permeability were more common in pericontusional areas after 2 days, and frequently did not become apparent until 6 days after injury.[79] This is consistent with the strong association between hemorrhage and activation of metalloproteinases, and the observation that persistent increases in metalloproteinases have been reported after 4 days in clinical TBI.[80]

An alternative to imaging studies is the use of CSF- and serum inflammatory biomarkers or albumin distribution to indirectly determine increased BBB permeability.[81, 82] These studies have confirmed the MRI findings indicating BBB dysfunction in the days following TBI. Irrespective of the technique used, it has been noted that patients with increased BBB permeability have poorer outcomes,[83] with those with more severe disruptions of BBB after 3 days being predisposed to developing substantially higher ICP values.[81] The development of increased ICP in the presence of a permeable BBB implies a continuing role for vasogenic edema after clinical TBI.[83] BBB permeability is generally thought to return to normal within several weeks, although persistent BBB dysfunction has been reported months to years later in patients who develop post-traumatic epilepsy and even after mild TBI. [84]

Consistent with experimental studies, increased transcytosis of vascular protein while endothelial tight junctions are intact has been reported in the first hours following human TBI.[85] However, this transcytosis persists for up to 2 years post-injury.[86] More severe edema at these later time points was also associated with opening of the tight junctions, perhaps reflecting the involvement of metabolic processes and inflammation initiated by the injury (once again) at these delayed time points.

Significance of identifying the temporal pattern of vascular permeability and edema

While the immediate functional vascular response of rodents to TBI is very similar to that of humans, subsequent metabolic and inflammatory responses that underlie BBB permeability and edema formation at later timepoints is far more persistent in human TBI.

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Indeed, the majority of studies in experimental animals describe BBB restoration within a month after CNS lesions. Conversely, increased BBB permeability and edema formation has been noted years after the traumatic event in humans. [87-89] Not only would this result in an extended period of injury development with a consequent lack of functional recovery in humans, but it also implies that the therapeutic opportunity for intervention at the metabolic and inflammatory level extends well beyond the weeks assumed from rodent studies. Important caveats include the type and severity of the injury. Severe TBI is rarely modeled in rodents and few studies focus on penetrating / ballistic injuries. Moreover, the methods used to monitor BBB function in rodents and humans are in most cases vastly dissimilar, contributing in part to the limited understanding of the temporal differences in vascular permeability that exist between the two. Additional difficulties include the unknown onset and extent of neovascularization after TBI observed in both rodent models and also in human TBI cases.[90] Newly formed, immature vessels have increased permeability and we currently do not know how long it takes until de novo vessels develop fully functional BBB in the rodent models versus in human TBI cases. Recent study has shown that in a rodent model of mild TBI, the inflammatory process can trigger revascularization as part of the endogenous repair program repair but only within a specific, delayed window period during which would-healing macrophages promote angiogenesis. [91] This finding further underlines the critical importance of understanding the differences between rodent and human pathological processes.

AXONAL INJURY

Axons in the CNS are especially vulnerable to the impact of physical forces; therefore, traumatic axonal injury (TAI) is a major pathological change after TBI.[86] Severe kinetic impact can shear or break axons resulting in primary axotomy. Less severe impact results in membrane disturbances, dislocated ion channels, and dislodging of the axoskeletal network with subsequent disruption of axonal transport. These initial (primary) changes, especially when combined with prolonged ionic and metabolic perturbations during the secondary injury process, may lead to axonal swelling, retraction bulbs, and to neuronal cell death. Diffuse axonal injury (DAI) that predominantly affects white matter (WM) structures is a clinical entity.[92-94] In experimental studies the term DAI is mostly used for

large animals with gyrencephalic brains. TBI-induced axonal pathology is more localized in the rodent brain and the term “traumatic axonal injury” (TAI) is frequently used. The pathomechanism of DAI and TAI is likely very similar if not identical so the difference between DAI and TAI is probably only in the semantics. DAI majorly contributes to the clinical manifestations of TBI, loss of consciousness and coma in the acute phase of severe TBI, and cognitive and functional impairments in survivors of severe TBI. While the initial physical impact and the associated metabolic changes result in acute disruption of axonal structure and functions, there are also delayed axonal pathologies. They evolve over time and may last weeks and months after the initial impact as other pathologies of the secondary injury processes, metabolic changes or inflammation can affect the evolution and resolution of axonal injuries.

When matching the temporal course of central axonal injury in rats and humans it must be understood that DAI is a large animal disease expression and the term generally used for gyrencephalic animals. The rodent has relatively less white matter and animal models producing TAI may not be immediately comparable to the distribution of local lesions in larger species. Additionally, the rotational forces required to produce human DAI like changes, may be hard to reproduce in small volume skulls and in animals with few or no gyri. Impact models of rats where brain parenchyma will have little rotational mass and possibly different and more transient loss of consciousness responses to DAI injury than humans, has led to debate on how comparable rodent models are to the human condition. [95]

Traditionally, axonal injuries are mostly identified by using various histopathological methods, using beta amyloid B precursor protein (APP) immunohistochemistry or silver impregnation. APP accumulates due to the disruption of axonal transport system and APP-immunoreactive structures are indicative of axonal injury.[96-98] Variations of silver impregnation methods visualize degenerating (or perturbed) membranes including axons and claimed to be more sensitive assay for TAI (or DAI) than APP immunohistochemistry.[99, 100] It should be noted that the two methodologies detect distinct perturbances in axonal morphology and function. In clinical settings, DAI is diagnosed by in vivo imaging, primarily by DTI which is not frequently used in rodent

studies making the comparisons between human and rodent timelines challenging. Various modalities of DTI indicate white matter changes and the oft accompanying micro-hemorrhage can be seen with Susceptibility Weighted Imaging (SWI).[101-103]

Structural elements of the axoskeleton, the low, medium and high molecular weight neurofilament proteins (NF-L, NF-M, and NF-H, respectively) and tau are highly enriched in the nervous tissue. Injury increased levels of these proteins in the blood (and CSF) have shown correlation with the onset and extent of axonal injury both experimentally and clinically enabling improved comparisons between clinical and experimental data.[104-108] Injury-induced disintegration of the axoskeleton is mediated by calcium-dependent enzymes calpain and caspase-3. These enzymes are activated by the sudden rise in intracellular calcium levels and the activated enzymes then breaking down spectrin. Axonal damage mediated by calpain and caspases is reflected in patterned breakdown of all spectrin breakdown characterized by known protease cleavage points, and calpain and caspase activity can be attributed based on predictable lengths of αII spectrin breakdown product (SBDP) produced after cleavage.[109, 110] αII spectrin is a component of the cytoskeleton and connects axolemmal components to the presynaptic terminal, stabilizing the nodal structure of myelinated axons. Calpain lyses the spectrin cytoskeleton into SBDP 145 and SBDP 150, and caspase-3 renders SBDP120.[111] Consequently, the relative contribution of each of these calcium-dependent proteases makes cytoskeletal breakdown potentially distinguishable. With significant increases in intracellular calcium immediately following injury, calpain activity rises between 8 and 12 h after injury peaking at 24 h before decreasing to lower levels by 3 days. The time course of primary axonal damage caused by the physical impact can be further extended by hypoxia [112] that -among its other effects- increases intracellular calcium levels. Relevant findings are illustrated on Fig. 4.

Experimental studies

Studies using rotational or acceleration/deceleration forces to cause axonal injury have shown that TAI occurs within minutes in the rat as shown by using dextrane as indicator of increased axolemmal permeability. Impaired axonal transport can be detected

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as early as 15-30 min after TBI using APP immune-fluorescence.[113, 114] Intra-axonal changes begin immediately after the impact and axonal swelling and detachment develops within the first day (12-24 h) after injury. APP accumulation in the rodent TBI model can be detected up to 14 days after injury.[115] Hellewell et al. demonstrated a maximum number of retraction bulbs at day one with a gradual decrease at 14 days post injury.[112] A key study by Mac Donald et al (2007) established the temporal pattern of TAI in a mouse model of CCI using a combination of histology, electronmicroscopy, and DTI.[116] The study showed that the numbers of both APP and NF-L positive axonal varicosities increased dramatically during the acute phase of injury defined as between 4-6 h and 4 days post impact followed by a substantial decline during the subacute phase -1 week to 1 month. Importantly, quantitative analysis of DTI parameters - axial diffusivity (AD), radial diffusivity (RD) relative anisotropy (RA) and mean diffusivity (ADC) - showed close correlation with the histological findings. AD decreased during the acute phase and returned to control levels by 1 month while RA levels remained reduced during both the acute and subacute phase.

Li et al. saw an increase in APP stained axonal varicosities up to 72 h. They also found that the temporal profile of the immunohistochemical staining correlated very closely with that of changes detected by DTI. Both fractional anisotropy (FA) and AD were significantly reduced in the brain regions affected by the trauma between 3 to 72 h.[117] These findings are important because they suggest that changes detected by DTI, reduced FA and AD can reflect the histological underpinning of TAI.[116, 118] There appears to be evidence of late demyelination in the rat at 4 weeks,[116] as compared to humans where demyelination is still detectable at 1-4 years after the injury. As discussed above, activated glia cells and macrophages, indicating inflammatory processes, have been shown to peak around day 3-7 post-injury in the rodent model.

