Modeling the Long-Term Consequences of Repeated Blast-Induced Mild Traumatic Brain Injuries

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Abstract

Repeated mild traumatic brain injury (rmTBI) caused by playing collision sports or by exposure to blasts during military operations can lead to late onset, chronic diseases such as chronic traumatic encephalopathy (CTE), a progressive neurodegenerative condition that manifests in increasingly severe neuropsychiatric abnormalities years after the last injury. Currently, because of the heterogeneity of the clinical presentation, confirmation of a CTE diagnosis requires post-mortem examination of the brain. The hallmarks of CTE are abnormal accumulation of phosphorylated tau protein, TDP-43 immunoreactive neuronal cytoplasmic inclusions, and astroglial abnormalities, but the pathomechanism leading to these terminal findings remains unknown. Animal modeling can play an important role in the identification of CTE pathomechanisms, the development of early stage diagnostic and prognostic biomarkers, and pharmacological interventions. Modeling the long-term consequences of blast rmTBI in animals is especially challenging because of the complexities of blast physics and animal-to-human scaling issues. This review summarizes current knowledge about the pathobiologies of CTE and rmTBI and discusses problems as well as potential solutions related to high-fidelity modeling of rmTBI and determining its long-term consequences.

Keywords: animal modeling; chronic neurodegenerative disorders; explosive blast; mild traumatic brain injury; pathomechanism; physics

Introduction

THE MILD FORM of traumatic brain injury (mTBI) that accounts for ~85% of all TBIs is generally viewed as an acute condition characterized by mild and transient symptoms. Indeed, the majority of injured persons show full functional recovery over time, but there is increasing evidence that even a single mTBI can cause lasting, if not permanent, changes without clinical symptoms.^{1,2} The terms mTBI and concussion are frequently used interchangeably. The Centers for Disease Control and Prevention (CDC) defines concussion as a “traumatic brain injury (TBI) caused by a sudden bump, blow, or jolt to the head altering the mental state,” although there are arguments that concussion is a subtype of mTBI. After recovery from a mTBI, most athletes return to play and soldiers return to duty where many of them sustain additional mTBIs. Despite the apparent functional recovery, studies have shown that repeated mTBIs (rmTBI) can have cumulative effects and long-term consequences.^{3,4} In fact, a history of rmTBI has been identified as the most important risk factor for late-onset neurodegenerative disease development, such as chronic traumatic encephalopathy (CTE).

Chronic traumatic encephalopathy is a progressive neurodegenerative disease, first described as “punch-drunk syndrome” or

“dementia pugilistica” in boxers in the 1920s and 1930s and later termed chronic traumatic encephalopathy by Critchley in 1949.^{5–9} Neurobehaviorally, CTE is characterized by increasing irritability, aggression, emotional lability, memory and cognitive deficits, and, finally, speech disturbances and dementia.^{10,11} Underlying these behavioral changes is a specific neuropathology, both at the gross and cellular levels. In the mild or early stages of CTE, gross changes, if present, usually include enlarged frontal and temporal horns of the lateral ventricles, as well as prominent perivascular spaces in white matter and cavum septum pellucidum. The later stages of CTE are characterized by decreased brain weight, widespread atrophy with thinning of the corpus callosum, and depigmentation of the locus coeruleus and substantia nigra.⁶

The National Institute of Neurological Disorders and Stroke/National Institute of Biomedical Imaging and Bioengineering consensus panel¹² defined the microscopic changes associated with CTE as an accumulation of abnormal hyperphosphorylated tau (p-tau) in neurons and astroglia distributed around small blood vessels at the depths of cortical sulci and in an irregular pattern. Additional, supportive but not diagnostic p-tau features include thorn-shaped astrocytes at the glia limitans of subpial and periventricular regions, TDP-43 immunoreactive cytoplasmic inclusions in neurons. The hypothesized pathomechanism of CTE includes

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chronic neuroinflammation, as indicated by studies that even a single TBI can trigger long-lasting inflammatory changes in the brain,¹³ but the risk of long-term pathological changes likely increases with increasing numbers of insults.