Clinical observations

DAI is a common pathological feature of closed head TBI, especially severe TBI.[92, 93, 119] Clinically, DAI is characterized by early loss of consciousness and often exhibits disproportionately limited focal lesions in the brain on computed tomography scans. Nonetheless, DAI can be the sole pathology that causes coma in severe TBI patients.[120, 121]

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The brain regions that most commonly show DAI injury are the subcortical grey-white matter interface near the frontal and temporal lobes, corpus callosum, and brainstem.[122] DAI results in impaired cognitive, autonomic, and sensory functions depending on the extent of lesions and affected brain regions, with clinical severity generally increasing the deeper the structure(s) involved. The severity of DAI can be classified from Grade I (mild) characterized by subcortical and internal/external capsule lesions; Grade II (moderate) in addition to the above regions the damage affects the corpus callosum (CC); Grade III (severe) with additional lesions in the brainstem.[120] As with other pathobiologies triggered by TBI, the primary DAI is instantaneous while the secondary DAI can last for an extended period of time.

It was long believed that DAI was part of the acute to subacute pathological response to TBI; however, there is strong indication that DAI can persist years after the insult. The original focus on immediate shearing forces and retraction bulbs of severed axons followed by Wallerian degeneration has been expanded by a growing appreciation of the extended metabolic disturbance initiated.[123, 124]. This is thought to involve calcium dysregulation, disruption of ionic homeostasis, energetics and cytoskeleton breakdown leading to axonal swelling and glial activation, followed by an extended period of inflammation, possibly lasting for years.[124] Histological evidence of DAI has been seen in human forensic material as early as 35 minutes after injury,[119] and axonal swelling and beading size has been seen to progress up to 24-48 h,[125] where milder trauma exhibits a slower swelling. Additionally, this has been seen to be a non-homogeneous process with much local variation. The temporal progression of DAI on MRI has been investigated but the great variability in timing within and between MRI studies makes a clear sequential picture hard to delineate. TBI is however associated with general WM loss over time, in excess of the natural loss seen in age matched controls. The time course of human DAI as seen on MRI has been suggested to be divided into four stages: acute (< 24 h), early sub-acute (1-13 days), late sub-acute (14-21), and chronic (> 21 days) where early lesions are generally superseded by local edema in the sub-acute phases and by general white matter loss in the late chronic stage.[126-129] DTI detection of DAI focuses on non-uniform or directional flow (anisotropy) along tracts and is quantified as fractional anisotropy or mean

diffusivity. Early changes over the first week may thus be confounded by edema, exhibiting either restricted (intracellular, days) or increased (extracellular, 1 to 2 weeks) water movement. Late chronic stages may show a continuation of WM loss for years. Grey matter loss may be a faster process and has been seen to diminish at 3 months,[130] whereas late volume loss appears to be WM-related.[131] Bendlin et. al. identified continued WM loss at one year post trauma.[132] In a follow-up study of this cohort of mild-severe TBI patients, WM changes in the corpus callosum using DTI were seen to progress most the first year after trauma but continue up to four years.[131] Importantly, reactive microglia were also detected in white matter tracts that showed axonal pathology, suggesting that persistent axonal / white matter pathology may be the result of a chronic neuroinflammatory process.[133] Interestingly, in this late follow-up, radial diffusivity (water movement perpendicular to axonal tracts) was more affected than axial diffusivity (along tracts), leading the authors to conclude that late pathology was driven more by myelin changes than axonal changes. However, a recent study that followed moderate to severe TBI patients longitudinally for at least at 3 time points,[134] from the acute through chronic phase (medians: 1 week, 7 and 21 months), found late alterations to be driven by changes in both radial and axonal diffusivity. Additionally, the inflammation process may persist for years.[62, 135] Although candidate protein biomarkers exist, none have yet been established to follow the evolution of DAI. Neurofilament light (NF-L) is seen to increase for days to weeks after injury and levels have been related to outcome. However, NF-L levels have not been clearly related to MRI findings. In aggregate, the time course of DAI in humans is a process with early and late pathophysiological components where changes can be ongoing for years.

Significance of identifying the temporal pattern of axonal injury

The temporal pattern of axonal injury appears to be comparable between rodents and humans during the acute phase of the injury. It is the sub-chronic and chronic phase of TBI where the temporal patterns differ. In the absence of a new physical impact, the late phase of axonal injury is likely caused and maintained by other TBI-induced metabolic and inflammatory abnormalities.[93, 97, 133] Mitochondrial abnormalities, oxidative stress, and lipid peroxidation can render structurally intact axons functionally impaired along with

elevated intra-axonal Ca++ level. Neuroinflammation that can persist for years in humans after TBI can cause similarly lasting, chronic axonal degeneration.[136]

Given the limited ability of the mammalian CNS to regrow lost or dysfunctional axons, the most beneficial therapeutic interventions are likely aimed at restoring/normalizing cerebral metabolism, buffering neurotoxicity, and modifying the neuroinflammatory process.[93] There are important caveats that include the relative low-fidelity modeling of axonal injury in rodents due to anatomical differences discussed above and the unknown contributions of the distinct secondary injury processes to the overall response to injury. These, in combination with the type of injury (i.e., closed head or penetrating, focal or diffuse) can majorly determine the relative contribution of axonal injury to the overall secondary injury process. DAI in clinical practice is a complex injury that is frequently associated with bleeding or microbleeding and often accompanied by other lesions. Bleeding, an indicator of vascular injury detectable by imaging, is an important (if not critical) comorbidity that further complicates the secondary injury process. Additionally, aging can affect the outcome of axonal injury. There are indications that the vulnerability of axons to physical impact is affected by age and age can affect recovery. [137-139]

The duration of ongoing axonal pathology reflects the period of increased cerebral vulnerability, i.e., when re-injury can trigger disproportionally severe consequences by rendering changes to axons irreversible and resulting in axonal losses. Rehabilitation therapies, when optimally timed, can result in substantial functional recovery. Better knowledge of the duration and trajectory of axonal pathologies in rodents versus humans will help to identify the window for such interventions. In summary, the experimental relationship between human and rodent DAI/TAI may not be immediately comparable, but evidence suggests that the temporal changes in the acute phase may be similar between rodents and human cases. However, the late and chronic phases of DAI suggest that the process may be 10-12 times faster in rodents than in humans.

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DISCUSSION and RECOMMENDATIONS

TBI is a dynamic condition even in its mildest form; clinical symptoms and underlying pathobiological responses substantially change over time. Neuromonitoring data best illustrate the dynamics of time-dependent changes in the onset and extent of select pathobiological responses after severe injury.[140-142] Despite the importance of identifying "therapeutic windows" for various interventions, only a fraction of studies addresses the temporal aspect of the TBI molecular sequelae. Of the approximately 38,000 publications found in PubMed, using "traumatic" AND "brain" AND "injury" as search terms, only 1,626 (4.3%) covered (some) aspect of time-dependent changes of the disease. Of the approximately 12,000 clinical studies, using the search terms "traumatic" AND "brain" AND "injury" AND "clinical," only 486 (~4%) addressed (some) aspect of time-dependent changes (using these additional search terms of "time" OR "temporal") of the TBI disease process. The proportion is similar in preclinical/experimental TBI, only ~450 of the total publications (~2%) addressed (some) temporal aspects of TBI. Because of the simplicity of the PubMed search, these percentages are not exact but clearly illustrate the relative lack of data that would help to determine the temporal profiles of pathobiological changes, and consequently help to identify the therapeutic windows for various pharmacological treatments.

Recommendation #1: We recommend conducting more longitudinal studies, collecting and reporting data at multiple post-injury time points. This will increase the data density necessary to characterize the profile and evolution of injury-induced changes. Specifically, data should be collected at 2-3 three times the rate of the expected information content of the temporal signal studied, as expected by the experimental hypotheses (Nyquist frequency).[143] In the absence of such a hypothesis, and without the possibility of high frequency sampling, an initial sample frequency along a geometric time series multiple of 2 (0.5, 1, 2, 4, 8, 16 time-units) can help to identify the required sampling frequencies/ time-points for a specific early biomarker.

Developing an algorithm for "translating time" between rodent and human TBI pathobiologies is further hindered by two interrelated issues: different classifications of

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injury severity and different outcome measures in rodent TBI studies vs. clinical cases. Classification has played an essential role in our understanding and treatment of diseases. Subdividing complex diseases into smaller, more homogeneous sets of pathologies has increasingly enabled accurate diagnosis, prognosis, and the development of specific treatments. However, TBI greatly lags behind other much better characterized diseases, such as cancer, when it comes to classification. Cancer is now subdivided into smaller subclasses, each with more homogenous molecular pathologies, increasingly enabling personalized, evidence-based treatments. TBI is an acquired, sudden-onset disease with dynamically changing pathobiological processes. Therefore, knowing the identity, onset, and extent of the various pathobiological processes is fundamental for developing a molecular-level classification system and evidence-based treatments for TBI. Current classification is still based on rather subjective clinical observations, and neuroimaging findings to a lesser extent, but not on molecular criteria. Experimental TBI studies generally lack even this subjective classification system. In clinical TBI, the Glasgow Coma Scale (GCS), length of loss of consciousness (LOC), alteration in mental/conscious sate (AOC), and post-traumatic amnesia (PTA) are used for classification. Due to their ease of use, practically every clinical TBI report contains GCS (and some LOC, AOC, and PTA) scores that are used for triaging, assessing disease progression, and selecting patients for clinical trials and epidemiology studies.[144-145] Similarly simple and subjective tests, such as the Glasgow Outcome Scale (GOS), are used to assess outcome and the efficacy of the pharmacological or other intervention in most clinical trials. In contrast, most experimental TBI studies do not report injury severity; available neurobehavioral classifications, for example the Neurology Severity Scale (NSS),[146] or the duration of post-injury apnea, regaining of the self-righting reflex, and so forth are rarely used and/or reported. Although the use of anesthesia is a major confounding factor in experimental TBI, classifying injury severity using standardized neurobehavioral assessments could provide critical information to better compare between experimental and clinical TBI cases.

Recommendation #2: We recommend that relevant organizations, such as funding agencies and scientific journals, encourage or even require the reporting of NSS or similar acute assessment of neurobehavioral deficits after experimental TBI. This simple step,

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combined with increased identification and utilization of clinically-relevant, molecular-level proxy outcome measures (e.g., biochemical or imaging markers (see Recommendation #3 below), will increase our ability to directly compare injury severity between clinical and experimental cases and will increase our ability to more directly compare the temporal patterns of various molecular pathologies as a function of injury severity.

TBI triggers an array of pathobiological responses, depending on the type and severity of impact. To better understand the temporal differences between rodent and human TBI-induced pathological processes, the identity, onset, intensity, and extent of the various injury-induced pathological changes should be determined using objective, quantifiable outcome measures that are identical (or at least comparable) to clinical TBI studies. In clinical TBI research, the severe form of TBI is the most studied, and the disease process is best documented. Severely injured patients are admitted to neurointensive care and are evaluated and constantly monitored by neurocritical care specialists.[147-149] Neurocritical Care monitoring provides real-time data on physiological parameters, such as blood pressure, breath, heart and pulse rates, blood oxygen saturation, and intracranial pressure, in addition to local and global methods of assessing cerebral blood flow (CBF), oxygenation, and metabolism. No equivalent preclinical data exists, although there are simple tools available for the physiological monitoring of rodents.[150, 151] For ethical reasons, modeling severe TBI in animal models is virtually non-existent; therefore, we cannot really compare the most data-rich clinical studies (severe TBI), where the main outcome measure is survival, to experimental TBI data. Conversely, experimental studies mostly model mild to moderate forms of TBI, making these types of preclinical studies the most data rich. Clinical studies are conducted on heterogeneous populations where confounders, such as age, gender, comorbidities, medications, and pre-hospital/hospital care, are not replicated in the animal-based experimental setting. This heterogeneity is hard to overcome and resolve in an experimental setting, but at least the effects of age and sex may be investigated. Moreover, the most clinically studied, albeit least frequent forms of TBI (i.e., moderate to severe) are typically part of polytraumas that cause other organ injuries, bone fractures, and so forth. In experimental TBI, however, practically every model only targets the head or brain.

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Recommendation #3: Funding agencies, scientific journals, and other regulatory bodies should encourage or even require the use of clinically-relevant outcome measures in experimental TBI such as neurobehavioral monitoring, structural imaging, and biochemical markers in the peripheral blood and/or cerebrospinal fluid (CSF) at multiple post-injury time points. In combination with outcome measures that are unique to experimental TBI research, e.g. histopathology these clinically relevant data will help to better identify the pathological processes and their temporal patterns.

Experimental and clinical TBI studies generally use different tools and methodologies to identify underlying pathologies. In the absence of identical or at least comparable outcome measures, the temporal patterns of pathobiological processes in rodents versus humans cannot be precisely determined. In vivo imaging, mostly computed tomography (CT) but also MRI/DTI, are key determinants of structural damage in the clinical setting. Serial neuroimaging is often used to assess disease progression. However, imaging is currently infrequently used in pre-clinical TBI studies to generate enough data for comparison, and serial imaging studies are extremely rare in experimental TBI research. Structural information in experimental TBI has been overwhelmingly obtained via terminal histopathology, which is not very useful for determining the evolution of pathological changes in clinical TBI. The proxy outcome measures that are most commonly used in both experimental and clinical TBI are blood-based protein biomarkers. However, serial sampling in experimental TBI is rarely performed and in the absence of comparable injury severity classifications in experimental and clinical TBI, and lack of established relations to functional outcome, the comparison of available protein biomarker data is challenging.

Translating time? Based on the available data, some of it discussed in this review, it appears that there is no universal "X factor" that can be used to "translate time" between rodent and human pathobiological changes. Normal biological and pathobiological processes seem to run faster in the rodent than in the human.[14] Normal metabolic, physiologic and developmental processes, such as RNA turnover, respiratory rate, gestation, and senescence, are about 2.5 to 84 times faster in rodents than in humans. Thus, 1 human day can roughly equal 2 rat hours during gestation or 0.3 rat hours during the period of sexual maturity. How can we then take this "time factor" into consideration

when attempting to model neurodegenerative diseases like Alzheimer's or CTE in rodents when the life expectancies of the two species are so vastly different? As illustrated above, the rate for rodents can be 15 or 100 times higher, respectively, in TBI-induced changes in CMRglc and neuroinflammation. In other words, there is no universal conversion rate.

The most probable way to successfully "translate time" is to use a big data (BD) approach.[152, 153] However, successful BD approaches are critically dependent on the volume of data. In theory, BD could generate new knowledge by mining the available ~40,000 legacy TBI publications. Neuro-linguistic programming (NLP) is rapidly improving and can be used to identify repeatable patterns such as relationships and correlations between the temporal patterns of rodent and human pathobiological processes. However, it appears that published TBI data is not (yet) sufficiently big for BD.[154] It has been calculated that only a fraction of the data generated during experimental or clinical TBI studies is ever published, deposited, or made available in databases or other archives.[155] Conservative estimates put the amount of unpublished, so-called "dark data," to more than 50% of all data generated. Accordingly, publications only represent a fraction of all data and importantly, the published data is typically "curated" (i.e. "selected" from a much larger pool of data) making the BD approach prone to subjectivity and bias. Efforts by the NIH and other agencies, requiring the use of common data elements and the deposition of all experimental data, will increase the availability of experimental data for mining and the accuracy of potential algorithms that could be used to "translate time." Such a BD approach could and will go beyond translating time, as it will enable more precise predictions of relationships between various pathological processes, species, and diseases, thus guiding hypothesis generation and clinical trials based on compounded and weighted evidence.

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REFERENCES

1. Skolnick, B.E., Maas, A.I., Narayan, R.K., van der Hoop, R.G., MacAllister, T., Ward, J.D., Nelson, N.R. and Stocchetti, N. (2014). A clinical trial of progesterone for severe traumatic brain injury. N Engl J Med 371, 2467-2476.

2. Wright, D.W., Yeatts, S.D., Silbergleit, R., Palesch, Y.Y., Hertzberg, V.S., Frankel, M., Goldstein, F.C., Caveney, A.F., Howlett-Smith, H., Bengelink, E.M., Manley, G.T., Merck, L.H., Janis, L.S. and Barsan, W.G. (2014). Very early administration of progesterone for acute traumatic brain injury. N Engl J Med 371, 2457-2466.

3. Schumacher, M., Denier, C., Oudinet, J.P., Adams, D. and Guennoun, R. (2015). Progesterone neuroprotection: The background of clinical trial failure. J Steroid Biochem Mol Biol.

4. Stein, D.G. (2015). Embracing failure: What the Phase III progesterone studies can teach about TBI clinical trials. Brain Inj 29, 1259-1272.

5. Kochanek, P.M., Bramlett, H.M., Dixon, C.E., Shear, D.A., Dietrich, W.D., Schmid, K.E., Mondello, S., Wang, K.K., Hayes, R.L., Povlishock, J.T. and Tortella, F.C. (2016). Approach to Modeling, Therapy Evaluation, Drug Selection, and Biomarker Assessments for a Multicenter Pre-Clinical Drug Screening Consortium for Acute Therapies in Severe Traumatic Brain Injury: Operation Brain Trauma Therapy. J Neurotrauma 33, 513-522.

6. Deselms, H., Maggio, N., Rubovitch, V., Chapman, J., Schreiber, S., Tweedie, D., Kim, D.S., Greig, N.H. and Pick, C.G. (2016). Novel pharmaceutical treatments for minimal traumatic brain injury and evaluation of animal models and methodologies supporting their development. Journal of neuroscience methods 272, 69-76.

7. Diaz-Arrastia, R., Kochanek, P.M., Bergold, P., Kenney, K., Marx, C.E., Grimes, C.J., Loh, L.T., Adam, L.T., Oskvig, D., Curley, K.C. and Salzer, W. (2014). Pharmacotherapy of traumatic brain injury: state of the science and the road forward: report of the Department of Defense Neurotrauma Pharmacology Workgroup. J Neurotrauma 31, 135-158.

8. McGonigle, P. and Ruggeri, B. (2014). Animal models of human disease: challenges in enabling translation. Biochem Pharmacol 87, 162-171.

9. Bayley, M.T., Teasell, R.W., Wolfe, D.L., Gruen, R.L., Eng, J.J., Ghajar, J., Tavender, E., Kua, A. and Bragge, P. (2014). Where to build the bridge between evidence and practice?: results of an international workshop to prioritize knowledge translation activities in traumatic brain injury care. J Head Trauma Rehabil 29, 268-276.

10. Talevi, A., Bellera, C.L., Di Ianni, M., Gantner, M., Bruno-Blanch, L.E. and Castro, E.A. (2012). CNS drug development - lost in translation? Mini Rev Med Chem 12, 959-970.

11. Agoston, D.V. and Skold, M.K. (2016). Editorial: When Physics Meets Biology; Biomechanics and Biology of Traumatic Brain Injury. Frontiers in neurology 7, 91.

12. Saatman, K.E., Duhaime, A.C., Bullock, R., Maas, A.I., Valadka, A. and Manley, G.T. (2008). Classification of traumatic brain injury for targeted therapies. J Neurotrauma 25, 719-738.

13. Vink, R. (2017). Large animal models of traumatic brain injury. J Neurosci Res.

14. Agoston, D.V. (2017). How to translate time? The temporal aspect of human and rodent biology. Frontiers in neurology 18, 1-5.

15. Ahmed, F., Plantman, S., Cernak, I. and Agoston, D.V. (2015). The temporal pattern of changes in serum biomarker levels reveals complex and dynamically changing pathologies after exposure toe a single low intensity blast in mice Frontiers in neurology 6, article 114.

16. Prins, M.L. and Hovda, D.A. (2009). The effects of age and ketogenic diet on local cerebral metabolic rates of glucose after controlled cortical impact injury in rats. J Neurotrauma 26, 1083-1093.