One of the difficulties in modeling rmTBI is determining the window of increased cerebral vulnerability (WICV) during which repeated insults can have a cumulative effect.¹⁴ It has been shown that if a second insult to the head takes place within the WICV, re-injury triggers a more severe, cumulative damage type of response relative to the initial injury. The cumulative effect of repeated insults within the WICV has been demonstrated for cerebral glucose metabolism,¹⁵ axonal damage,¹⁶ and inflammation.¹⁷ These studies also indicated that WICV differs for the various pathobiological processes. A cumulative effect on cerebral glucose metabolism was detected with a 24 h interval between insults.¹⁵ For axonal and vascular changes, only a 1.5–3 h interval was necessary.¹⁶ Conversely, a much longer interinjury period (five days) was found to elicit a cumulative neuroinflammatory response that lasted for eight weeks after the last injury.¹⁷ The most intense inflammatory response was seen in animals that received five insults at five-day intervals.

Studies using rodents and varying survival times (up to six months) have shown that a single injury can trigger chronic neuropathology resembling CTE and in association with neurobehavioral deficits in some cases (e.g.¹⁸). Some studies showed that repeated insults can increase the extent of tau pathologies; however, the interinjury intervals were highly variable (2–48 h).^{18–21} Not surprisingly, increases in p-tau and astroglial pathologies also varied from negative to significant at six months after injury. These studies suggest that the WICV may vary depending on: (1) the type of injury or animal model used (e.g., controlled cortical impact, fluid percussion, or impact acceleration); and (2) the pathology/outcome measures (metabolic, axonal changes, or inflammation).

Blast Scenarios

The short- and long-term consequences of TBI represent one of the most significant and complex challenges for the military healthcare system.^{22,23} A TBI impacts force readiness in the short term, while a substantial number of veterans experience the consequences of TBI (and post-traumatic stress disorder [PTSD]) in the long term.²⁴ Dominant use of improvised explosive devices (IEDs) by insurgents against United States (US) military personnel during the latest military conflicts (and their continued use) have resulted in a staggering number of blast-induced TBI (bTBI) cases.^{25–29} It was estimated that the incidence of TBI during the conflicts in Iraq and Afghanistan ranged from 12–35% with approximately 80% of the injuries involving blast exposure. Accordingly, between 200,000 and 500,000 military personnel have required various levels of medical care because of TBI.^{30–32}

Military TBI occurs in combination with a multitude of environmental and physical factors, including psychological hazards.³³ Several symptoms observed in mbTBI or repeated mbTBI (rmbTBI) can be triggered by psychological trauma(s) alone without organic injury, diagnosed as PTSD. Persons experiencing PTSD re-experience the stressful event/trauma, thereby reinforcing the negative association of certain triggers and causing them to become/remain reactive for an extended period. Up to 50% of service members with a history of bTBI meet the diagnostic criteria for PTSD even after ~ two years of exposure(s); thus, comorbidity with PTSD is one of the main discriminators between civilian and military (blast) TBIs.

The majority of military TBIs, similar to civilian TBI cases, are mild in severity and repetitive. The similarities or dissimilarities

between pathomechanisms of civilian mTBI, typically caused by kinetic forces, and blast-induced mTBI (mbTBI) are currently unclear because to, in part, a highly complex injurious environment created by blast. An explosion is created when solid (or liquid) substances are rapidly (i.e., within a few microseconds) converted into gases at extreme pressures and temperatures.^{34–36} The resulting blast consists of shockwaves (SWs), leading elements of the sudden disturbance in atmospheric pressure (hence the term blast overpressure), and the blast wind, a high velocity (supersonic) air movement.