17. Chugani, H.T. (1998). A critical period of brain development: studies of cerebral glucose utilization with PET. Prev Med 27, 184-188.

18. Harada, M., Okuda, C., Sawa, T. and Murakami, T. (1992). Cerebral extracellular glucose and lactate concentrations during and after moderate hypoxia in glucose- and saline-infused rats. Anesthesiology 77, 728-734.

19. Vespa, P. (2005). Continuous EEG monitoring for the detection of seizures in traumatic brain injury, infarction, and intracerebral hemorrhage: "to detect and protect". J Clin Neurophysiol 22, 99-106.

20. Kilbaugh, T.J., Bhandare, S., Lorom, D.H., Saraswati, M., Robertson, C.L. and Margulies, S.S. (2011). Cyclosporin A preserves mitochondrial function after traumatic brain injury in the immature rat and piglet. J Neurotrauma 28, 763-774.

21. Sanchez, J.J., Bidot, C.J., O'Phelan, K., Gajavelli, S., Yokobori, S., Olvey, S., Jagid, J., Garcia, J.A., Nemeth, Z. and Bullock, R. (2013). Neuromonitoring with microdialysis in severe traumatic brain injury patients. Acta Neurochir Suppl 118, 223-227.

22. Stein, N.R., McArthur, D.L., Etchepare, M. and Vespa, P.M. (2012). Early cerebral metabolic crisis after TBI influences outcome despite adequate hemodynamic resuscitation. Neurocrit Care 17, 49-57.

23. Yoshino, A., Hovda, D.A., Kawamata, T., Katayama, Y. and Becker, D.P. (1991). Dynamic changes in local cerebral glucose utilization following cerebral conclusion in rats: evidence of a hyper- and subsequent hypometabolic state. Brain Res 561, 106-119.

24. Bergsneider, M., Hovda, D.A., Shalmon, E., Kelly, D.F., Vespa, P.M., Martin, N.A., Phelps, M.E., McArthur, D.L., Caron, M.J., Kraus, J.F. and Becker, D.P. (1997). Cerebral hyperglycolysis following severe traumatic brain injury in humans: a positron emission tomography study. Journal of neurosurgery 86, 241-251.

25. Bartnik, B.L., Sutton, R.L., Fukushima, M., Harris, N.G., Hovda, D.A. and Lee, S.M. (2005). Upregulation of pentose phosphate pathway and preservation of tricarboxylic acid cycle flux after experimental brain injury. J Neurotrauma 22, 1052-1065.

26. Cosi, C. and Marien, M. (1998). Decreases in mouse brain NAD+ and ATP induced by 1-methyl-4-phenyl-1, 2,3,6-tetrahydropyridine (MPTP): prevention by the poly(ADP-ribose) polymerase inhibitor, benzamide. Brain Res 809, 58-67.

27. Prins, M.L. (2012). Cerebral ketone metabolism during development and injury. Epilepsy research 100, 218-223.

28. Lee, S.M., Wong, M.D., Samii, A. and Hovda, D.A. (1999). Evidence for energy failure following irreversible traumatic brain injury. Ann N Y Acad Sci 893, 337-340.

29. Deng-Bryant, Y., Prins, M.L., Hovda, D.A. and Harris, N.G. (2011). Ketogenic diet prevents alterations in brain metabolism in young but not adult rats after traumatic brain injury. J Neurotrauma 28, 1813-1825.

30. Byrnes, K.R., Wilson, C.M., Brabazon, F., von Leden, R., Jurgens, J.S., Oakes, T.R. and Selwyn, R.G. (2014). FDG-PET imaging in mild traumatic brain injury: a critical review. Front Neuroenergetics 5, 13.

31. Sutton, R.L., Hovda, D.A., Adelson, P.D., Benzel, E.C. and Becker, D.P. (1994). Metabolic changes following cortical contusion: relationships to edema and morphological changes. Acta Neurochir Suppl (Wien) 60, 446-448.

32. Liu, Y.R., Cardamone, L., Hogan, R.E., Gregoire, M.C., Williams, J.P., Hicks, R.J., Binns, D., Koe, A., Jones, N.C., Myers, D.E., O'Brien, T.J. and Bouilleret, V. (2010). Progressive metabolic and structural cerebral perturbations after traumatic brain injury: an in vivo imaging study in the rat. Journal of nuclear medicine : official publication, Society of Nuclear Medicine 51, 1788-1795.

33. Prins, M.L. and Hovda, D.A. (2001). Mapping cerebral glucose metabolism during spatial learning: interactions of development and traumatic brain injury. J Neurotrauma 18, 31-46.

34. Humayun, M.S., Presty, S.K., Lafrance, N.D., Holcomb, H.H., Loats, H., Long, D.M., Wagner, H.N. and Gordon, B. (1989). Local cerebral glucose abnormalities in mild closed head injured patients with cognitive impairments. Nucl Med Commun 10, 335-344.

Journal of Neurotrauma
How to Translate Time; the Temporal Aspects of Rodent and Human Pathobiological Processes in Traumatic Brain Injury (DOI: 10.1089/neu.2018.6261)

35. Chen, S., Pickard, J.D. and Harris, N.G. (2003). Time course of cellular pathology after controlled cortical impact injury. Experimental neurology 182, 87-102.

36. Peskind, E.R., Petrie, E.C., Cross, D.J., Pagulayan, K., McCraw, K., Hoff, D., Hart, K., Yu, C.E., Raskind, M.A., Cook, D.G. and Minoshima, S. (2011). Cerebrocerebellar hypometabolism associated with repetitive blast exposure mild traumatic brain injury in 12 Iraq war Veterans with persistent post-concussive symptoms. NeuroImage 54 Suppl 1, S76-82.

37. Meabon, J.S., Huber, B.R., Cross, D.J., Richards, T.L., Minoshima, S., Pagulayan, K.F., Li, G., Meeker, K.D., Kraemer, B.C., Petrie, E.C., Raskind, M.A., Peskind, E.R. and Cook, D.G. (2016). Repetitive blast exposure in mice and combat veterans causes persistent cerebellar dysfunction. Sci Transl Med 8, 321ra326.

38. Petrie, E.C., Cross, D.J., Yarnykh, V.L., Richards, T., Martin, N.M., Pagulayan, K., Hoff, D., Hart, K., Mayer, C., Tarabochia, M., Raskind, M., Minoshima, S. and Peskind, E. (2013). Neuroimaging, Behavioral, and Psychological Sequelae of Repetitive Combined Blast/Impact Mild Traumatic Brain Injury in Iraq and Afghanistan War Veterans. J Neurotrauma.

39. Woodcock, T. and Morganti-Kossmann, M.C. (2013). The role of markers of inflammation in traumatic brain injury. Frontiers in neurology 4, 18.

40. Zeiler, F.A., Thelin, E.P., Czosnyka, M., Hutchinson, P.J., Menon, D.K. and Helmy, A. (2017). Cerebrospinal Fluid and Microdialysis Cytokines in Severe Traumatic Brain Injury: A Scoping Systematic Review. Frontiers in neurology 8, 331.

41. Seok, J., Warren, H.S., Cuenca, A.G., Mindrinos, M.N., Baker, H.V., Xu, W., Richards, D.R., McDonald-Smith, G.P., Gao, H., Hennessy, L., Finnerty, C.C., Lopez, C.M., Honari, S., Moore, E.E., Minei, J.P., Cuschieri, J., Bankey, P.E., Johnson, J.L., Sperry, J., Nathens, A.B., Billiar, T.R., West, M.A., Jeschke, M.G., Klein, M.B., Gamelli, R.L., Gibran, N.S., Brownstein, B.H., Miller-Graziano, C., Calvano, S.E., Mason, P.H., Cobb, J.P., Rahme, L.G., Lowry, S.F., Maier, R.V., Moldawer, L.L., Herndon, D.N., Davis, R.W., Xiao, W. and Tompkins, R.G. (2013). Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc Natl Acad Sci U S A 110, 3507-3512.

42. Wang, Y., Yue, X., Kiesewetter, D.O., Niu, G., Teng, G. and Chen, X. (2014). PET imaging of neuroinflammation in a rat traumatic brain injury model with radiolabeled TSPO ligand DPA-714. Eur J Nucl Med Mol Imaging 41, 1440-1449.

43. Lavisse, S., Guillermier, M., Herard, A.S., Petit, F., Delahaye, M., Van Camp, N., Ben Haim, L., Lebon, V., Remy, P., Dolle, F., Delzescaux, T., Bonvento, G., Hantraye, P. and Escartin, C. (2012). Reactive astrocytes overexpress TSPO and are detected by TSPO positron emission tomography imaging. The Journal of neuroscience : the official journal of the Society for Neuroscience 32, 10809-10818.

44. Vivash, L. and O'Brien, T.J. (2016). Imaging Microglial Activation with TSPO PET: Lighting Up Neurologic Diseases? Journal of nuclear medicine : official publication, Society of Nuclear Medicine 57, 165-168.

45. Shitaka, Y., Tran, H.T., Bennett, R.E., Sanchez, L., Levy, M.A., Dikranian, K. and Brody, D.L. (2011). Repetitive closed-skull traumatic brain injury in mice causes persistent multifocal axonal injury and microglial reactivity. J Neuropathol Exp Neurol 70, 551-567.

46. Thelin, E.P., Tajsic, T., Zeiler, F.A., Menon, D.K., Hutchinson, P.J.A., Carpenter, K.L.H., Morganti-Kossmann, M.C. and Helmy, A. (2017). Monitoring the Neuroinflammatory Response Following Acute Brain Injury. Frontiers in neurology 8, 351.

47. Helmy, A., De Simoni, M.G., Guilfoyle, M.R., Carpenter, K.L. and Hutchinson, P.J. (2011). Cytokines and innate inflammation in the pathogenesis of human traumatic brain injury. Prog Neurobiol 95, 352-372.

48. Shore, P.M., Thomas, N.J., Clark, R.S., Adelson, P.D., Wisniewski, S.R., Janesko, K.L., Bayir, H., Jackson, E.K. and Kochanek, P.M. (2004). Continuous versus intermittent cerebrospinal fluid drainage after severe traumatic brain injury in children: effect on biochemical markers. Journal of neurotrauma 21, 1113-1122.

49. Helmy, A., Carpenter, K.L., Skepper, J.N., Kirkpatrick, P.J., Pickard, J.D. and Hutchinson, P.J. (2009). Microdialysis of cytokines: methodological considerations, scanning electron microscopy, and determination of relative recovery. J Neurotrauma 26, 549-561.

50. Frugier, T., Morganti-Kossmann, M.C., O'Reilly, D. and McLean, C.A. (2010). In situ detection of inflammatory mediators in post mortem human brain tissue after traumatic injury. J Neurotrauma 27, 497-507.

51. Maier, B., Schwerdtfeger, K., Mautes, A., Holanda, M., Muller, M., Steudel, W.I. and Marzi, I. (2001). Differential release of interleukines 6, 8, and 10 in cerebrospinal fluid and plasma after traumatic brain injury. Shock 15, 421-426.

52. Buttram, S.D., Wisniewski, S.R., Jackson, E.K., Adelson, P.D., Feldman, K., Bayir, H., Berger, R.P., Clark, R.S. and Kochanek, P.M. (2007). Multiplex assessment of cytokine and chemokine levels in cerebrospinal fluid following severe pediatric traumatic brain injury: effects of moderate hypothermia. J Neurotrauma 24, 1707-1717.

53. Hayakata, T., Shiozaki, T., Tasaki, O., Ikegawa, H., Inoue, Y., Toshiyuki, F., Hosotubo, H., Kieko, F., Yamashita, T., Tanaka, H., Shimazu, T. and Sugimoto, H. (2004). Changes in CSF S100B and cytokine concentrations in early-phase severe traumatic brain injury. Shock 22, 102-107.

54. Schmidt, O.I., Heyde, C.E., Ertel, W. and Stahel, P.F. (2005). Closed head injury--an inflammatory disease? Brain Res Brain Res Rev 48, 388-399.

55. Helmy, A., Antoniades, C.A., Guilfoyle, M.R., Carpenter, K.L. and Hutchinson, P.J. (2012). Principal component analysis of the cytokine and chemokine response to human traumatic brain injury. PLoS One 7, e39677.

56. Perez-Barcena, J., Ibanez, J., Brell, M., Crespi, C., Frontera, G., Llompart-Pou, J.A., Homar, J. and Abadal, J.M. (2011). Lack of correlation among intracerebral cytokines, intracranial pressure, and brain tissue oxygenation in patients with traumatic brain injury and diffuse lesions. Crit Care Med 39, 533-540.

57. Roberts, D.J., Jenne, C.N., Leger, C., Kramer, A.H., Gallagher, C.N., Todd, S., Parney, I.F., Doig, C.J., Yong, V.W., Kubes, P. and Zygun, D.A. (2013). Association between the cerebral inflammatory and matrix metalloproteinase responses after severe traumatic brain injury in humans. J Neurotrauma 30, 1727-1736.

58. Juengst, S.B., Kumar, R.G., Failla, M.D., Goyal, A. and Wagner, A.K. (2015). Acute inflammatory biomarker profiles predict depression risk following moderate to severe traumatic brain injury. J Head Trauma Rehabil 30, 207-218.

59. Juengst, S.B., Kumar, R.G., Failla, M.D., Goyal, A. and Wagner, A.K. (2014). Acute Inflammatory Biomarker Profiles Predict Depression Risk Following Moderate to Severe Traumatic Brain Injury. J Head Trauma Rehabil.

60. Kumar, R.G., Boles, J.A. and Wagner, A.K. (2015). Chronic Inflammation After Severe Traumatic Brain Injury: Characterization and Associations With Outcome at 6 and 12 Months Postinjury. J Head Trauma Rehabil 30, 369-381.

61. Kumar, R.G., Diamond, M.L., Boles, J.A., Berger, R.P., Tisherman, S.A., Kochanek, P.M. and Wagner, A.K. (2015). Acute CSF interleukin-6 trajectories after TBI: associations with neuroinflammation, polytrauma, and outcome. Brain Behav Immun 45, 253-262.

62. Ramlackhansingh, A.F., Brooks, D.J., Greenwood, R.J., Bose, S.K., Turkheimer, F.E., Kinnunen, K.M., Gentleman, S., Heckemann, R.A., Gunanayagam, K., Gelosa, G. and Sharp, D.J. (2011). Inflammation after trauma: microglial activation and traumatic brain injury. Ann Neurol 70, 374-383.

63. Cox, A.L., Coles, A.J., Nortje, J., Bradley, P.G., Chatfield, D.A., Thompson, S.J. and Menon, D.K. (2006). An investigation of auto-reactivity after head injury. Journal of neuroimmunology 174, 180-186.

64. Helmy, A., Guilfoyle, M.R., Carpenter, K.L., Pickard, J.D., Menon, D.K. and Hutchinson, P.J. (2016). Recombinant human interleukin-1 receptor antagonist promotes M1 microglia biased cytokines and chemokines following human traumatic brain injury. J Cereb Blood Flow Metab 36, 1434-1448.

65. Beaumont, A., Marmarou, A., Hayasaki, K., Barzo, P., Fatouros, P., Corwin, F., Marmarou, C. and Dunbar, J. (2000). The permissive nature of blood brain barrier (BBB) opening in edema formation following traumatic brain injury. Acta Neurochir Suppl 76, 125-129.

66. Ayata, C. and Ropper, A.H. (2002). Ischaemic brain oedema. J Clin Neurosci 9, 113-124.

67. Hanstock, C.C., Faden, A.I., Bendall, M.R. and Vink, R. (1994). Diffusion-weighted imaging differentiates ischemic tissue from traumatized tissue. Stroke 25, 843-848.

68. Barzo, P., Marmarou, A., Fatouros, P., Hayasaki, K. and Corwin, F. (1997). Contribution of vasogenic and cellular edema to traumatic brain swelling measured by diffusion-weighted imaging. Journal of neurosurgery 87, 900-907.

69. Barzo, P., Marmarou, A., Fatouros, P., Hayasaki, K. and Corwin, F. (1997). Biphasic pathophysiological response of vasogenic and cellular edema in traumatic brain swelling. Acta Neurochir Suppl 70, 119-122.

70. Habgood, M.D., Bye, N., Dziegielewska, K.M., Ek, C.J., Lane, M.A., Potter, A., Morganti-Kossmann, C. and Saunders, N.R. (2007). Changes in blood-brain barrier permeability to large and small molecules following traumatic brain injury in mice. Eur J Neurosci 25, 231-238.

71. Pop, V. and Badaut, J. (2011). A Neurovascular Perspective for Long-Term Changes After Brain Trauma. Translational stroke research 2, 533-545.

72. Baldwin, S.A., Fugaccia, I., Brown, D.R., Brown, L.V. and Scheff, S.W. (1996). Blood-brain barrier breach following cortical contusion in the rat. Journal of neurosurgery 85, 476-481.

73. Nag, S., Venugopalan, R. and Stewart, D.J. (2007). Increased caveolin-1 expression precedes decreased expression of occludin and claudin-5 during blood-brain barrier breakdown. Acta neuropathologica 114, 459-469.

74. Chodobski, A., Zink, B.J. and Szmydynger-Chodobska, J. (2011). Blood-brain barrier pathophysiology in traumatic brain injury. Translational stroke research 2, 492-516.

75. Povlishock, J.T., Becker, D.P., Sullivan, H.G. and Miller, J.D. (1978). Vascular permeability alterations to horseradish peroxidase in experimental brain injury. Brain Research 153, 223-239.

76. Shlosberg, D., Benifla, M., Kaufer, D. and Friedman, A. (2010). Blood-brain barrier breakdown as a therapeutic target in traumatic brain injury. Nat Rev Neurol 6, 393-403.

77. Hudak, A.M., Peng, L.F., de la Plata, C.M., Thottakara, J., Moore, C., Harper, C., McColl, R., Babcock, E. and Diaz-Arrastia, R. (2014). Cytotoxic and vasogenic cerebral oedema in traumatic brain injury: Assessment with FLAIR and DWI imaging. Brain Injury 28, 1602-1609.

78. Marmarou, A., Signoretti, S., Fatouros, P.P., Portella, G., Aygok, G.A. and Bullock, M.R. (2006). Predominance of cellular edema in traumatic brain swelling in patients with severe head injuries. Journal of neurosurgery 104, 720-730.

79. Bullock, R., Statham, P., Patterson, J., Wyper, D., Hadley, D. and Teasdale, E. (1990). The time course of vasogenic oedema after focal human head injury--evidence from SPECT mapping of blood brain barrier defects. Acta Neurochir Suppl (Wien) 51, 286-288.

80. Vilalta, A., Sahuquillo, J., Rosell, A., Poca, M.A., Riveiro, M. and Montaner, J. (2008). Moderate and severe traumatic brain injury induce early overexpression of systemic and brain gelatinases. Intensive care medicine 34, 1384-1392.

81. Saw, M.M., Chamberlain, J., Barr, M., Morgan, M.P.G., Burnett, J.R. and Ho, K.M. (2014). Differential Disruption of Blood-Brain Barrier in Severe Traumatic Brain Injury. Neurocritical Care 20, 209-216.