It has been hypothesized that the uniqueness of primary bTBI is because of the former, SW component of explosive blast, and that SWs are injurious particularly to the brain.^{34,36–38} The latter is responsible mainly for damage to external tissues and air-filled organs such as the lungs, but it can also trigger kinetic motion depending on the blast environment. In addition to these main components, there are toxic gases, high temperatures, and penetrating projectiles that result from explosive blast. The physical environment of the blast—open field versus walled structures, etc.—can modify majorly the physical forces and the components of explosive blast that interact with the body.

Previous studies focusing on the interaction between SWs and biological structures have shown that SWs can propagate through structures of similar material densities/acoustic impedance without substantial loss of energy. The SWs, however, dissipate more energy at boundaries with differing acoustic impedances such as air and skull, blood, and/or cerebrospinal fluid (CSF) and brain parenchyma, causing a unique type of injury.^{39,40}

An open field explosion results in rapidly developing SWs that uniformly expand outward in three dimensions from the center of the explosion.^{34,36} The dimensionality and directionality of blast-generated pressure waves within the blast tube are similar to those observed in free field explosions—albeit, blast tubes require significantly smaller quantities of explosives to generate target peak pressures.^{34–36,41} Exposing animal(s) inside a blast tube is considered to mimic primary bTBI because the SW is the primary physical component interacting with the model. Military vehicles, a frequent target of blast from IEDs, create a different blast environment than open field explosions because of their enclosed spaces and varying configurations.^{42–45} Underbody explosions, frequent in combat and patrol situations, create heterogeneous physical forces of which hyper-acceleration of the body and/or ejecta is an important component.^{46,47}

Another combat-relevant scenario: explosion taking place within a walled structure (e.g., house or building). Explosions within walled structures create an extremely complex blast environment because pressure waves are reflected from walls, the floor, and other surfaces, thereby exposing subjects to highly variable waveforms of differing intensities.^{34–36}

In primary bTBI, it is the energy of SWs, dissipated at boundaries with differing acoustic impedance, that cause the substantial damage seen in acute malignant cerebral edema and severe hyperemia cases during Operation Iraqi Freedom.^{48–52} The onset of edema was rapid and life threatening; consequently, hemicraniectomy was performed as soon as possible after severe blast exposure. In addition, (delayed) vasospasm was observed frequently in those severe bTBI patients, and the incidence of vasospasm was unusually high compared with civilian TBI. At the moderate to mild levels of bTBI, exposure to blast results in distinct structural, functional, and molecular changes detected by diffusion tensor imaging (DTI), functional magnetic resonance imaging (fMRI),^{53–55} positron emission tomography,^{56,57} and magnetic resonance spectroscopy imaging.⁵⁸

In addition to well-documented white matter abnormalities,^{26,59} studies have found increased amygdala activation to fear in

veterans with major depressive disorder as a consequence of bTBI,⁵⁵ reduced right hippocampal volume, and decreased N-acetyl-aspartate levels.⁵⁸ Reductions in cerebral glucose metabolism were also detected in several cortical areas and in the cerebellum of veterans with a history of mbTBI, especially with rmbTBI, but not with PTSD.^{56,60} Importantly, these changes were found to have lasting behavioral and functional effects.^{57,61,62} Neurobehavioral abnormalities observed years (4–5) after the last exposure included anxiety, depression, and irritability, deficits in memory and cognition, and sleep disturbances that coincided with specific structural and molecular changes detected by the various imaging modalities. The pathomechanism of how mbTBI and rmbTBI can cause lasting structural and neurobehavioral changes is unknown currently. Similarly unknown is whether or not these changes are part of the pathomechanism that eventually results in CTE.

It has been hypothesized that because of the distinct physical nature of explosive blast, the neuropathology of mbTBI- and/or rmbTBI-induced CTE can be different. A recent histopathological study that analyzed the brains of service members who had experienced acute and chronic blast exposures found a neuropathology that appears distinct from that of civilian, impact-induced CTE cases.⁶³ Consistent with the known biological effects of SWs, the main pathological feature—extensive astroglial scarring—was found at boundaries between the brain parenchyma and fluids: subpial glial plate, penetrating cortical blood vessels, gray–white matter junctions, and structures lining the ventricles. The authors noted that astroglial scarring was not present in the same brain regions analyzed in the civilian (control) cases, with or without a history of impact TBI. It should be noted that astroglial scarring, a general indicator of degenerative conditions, has been observed in age-related astrogliopathy^{64–66} without TBI. The locations of blast-induced astrogliopathy at the interface of structures with differing acoustic impedance, however, seem to be specific to primary bTBI and therefore may be of diagnostic value.