82. Stahel, P.F., Morganti-Kossmann, M.C., Perez, D., Redaelli, C., Gloor, B., Trentz, O. and Kossmann, T. (2001). Intrathecal levels of complement-derived soluble membrane attack complex (sC5b-9) correlate with blood-brain barrier dysfunction in patients with traumatic brain injury. J Neurotrauma 18, 773-781.

83. Ho, K.M., Honeybul, S., Yip, C.B. and Silbert, B.I. (2014). Prognostic significance of blood-brain barrier disruption in patients with severe nonpenetrating traumatic brain injury requiring decompressive craniectomy. Journal of neurosurgery 121, 674-679.

84. Tomkins, O., Shelef, I., Kaizerman, I., Eliushin, A., Afawi, Z., Misk, A., Gidon, M., Cohen, A., Zumsteg, D. and Friedman, A. (2008). Blood-brain barrier disruption in post-traumatic epilepsy. Journal of Neurology Neurosurgery and Psychiatry 79, 774-777.

85. Korn, A., Golan, H., Melamed, I., Pascual-Marqui, R. and Friedman, A. (2005). Focal cortical dysfunction and blood-brain barrier disruption in patients with postconcussion syndrome. Journal of Clinical Neurophysiology 22, 1-9.

86. Castejon, O.J. (2013). Increased vesicular and vacuolar transendothelial transport in traumatic human brain oedema. A review. Folia Neuropathologica 51, 93-102.

87. Glushakova, O.Y., Johnson, D. and Hayes, R.L. (2014). Delayed increases in microvascular pathology after experimental traumatic brain injury are associated with prolonged inflammation, blood-brain barrier disruption, and progressive white matter damage. J Neurotrauma 31, 1180-1193.

88. Schoknecht, K. and Shalev, H. (2012). Blood-brain barrier dysfunction in brain diseases: Clinical experience. Epilepsia 53 Suppl 6, 7-13.

89. Shetty, A.K., Mishra, V., Kodali, M. and Hattiangady, B. (2014). Blood brain barrier dysfunction and delayed neurological deficits in mild traumatic brain injury induced by blast shock waves. Frontiers in cellular neuroscience 8, 232.

90. Prakash, R. and Carmichael, S.T. (2015). Blood-brain barrier breakdown and neovascularization processes after stroke and traumatic brain injury. Curr Opin Neurol 28, 556-564.

91. Russo, M.V., Latour, L.L. and McGavern, D.B. (2018). Distinct myeloid cell subsets promote meningeal remodeling and vascular repair after mild traumatic brain injury. Nature immunology 19, 442-452.

92. Ma, J., Zhang, K., Wang, Z. and Chen, G. (2016). Progress of Research on Diffuse Axonal Injury after Traumatic Brain Injury. Neural Plast 2016, 9746313.

93. Smith, D.H., Hicks, R. and Povlishock, J.T. (2013). Therapy development for diffuse axonal injury. J Neurotrauma 30, 307-323.

94. Su, E. and Bell, M. (2016). Diffuse Axonal Injury. In: *Translational Research in Traumatic Brain Injury*. Laskowitz, D., Grant, G. (eds). CRC Press/Taylor and Francis Group

(c) 2016 by Taylor & Francis Group, LLC.: Boca Raton (FL).

95. Johnson, V.E., Meaney, D.F., Cullen, D.K. and Smith, D.H. (2015). Animal models of traumatic brain injury. Handbook of clinical neurology 127, 115-128.

96. Sherriff, F.E., Bridges, L.R. and Sivaloganathan, S. (1994). Early detection of axonal injury after human head trauma using immunocytochemistry for beta-amyloid precursor protein. Acta neuropathologica 87, 55-62.

97. Smith, D.H., Chen, X.H., Iwata, A. and Graham, D.I. (2003). Amyloid beta accumulation in axons after traumatic brain injury in humans. Journal of neurosurgery 98, 1072-1077.

98. Yang, S., Sun, R., Zhou, Z., Zhou, J., Liang, J. and Mu, H. (2014). Expression of amyloid-beta protein and amyloid-beta precursor protein after primary brain-stem injury in rats. Am J Forensic Med Pathol 35, 201-205.

99. Grafe, M.R. and Leonard, C.M. (1980). Successful silver impregnation of degenerating axons after long survivals in the human brain. J Neuropathol Exp Neurol 39, 555-574.

100. Tenkova, T.I. and Goldberg, M.P. (2007). A modified silver technique (de Olmos stain) for assessment of neuronal and axonal degeneration. Methods in molecular biology (Clifton, N.J.) 399, 31-39.

101. Shenton, M.E., Hamoda, H.M., Schneiderman, J.S., Bouix, S., Pasternak, O., Rathi, Y., Vu, M.A., Purohit, M.P., Helmer, K., Koerte, I., Lin, A.P., Westin, C.F., Kikinis, R., Kubicki, M., Stern, R.A. and Zafonte, R. (2012). A review of magnetic resonance imaging and diffusion tensor imaging findings in mild traumatic brain injury. Brain Imaging Behav 6, 137-192.

102. Bigler, E.D. (2013). Traumatic brain injury, neuroimaging, and neurodegeneration. Front Hum Neurosci 7, 395.

103. Sharp, D.J. and Ham, T.E. (2011). Investigating white matter injury after mild traumatic brain injury. Curr Opin Neurol 24, 558-563.

Journal of Neurotrauma
How to Translate Time; the Temporal Aspects of Rodent and Human Pathobiological Processes in Traumatic Brain Injury (DOI: 10.1089/neu.2018.6261)

104. Al Nimer, F., Thelin, E., Nystrom, H., Dring, A.M., Svenningsson, A., Piehl, F., Nelson, D.W. and Bellander, B.M. (2015). Comparative Assessment of the Prognostic Value of Biomarkers in Traumatic Brain Injury Reveals an Independent Role for Serum Levels of Neurofilament Light. PLoS One 10, e0132177.

105. Gatson, J.W., Barillas, J., Hynan, L.S., Diaz-Arrastia, R., Wolf, S.E. and Minei, J.P. (2014). Detection of neurofilament-H in serum as a diagnostic tool to predict injury severity in patients who have suffered mild traumatic brain injury. Journal of neurosurgery 121, 1232-1238.

106. Ljungqvist, J., Zetterberg, H., Mitsis, M., Blennow, K. and Skoglund, T. (2017). Serum Neurofilament Light Protein as a Marker for Diffuse Axonal Injury: Results from a Case Series Study. J Neurotrauma 34, 1124-1127.

107. Martinez-Morillo, E., Childs, C., Garcia, B.P., Alvarez Menendez, F.V., Romaschin, A.D., Cervellin, G., Lippi, G. and Diamandis, E.P. (2015). Neurofilament medium polypeptide (NFM) protein concentration is increased in CSF and serum samples from patients with brain injury. Clin Chem Lab Med 53, 1575-1584.

108. Shibahashi, K., Doi, T., Tanaka, S., Hoda, H., Chikuda, H., Sawada, Y., Takasu, Y., Chiba, K., Nozaki, T., Hamabe, Y. and Ogata, T. (2016). The Serum Phosphorylated Neurofilament Heavy Subunit as a Predictive Marker for Outcome in Adult Patients after Traumatic Brain Injury. J Neurotrauma 33, 1826-1833.

109. Wang, K.K., Ottens, A.K., Liu, M.C., Lewis, S.B., Meegan, C., Oli, M.W., Tortella, F.C. and Hayes, R.L. (2005). Proteomic identification of biomarkers of traumatic brain injury. Expert Rev Proteomics 2, 603-614.

110. Wang, K.K., Yang, Z., Zhu, T., Shi, Y., Rubenstein, R., Tyndall, J.A. and Manley, G.T. (2018). An update on diagnostic and prognostic biomarkers for traumatic brain injury. Expert Rev Mol Diagn 18, 165-180.

111. McGinn, M.J., Kelley, B.J., Akinyi, L., Oli, M.W., Liu, M.C., Hayes, R.L., Wang, K.K. and Povlishock, J.T. (2009). Biochemical, structural, and biomarker evidence for calpain-

mediated cytoskeletal change after diffuse brain injury uncomplicated by contusion. J Neuropathol Exp Neurol 68, 241-249.

112. Hellewell, S.C., Yan, E.B., Agyapomaa, D.A., Bye, N. and Morganti-Kossmann, M.C. (2010). Post-traumatic hypoxia exacerbates brain tissue damage: analysis of axonal injury and glial responses. J Neurotrauma 27, 1997-2010.

113. Stone, J.R., Okonkwo, D.O., Dialo, A.O., Rubin, D.G., Mutlu, L.K., Povlishock, J.T. and Helm, G.A. (2004). Impaired axonal transport and altered axolemmal permeability occur in distinct populations of damaged axons following traumatic brain injury. Experimental neurology 190, 59-69.

114. Dikranian, K., Cohen, R., Mac Donald, C., Pan, Y., Brakefield, D., Bayly, P. and Parsadanian, A. (2008). Mild traumatic brain injury to the infant mouse causes robust white matter axonal degeneration which precedes apoptotic death of cortical and thalamic neurons. Experimental neurology 211, 551-560.

115. Hamberger, A., Huang, Y.L., Zhu, H., Bao, F., Ding, M., Blennow, K., Olsson, A., Hansson, H.A., Viano, D. and Haglid, K.G. (2003). Redistribution of neurofilaments and accumulation of beta-amyloid protein after brain injury by rotational acceleration of the head. J Neurotrauma 20, 169-178.

116. Mac Donald, C.L., Dikranian, K., Bayly, P., Holtzman, D. and Brody, D. (2007). Diffusion tensor imaging reliably detects experimental traumatic axonal injury and indicates approximate time of injury. The Journal of neuroscience : the official journal of the Society for Neuroscience 27, 11869-11876.