While the number of existing chronic rmbTBI histopathological analyses is small, the neuropathological hallmarks appear to be somewhat different than those observed in civilian CTE cases. Importantly, it is possible that repeated mbTBI could trigger a distinct and maybe more aggressive neuropathological process. In the absence of detailed temporal analyses—e.g., by noninvasive imaging technologies—we cannot distinguish currently between aggressive midstage versus delayed end-stage changes related to the condition.

Animal Modeling

Rodent models

Given the limitations of clinical studies, some of which are listed above, animal modeling can play a key role in identifying the pathomechanism and related long-term consequences of rmbTBI. Shock tubes and small animals are the models of choice for studying bTBI for reasons of cost, logistics, and safety.⁶⁷ Using various rodent models of bTBI, studies have generated significant amounts of data about the behavioral, morphological, cellular, and molecular changes in bTBI using various outcome measures at the acute and subacute phase of the injury.^{60,68–93} These studies provide experimental evidence for inflammation,^{70,83,84,90,94–97} metabolic dysfunction,^{68,69,83,98} axonal changes,^{68,80,99,100} vascular changes^{82,90,96,101–104} as well as neuronal and glial damage and/or loss.^{86,89,90,96,100,102,103,105–107,108–110}

The overwhelming majority of these studies analyzed short-term changes after the injury; only a few studies explored the effects of a single exposure to mild blast beyond the acute/subacute phase of injury.^{96,111,112} These studies found that a single exposure to mild

blast causes lasting memory impairment in rats, up to 71 days post-injury, and a transient, early-onset elevation in anxiety. Proteomic analysis of brain tissue from the same behaviorally tested rats showed changes in specific brain regions (dorsal and ventral hippocampus, amygdala, and prefrontal cortex) that implicate neuroinflammation in the long-term pathology of mbTBI.¹¹² Importantly, acute treatment with the anti-inflammatory drug minocycline prevented the onset of neurobehavioral abnormalities and normalized serum levels of select biomarkers, including tau, further supporting the hypothesis that neuroinflammation is a major long-term pathological response to single mbTBI.^{83,96}

In reality, most service members are exposed to multiple mild explosive blasts⁵⁷; therefore, modeling the long-term consequences of rmbTBI is critical to understanding its pathomechanism. Identifying the WICV after mbTBI is especially critical for determining the time for “safe return to duty,” a highly important issue for force readiness as well as for the short- and long-term health of service members. In a set of experiments using the rat model of blast, the effects of single and multiple (five total, one per 24 h interval) mild blast overpressure (BOP) exposure on neurobehavioral outcomes, plasma and brain tissue levels of select protein biomarkers, and cellular changes were determined at 2 h and 22 days post-injury.¹⁰³

Repeated exposure resulted in increased anxiety but no significant memory impairments at the acute time point (2 h), and there were no changes in the plasma levels of select protein biomarkers (vascular endothelial growth factor [VEGF], neurofilament-heavy chain [NF-H], neuron-specific enolase [NSE], and glial fibrillary acidic protein [GFAP]) after single or repeated BOP exposure. At 22 days post-injury, all four markers were significantly elevated after single and repeated exposure, although there were no significant differences between values obtained from the single versus multi-injured animals, suggesting an absence of a cumulative effect of repeated exposures at this time interval.

Histological analysis showed significantly increased numbers of TUNEL+ (terminal-deoxy-transferase-mediated dUTP nick-end labeling)-positive cells in the dorsal and ventral hippocampus of both injured groups as early as 2 h after injury. At 22 days, the increase in apoptotic cells was limited to the ventral hippocampus of multi-injured animals. These findings suggest that the exposure to mild BOP triggers early hippocampal cell death, as well as neuronal, glial, and vascular damage and transient increases in anxiety-related behaviors. There were no significant differences in the severity of pathological outcomes, however, between single and multi-injured animals at the 24 h interinjury interval. Explanations include a potential conditioning phenomenon that is triggered after the first injury, thereby counteracting/mitigating subsequent damage at this rate of injury.^{113,114} Daily mild blast exposure (for three days) increased conditioned fear in a rodent model of rmbTBI, which the authors interpreted as a decrease in inhibitory behavioral control because of repeated mild blast exposure.⁸¹ A more detailed analysis of the effects of single versus repeated blast exposure, using the same injury paradigm in rats, also showed no significant group differences in the serum levels of 13 protein biomarkers associated with vascular changes, inflammation, neuronal and glial damage at 42 days post-injury.⁹⁰ These findings further support the conclusion that under the blast conditions used and outcome measures tested, there is no cumulative effect of multiple injuries if they take place 24 h apart.

We also analyzed a subset of single and multi-injured brains from the same set of experiments using DTI.⁹² The brains were analyzed by *ex vivo* DTI at 2 h and 42 days after single and multiple blast (or sham) exposure(s). We found significant interactions in

axial and radial diffusivity in a number of subcortical structures only at 2 h post-injury, and differences between single- and multi-injured rats were largely in the thalamus but not in the hippocampus. Neither hippocampal volume nor hippocampal fractional anisotropy were significantly changed by single or repeated blast injury. These findings further demonstrate that the frequency of blast exposures relative to the WICV play a critical role in inducing the pathological changes that culminate in chronic neurodegenerative conditions.

The rodent timeline, pertaining to specific physiological and pathological processes, can be different vastly from that of humans. Therefore, we shortened the interinjury interval to determine whether blast exposures repeated at a higher frequency would result in a cumulative effect.⁹¹ We exposed rats to mild BOP at a rate of one per 30 min (five total), monitored select physiological parameters (heart and breath rates, arterial oxygen saturation, and pulse distension) and behavior, and determined injury-induced changes in protein biomarkers in brain tissue and plasma. At this injury rate, oxygen saturation and heart rate were decreased transiently in multi-injured animals (resolved by 24 h) while anxiety- and depression-related behaviors were increased. Proteomic analysis of brain tissue indicated significant oxidative stress, vascular changes, altered cell adhesion, and inflammation that was most pronounced in the prefrontal cortex. Injury-induced changes detected in the various brain regions were also reflected in injured rats' plasma biomarker levels, suggesting robust and complex molecular changes that include inflammation.

In another rat experiment where the interinjury interval was shortened to one min, DTI showed a significant increase in microstructural damage with a second injury, primarily in white matter tracts, suggesting that the first mTBI can sensitize the brain to subsequent injury.⁷⁸ These and other studies^{75,115} suggest that the WICV is much shorter in the rat than in humans.

While these intervals seem (and are) unrealistic for humans, available experimental evidence show that in addition to normal biological processes, such as gestation, reaching sexual maturity, and life span among others,¹¹⁶ TBI-induced pathological processes (e.g., altered cerebral glucose metabolism, inflammation, axonal pathology, vascular changes/water transport) may run five to 100 times faster in rats than in humans. Accordingly, when it comes to complex pathobiological processes, as opposed to simple enzyme kinetics, rat hours seem to be more comparable to human days than to human hours.