117. Li, S., Sun, Y., Shan, D., Feng, B., Xing, J., Duan, Y., Dai, J., Lei, H. and Zhou, Y. (2013). Temporal profiles of axonal injury following impact acceleration traumatic brain injury in rats--a comparative study with diffusion tensor imaging and morphological analysis. Int J Legal Med 127, 159-167.

118. Mac Donald, C.L., Dikranian, K., Song, S.K., Bayly, P.V., Holtzman, D.M. and Brody, D.L. (2007). Detection of traumatic axonal injury with diffusion tensor imaging in a mouse model of traumatic brain injury. Experimental neurology 205, 116-131.

119. Davceva, N., Basheska, N. and Balazic, J. (2015). Diffuse Axonal Injury-A Distinct Clinicopathological Entity in Closed Head Injuries. Am J Forensic Med Pathol 36, 127-133.

120. Adams, J.H., Doyle, D., Ford, I., Gennarelli, T.A., Graham, D.I. and McLellan, D.R. (1989). Diffuse axonal injury in head injury: definition, diagnosis and grading. Histopathology 15, 49-59.

121. Gennarelli, T.A., Thibault, L.E., Adams, J.H., Graham, D.I., Thompson, C.J. and Marcincin, R.P. (1982). Diffuse axonal injury and traumatic coma in the primate. Ann Neurol 12, 564-574.

122. Smith, D.H., Nonaka, M., Miller, R., Leoni, M., Chen, X.H., Alsop, D. and Meaney, D.F. (2000). Immediate coma following inertial brain injury dependent on axonal damage in the brainstem. Journal of neurosurgery 93, 315-322.

123. Bramlett, H.M. and Dietrich, W.D. (2015). Long-Term Consequences of Traumatic Brain Injury: Current Status of Potential Mechanisms of Injury and Neurological Outcomes. J Neurotrauma 32, 1834-1848.

124. McGinn, M.J. and Povlishock, J.T. (2015). Cellular and molecular mechanisms of injury and spontaneous recovery. Handbook of clinical neurology 127, 67-87.

125. Smith, C. (2013). Review: the long-term consequences of microglial activation following acute traumatic brain injury. Neuropathology and applied neurobiology 39, 35-44.

126. Weiss, N., Galanaud, D., Carpentier, A., Naccache, L. and Puybasset, L. (2007). Clinical review: Prognostic value of magnetic resonance imaging in acute brain injury and coma. Crit Care 11, 230.

127. Tshibanda, L., Vanhaudenhuyse, A., Galanaud, D., Boly, M., Laureys, S. and Puybasset, L. (2009). Magnetic resonance spectroscopy and diffusion tensor imaging in coma survivors: promises and pitfalls. Prog Brain Res 177, 215-229.

128. Perez, A.M., Adler, J., Kulkarni, N., Strain, J.F., Womack, K.B., Diaz-Arrastia, R. and Marquez de la Plata, C.D. (2014). Longitudinal white matter changes after traumatic axonal injury. J Neurotrauma 31, 1478-1485.

129. Sidaros, A., Engberg, A.W., Sidaros, K., Liptrot, M.G., Herning, M., Petersen, P., Paulson, O.B., Jernigan, T.L. and Rostrup, E. (2008). Diffusion tensor imaging during recovery from severe traumatic brain injury and relation to clinical outcome: a longitudinal study. Brain 131, 559-572.

130. Brezova, V., Moen, K.G., Skandsen, T., Vik, A., Brewer, J.B., Salvesen, O. and Haberg, A.K. (2014). Prospective longitudinal MRI study of brain volumes and diffusion changes during the first year after moderate to severe traumatic brain injury. Neuroimage Clin 5, 128-140.

131. Farbota, K.D., Sodhi, A., Bendlin, B.B., McLaren, D.G., Xu, G., Rowley, H.A. and Johnson, S.C. (2012). Longitudinal volumetric changes following traumatic brain injury: a tensor-based morphometry study. J Int Neuropsychol Soc 18, 1006-1018.

132. Bendlin, B.B., Ries, M.L., Lazar, M., Alexander, A.L., Dempsey, R.J., Rowley, H.A., Sherman, J.E. and Johnson, S.C. (2008). Longitudinal changes in patients with traumatic brain injury assessed with diffusion-tensor and volumetric imaging. NeuroImage 42, 503-514.

133. Johnson, V.E., Stewart, W. and Smith, D.H. (2013). Axonal pathology in traumatic brain injury. Experimental neurology 246, 35-43.

134. Newcombe, V.F., Correia, M.M., Ledig, C., Abate, M.G., Outtrim, J.G., Chatfield, D., Geeraerts, T., Manktelow, A.E., Garyfallidis, E., Pickard, J.D., Sahakian, B.J., Hutchinson, P.J., Rueckert, D., Coles, J.P., Williams, G.B. and Menon, D.K. (2016). Dynamic Changes in White Matter Abnormalities Correlate With Late Improvement and Deterioration Following TBI: A Diffusion Tensor Imaging Study. Neurorehabil Neural Repair 30, 49-62.

135. Johnson, V.E., Stewart, J.E., Begbie, F.D., Trojanowski, J.Q., Smith, D.H. and Stewart, W. (2013). Inflammation and white matter degeneration persist for years after a single traumatic brain injury. Brain 136, 28-42.

136. Davalos, D., Ryu, J.K., Merlini, M., Baeten, K.M., Le Moan, N., Petersen, M.A., Deerinck, T.J., Smirnoff, D.S., Bedard, C., Hakozaki, H., Gonias Murray, S., Ling, J.B., Lassmann, H., Degen, J.L., Ellisman, M.H. and Akassoglou, K. (2012). Fibrinogen-induced perivascular microglial clustering is required for the development of axonal damage in neuroinflammation. Nat Commun 3, 1227.

137. Byrne, A.B., Walradt, T., Gardner, K.E., Hubbert, A., Reinke, V. and Hammarlund, M. (2014). Insulin/IGF1 signaling inhibits age-dependent axon regeneration. Neuron 81, 561-573.

138. Girouard, M.P., Bueno, M., Julian, V., Drake, S., Byrne, A.B. and Fournier, A.E. (2018). The Molecular Interplay between Axon Degeneration and Regeneration. Developmental neurobiology 78, 978-990.

139. Jaerve, A., Schiwy, N., Schmitz, C. and Mueller, H.W. (2011). Differential effect of aging on axon sprouting and regenerative growth in spinal cord injury. Experimental neurology 231, 284-294.

140. Smith, M. (2017). Multimodality Neuromonitoring in Adult Traumatic Brain Injury: A Narrative Review. Anesthesiology.

141. Stocchetti, N., Le Roux, P., Vespa, P., Oddo, M., Citerio, G., Andrews, P.J., Stevens, R.D., Sharshar, T., Taccone, F.S. and Vincent, J.L. (2013). Clinical review: neuromonitoring - an update. Crit Care 17, 201.

142. Bhatia, A. and Gupta, A.K. (2007). Neuromonitoring in the intensive care unit. I. Intracranial pressure and cerebral blood flow monitoring. Intensive Care Med 33, 1263-1271.

143. Grenander, U. (1959). The Nyquist frequency of sampling Vol 1. Wiley: New York.

144. Lollis, S.S., Quebada, P.B. and Friedman, J.A. (2008). Traumatic brain injury. Handbook of clinical neurology 90, 217-229.

145. Young, G.B. (2010). Traumatic brain injury: the continued quest for early prognostic determination. Crit Care Med 38, 325-326.

146. Yarnell, A.M., Barry, E.S., Mountney, A., Shear, D., Tortella, F. and Grunberg, N.E. (2016). The Revised Neurobehavioral Severity Scale (NSS-R) for Rodents. Curr Protoc Neurosci 75, 9.52.51-59.52.16.

147. Dhawan, V. and DeGeorgia, M. (2012). Neurointensive care biophysiological monitoring. J Neurointerv Surg 4, 407-413.

148. Ko, S.B. (2013). Multimodality monitoring in the neurointensive care unit: a special perspective for patients with stroke. J Stroke 15, 99-108.

149. Rostami, E. (2014). Glucose and the injured brain-monitored in the neurointensive care unit. Frontiers in neurology 5, 91.

150. Agoston, D. (2015). Bench-to-bedside and bedside back to the bench: seeking a better understanding of the acute pathophysiological process in severe traumatic brain injury. Frontiers in neurology 6, 1-6.

151. Agoston, D.V., Risling, M. and Bellander, B.M. (2012). Bench-to-bedside and bedside back to the bench; coordinating clinical and experimental traumatic brain injury studies. Frontiers in neurology 3, 3.

152. Agoston, D.V. and Langford, D. (2017). Big Data in traumatic brain injury; promise and challenges. Concussion 2, Cnc45.

153. Agoston, D.V. (2017). Understanding the complexities of traumatic brain injury: A big data approach to a big disease. Behav Brain Res 340, 172-173.

154. Horvat, C.M. and Kochanek, P.M. (2017). Big Data Not Yet Big Enough to Determine the Influence of Intracranial Pressure Monitoring on Outcome in Children With Severe Traumatic Brain Injury. JAMA Pediatr 171, 942-943.