Porcine models

The apparent bTBI-specific location of astroglial pathology at the depths of cortical sulci,⁶³ if confirmed by additional histopathological studies in humans, represents a major challenge for the experimental modeling of blast-induced CTE because it would require the use of gyrencephalic animals (e.g., pigs) as opposed to lissencephalic rodents.¹¹⁷ Available data show that TBI can have different consequences in the lissencephalic rodent brain versus gyrencephalic brains of humans, nonhuman primates, and pigs.¹¹⁸ Pigs have already been used successfully in TBI research.^{119–128} Several studies have shown that a single, nonblast injury can increase tau and p-tau levels in pigs. The most compelling and carefully controlled study used a rotational injury model for pigs and showed that rotational injury in the coronal plate induced tau and amyloid- β accumulation, and neurofilament inclusions.¹²⁷ Although the survival time was relatively short (up to 10 days), these findings may represent the precursor of CTE neuropathology.

Using swine to model civilian TBI, caused by kinetic and/or rotational forces, has provided a wealth of clinically relevant information about systemic changes and neuropathological responses to insults that strongly support using the pig model in blast-induced TBI. The Defense Advanced Research Project Agency-funded program, Preventing Violent Explosive Neurotrauma, modeled various military field scenarios and exposed Yorkshire and Yucatan pigs to explosive blast of differing intensities.⁴¹ This model has recapitulated successfully most of the clinically observed features of explosive bTBI, including systemic changes and vascular reactivity, and shed light on the effect of blast exposure(s) on intracranial, intraventricular, and intravascular pressures. Proteomic analysis of serum obtained at multiple time points (up to two weeks) after blast showed that a specific temporal profile of NF-H in serum is associated with injury severity and poor outcome.¹²⁹ All tested markers of neuron and glia damage showed time-dependent changes after blast exposure, but only the rapid peaking of NF-H in serum at 6 h post-injury correlated with poor clinical and pathological outcomes.

Analyzing injury-induced changes in the CSF enables us to identify true intracranial pathologies by determining CSF levels of not only markers specific to the nervous system, but also markers of inflammation, vascular injury, etc. Proteomic analysis of CSF samples, obtained from the same pigs and identical time points, also showed dynamically changing protein marker levels. After peaking at 6 h post-injury, all markers (except S100 β) showed a biphasic response with values peaking again at 72 h post-injury, indicating ongoing intracranial processes including vascular pathologies.⁹⁵ Indeed, Claudin-5, a major cell adhesion molecule involved in tight junction formation of endothelial cells, and VEGF, involved in the regulation of vascular function, were both significantly elevated in the CSF at 72 h after injury.

How these early pathobiological responses develop into chronic changes that ultimately result in CTE remains unknown. This unfortunate outcome is mostly because of limiting the analysis of single- and multi-injured pigs to neuropathology using basic markers, without obtaining blood and/or CSF samples at various post-injury time points to be analyzed by proteomics. Sadly, the opportunity to determine the temporal pattern of molecular changes that could contribute to the development of the observed terminal neuropathologies and elucidate the long-term effects of rmbTBI was missed.

Despite the unfortunate limitations, neuropathologic analysis of porcine brains provided some useful information about the long-term effects of single and repeated blast exposure. Histology showed strong evidence for astrogliosis, as indicated by increased GFAP immunoreactivity, and axonal injury, as indicated by β -amyloid precursor accumulation in axons.¹³⁰ Importantly, axonal injury was detected predominantly, if not exclusively, in periventricular regions while astrogliosis was found mainly in the hippocampus. Both areas, especially the periventricular region, are located at the interface of structures with differing acoustic impedance, a prime target for SWs.^{34–36,39,40}

Long-term neuropathological changes, nine months after single or repeated blast exposure, include microglial activation in addition to astrogliosis.¹³¹ Unfortunately, the authors did not spatially compare pig and human neuropathology in terms of assessing astroglial pathology deep in sulci for the presence of GFAP-positive astrocytes. Nonetheless, the tested markers showed a dose-response effect whereby numbers of immunoreactive astroglia and microglia were significantly higher in the brains of animals exposed to two blasts (one week apart). Interestingly, the authors did not find

evidence of tau-immunoreactive neurofibrillary tangles, astrocytic tangles, or tau-reactive astrocytes at this late time point.