155. Alberts, B. and Kamb, A. (2016). Publishing confirming and non-confirming data. F1000Res 5, 135.

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Figure legends

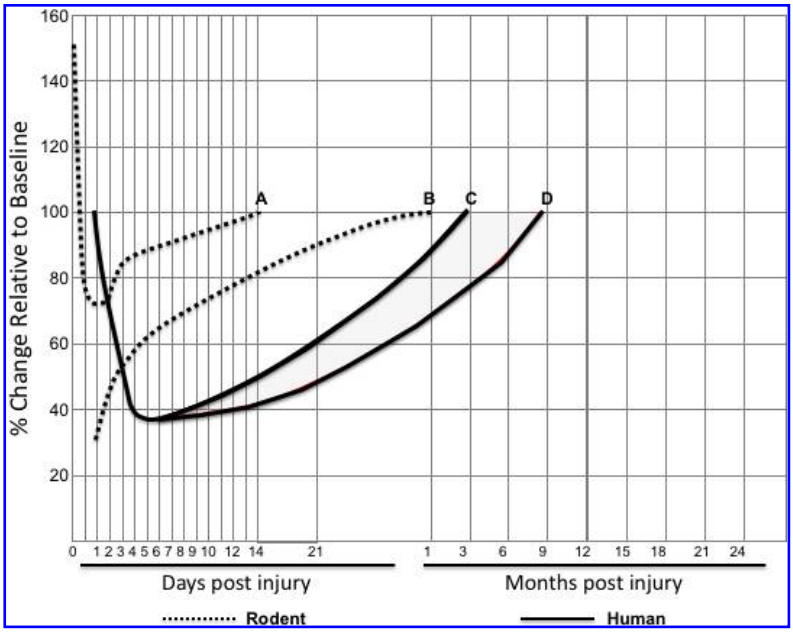

FIG. 1. Time-related changes in cerebral glucose metabolism after different types of traumatic brain injury (TBI) in rats (dashed lines) and humans (solid lines). Data are expressed as a percentage of baseline (either sham or control) values. (**A**) Data from adult rats with unilateral fluid percussion injury using in vitro autoradiography of [t4C]2-deoxy-D-glucose to determine changes in the rate of cerebral glucose utilization.[26] (**B**) Summary of in vitro autoradiography of [t4C]2-deoxy-D-glucose data following cortical contusion in adult rats showing changes in the rate of cerebral glucose utilization.[16,36] Data summarizing the effect of (**C**) moderate and (**D**) severe TBI in humans using FDG-P to determine changes in the rate of global cerebral metabolic rate of glucose.[150]

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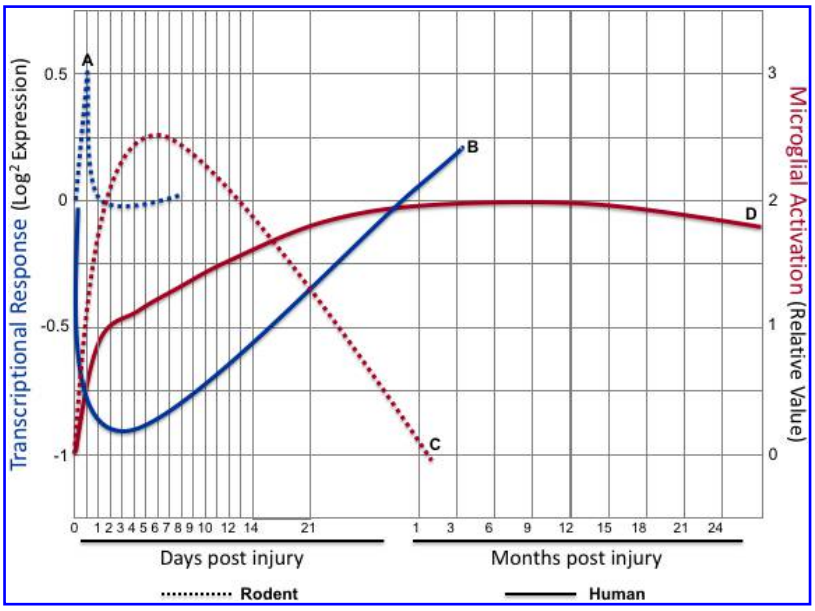

FIG. 2. A comparison of the temporal changes in inflammatory response after different types of insults in rodents (dashed lines) and humans (solid lines). Transcriptional response after (general) trauma in (**A**) rodents and in (**B**) humans. Data are expressed on a log2 expression vs. time scale to illustrate instances where genes significantly changed over time in human injuries, albeit minimally in murine models.[45] (**C**) Microglial activation after controlled cortical impact in rats as detected by positron emission tomography (PET) imaging, ex vivo autoradiography, and verified by immunohistochemistry.[46] (**D**) Microglial activation was detected by PET imaging up to 17 years after moderate to severe TBI in humans.[63]

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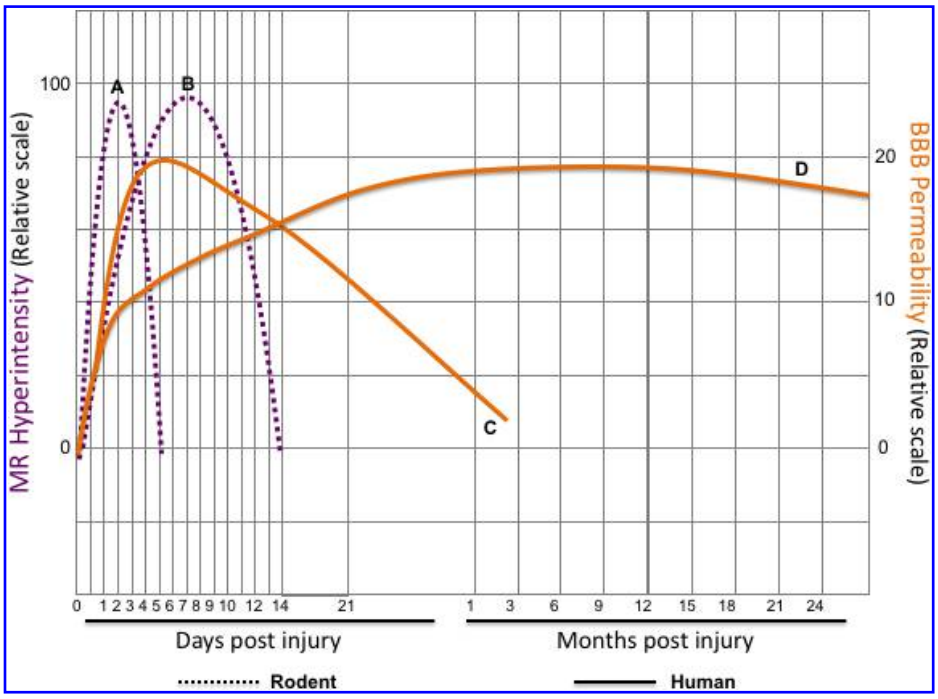

FIG. 3. Comparisons of the time course of changes in cerebral edema detected by neuroimaging and/or by biochemical methods after different types of insults in rodents and humans. (**A**) Diffusion-weighted magnetic resonance (MR) imaging showed increased hyperintensity after moderate fluid percussion TBI,[68] likely the result of vasogenic cerebral edema. (**B**) Cytogenic edema peaked around day 7 following impact acceleration (diffuse, closed-head) injury, and normalized around 2 weeks.[69] (**C**) Clinical studies showed evidence of BBB dysfunction reaching its max around 3-6 days after injury – depending on the type of insult and other factors – and returning to normal within several weeks.[85] (**D**) However, there is evidence that BBB dysfunction can last for months to years and is associated with an increased risk for developing post-traumatic epilepsy.[85]

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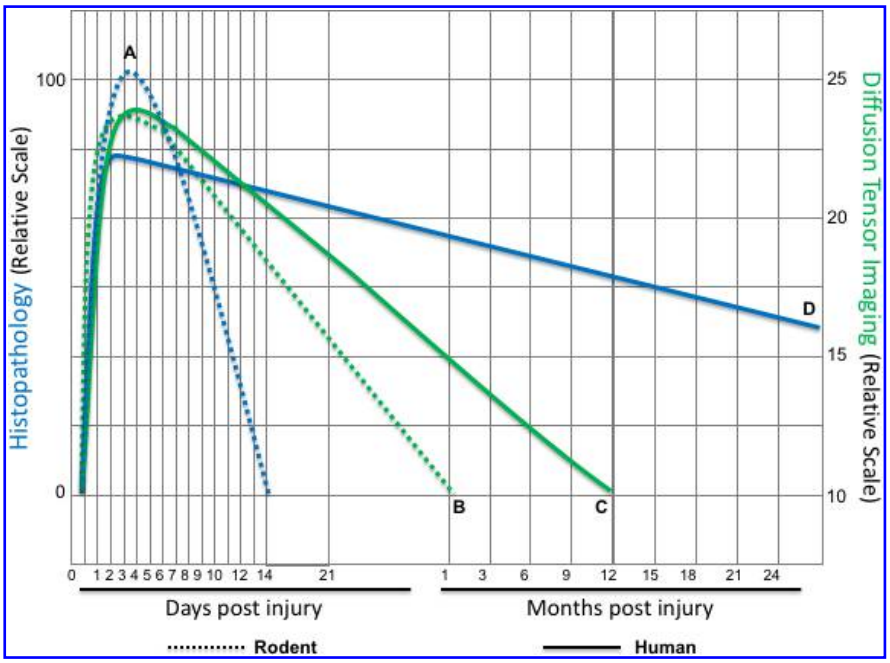

FIG. 4. The time course of axonal damage in rodents and humans measured by histopathology and in vivo imaging. (**A**) Following rotational acceleration injury in rats, APP histology showed rapid appearance of retraction bulbs and swollen axons in the corpus callosum, which gradually decreased and completely disappeared by day 14.[108] (**B**) A similar time course was observed in mice after controlled cortical impact, but axonal varicosities were still detectable at 1 month post-injury using histology; diffusion tensor imaging also showed axonal abnormalities at the 1-month time point.[112] (**C**) Human studies show a protracted APP histopathology with moderate to extensive axonal pathology up to 1 year post-injury in 80% of cases investigated. (**D**) In fact, moderate axonal pathology was observed several years (up to 18) post-injury in 50% of clinical cases.[129, 131]