Summary, Caveats, and Recommendations

In light of the existing studies that delivered very limited information,^{130,131} there is an urgent need to perform additional longitudinal studies using high-fidelity modeling of explosive bTBI with gyrencephalic animals (e.g., mini-pigs). These studies should not only use terminal neuropathologic analysis, but also functional/neurobehavioral, imaging, as well as blood- and CSF-based proteomic assays at multiple post-injury time points. These methods all have high clinical relevance, and data will be translatable readily into human use.

The use of pigs as model animals for bTBI has, however, several caveats. Cadaver studies have shown that the human head has a much different biomechanical response to blast compared with the head of other species.³⁴ This is because of a number of factors that include head size and shape as well as skull thickness and elasticity. A particular challenge to model blast TBI in pigs (as well as in rodents) is to determine how to scale blast conditions experienced by humans to the experimental species.

Computational modeling indicated that the human brain is more vulnerable to blast than any other mammalian species including rodents and pigs. The differing vulnerability is because of the differences in the mass of protective tissues (hair and skull) relative to the mass of the brain and because of difference in acoustic impedance of tissues between species.¹³² The impact of the shock front on the skull, the transfer of the SW across the skull, and the movement of this wave within the cranium are all affected by the size, geometry, and thickness of the skull and the directionality of the blast wave relative to the subject.^{34–36,40,133,134}

Using pigs in blast TBI research presents some unique challenges such as the logistics of handling, housing, and preparing the animals for exposures, performing the exposures with real explosives, and post-injury animal care, especially long term.⁴¹ Using pigs (even mini-pigs) would render some of the existing blast facilities inadequate, because most of the shock tubes are too small to allow positioning of the animal inside the tube without causing blockage.^{34–36}

It is critical to remember that in most real case scenarios, blast affects the entire body, not only the head.³⁷ The energy from blast waves can travel to the brain from other body parts via (large) blood vessels. This “vascular load” can also contribute to the pathogenesis of bTBI.⁹⁸ Importantly, sensory organs, eyes, and ears are most frequently affected by exposure to explosive blast.^{135–142} Understanding how injuries to the visual and auditory/vestibular systems influence the pathomechanism of bTBI, especially long-term effects, is important to better understand the pathomechanisms of bTBI, but it is outside of the scope of this review.

Careful experimental designs taking into account the physics of blast modeling can help to address key and still unresolved issues of (1) what is the pathomechanism of primary bTBI and rmbTBI (caused by SW) and (2) is the pathomechanism of primary bTBI different from that caused by kinetic forces. Simple designs can help to identify the pathomechanism of primary bTBI (caused by SW) versus the pathomechanism of kinetic forces generated by the blast. Placing the animals inside the shock or blast tube exposes them to SW, whereas placing animals outside or at the end of the tube will expose them to kinetic forces.

Many experiments (using rodents) inadvertently studied the effects of the kinetic forces but interpreted the data as the effect of

blast.³⁵ An example is the study that has drawn similarities between the neuropathology and the neurobehavioral deficits observed in single blast exposed mice, athletes, and blast exposed veterans.¹⁴³ The fundamental flaw in the experimental design was that the animals were incorrectly positioned and exposed only to kinetic forces. Not surprisingly, the effects the authors found were very similar to the civilian type of TBI caused kinetic forces.

Of particular importance in studying the pathomechanism of rmbTBI is to know the window of cerebral vulnerability after the insult in the experimental species. Determining how to “scale” time between different species e.g. rodents vs. human or pigs vs. humans is challenging because available experimental data suggest that the WICV may be different for different pathologies, inflammation, metabolism, vascular and axonal pathologies (Agoston et al, to be submitted). Determining the “scalability” of time is important particularly for the neuroinflammatory process that appears to play a key role in CTE pathomechanism.^{144,145} Additional challenges in high fidelity modeling of the long-term effects of rmbTBI are to incorporate conditions typically accompanying mbTBI, such as stress, sleep deprivation, medications, comorbidities, etc., into experimental modeling.

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