



Animal Models of Traumatic Brain Injury and Assessment of Injury Severity

Xiaotang Ma¹ · Aswati Aravind¹ · Bryan J. Pfister¹ · Namas Chandra¹ · James Haorah¹ 

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Abstract

Traumatic brain injury (TBI) contributes a major cause of death, disability, and mental health disorders. Most TBI patients suffer long-term post-traumatic stress disorder, cognitive dysfunction, and disability. The underlying molecular and cellular mechanisms of such neuropathology progression in TBI remain elusive. In part, it is due to non-standardized classification of mild, moderate, and severe injury in various animal models of TBI. Thus, a better diagnosis and treatment requires a better understanding of the injury mechanisms in a well-defined severity of mild, moderate, and severe injury in different models that may potentially reflect the various types of human brain injuries. The purpose of this review article is to highlight the classification of mild, moderate, and severe injury in various animal models of TBI with special focus on mixed injury that represents a translational concussive head injury. We will classify animal models of TBI broadly into focal injury, diffuse injury, and mixed injury. Focal injury, a localized injury, is represented by animal models of controlled cortical impact, penetrating ballistic-like brain injury, and Feeney or Shohami weight drop injury. A global diffuse injury is best represented by shock tube model of primary blast injury, and Marmarou or Maryland weight drop model. A mixed injury consists of focal and diffuse injury which reproduces the concussive clinical syndrome, and it is best studied in animal model of lateral fluid percussion injury.

Keywords Traumatic brain injury · Animal models · Injury severity · Mixed injury · Focal injury · Diffuse injury

Introduction

Traumatic brain injury (TBI) is caused by an external mechanical force such as concussive head injury, penetration of objects, sudden acceleration-deceleration of motor vehicle accidents, and propagation of supersonic blast waves [1]. There are about 1.7 million people who suffer TBI each year in the USA, which accounts for more than 3 million people living with a life-long disability [2]. The number of TBI incidence is still rising with 2.8 million TBI-related emergency hospital visits and deaths in 2013 in the USA, consisting of about 54% children and 79% seniors that resulted from falls [3]. The incidence of motor vehicle accidents, athletic sports head injury, and blast-wave diffuse injury is also a major cause of TBI [4]. Many of these brain injuries when

left untreated properly lead to development of cognitive/behavioral impairment [5, 6], PTSD [7–9], chronic encephalopathy [10–12], epileptic seizure [13, 14], and neurodegenerative Alzheimer's disease [15–17]. The prevention of TBI-related neurodegenerative disorders has not been satisfactorily successful despite the number of clinical trials and treatment [18, 19], which was partly attributed to clinical irrelevance of the animal models [20]. Yet, we have to consider the fact that TBI in itself involves complex mechanisms of brain injuries in human, which poses challenges for accurate replication of human TBI in animal models. Thus, there is not a single animal model that can accurately mimic human brain injury [21]. However, animal models of TBI offer the best alternative to examine the biomechanical, cellular, and molecular mechanisms of TBI-associated neuropathological progression and the time-dependent effects.

The assessment of injury severity post-TBI has played a critical role for an effective evaluation of therapeutic treatments. In clinical settings, the Glasgow Coma Scale (GCS) [22, 23] or improved/extended GCS (GCSE) [23] is used for the severity of TBI diagnosis and prognosis. The GCS is based on a 15-point neurological scale according to eye, verbal, and motor movements. The clinical guidelines incorporating duration of loss of

✉ James Haorah
james.haorah@njit.edu

¹ Laboratory of Neurovascular Inflammation and Neurodegeneration, Department of Biomedical Engineering, Center for Injury Bio Mechanics, Materials and Medicine, New Jersey Institute of Technology, Newark, NJ 07102, USA

unconsciousness and structural imaging with GSC classify human TBI into mild, moderate, and severe level. However, there is no established guideline or standard to assess injury severity in experimental TBI, which makes it challenging for animal model findings to a clinical translation. Such differential parameters for a consensus standardization of injury severity in various TBI models include the mechanical parameters like the peak pressure and duration [24]; neurological changes like apnea, righting reflex time, and pinna reflex [25]; physiological changes like weight loss and intracranial pressure increment [26]; histological changes like infarct volume and neuronal loss [27]; and behavioral changes like the neurological severity score (NSS) and motor function tests [28]. Even though there is no consensus on grading standard injury severity, this review article provides the up-to-date knowledge on severity of injury criteria for each model based on focal, diffuse, and mixed injury. We also discuss on the use of mortality rate and brain infarct volume as possible standard criteria for defining mild, moderate, and severe injury.

Table 1 shows the animal models of TBI, the types of injury, the clinical relevance, and the strengths or the weaknesses of each TBI model within the category of focal, diffuse, and mixed mechanisms of injury. The principal mechanisms of focal brain injury known as the localized tissue damage resulted from a blow to the head, car accidents, or violent assaults, while diffuse injury is caused by acceleration or deceleration impact including the unrestricted head movement in motor vehicle accidents or blast-wave propagation [29]. The mixed injury model represents the types of head injury resulted from falls or athletic sports injury. In this review article, the rat model of fluid percussion injury (FPI) typifies the mixed injury, the open-skull controlled cortical impact (CCI) model mimics the focal injury, and shock-wave model represents the mechanisms of diffuse injury. Irrespective of focal, diffuse, or mixed type of injury, the nature of TBI involves the damage mechanisms of primary and secondary injury. Primary injury is directly caused by mechanical pulse/force that includes surface contusion, blood vessel damage, laceration, hemorrhage, and perhaps axonal shearing [26]. The secondary injury is a consequential event of the primary injury that involves the cascade of biochemical activation including molecular, cellular, and metabolic events that lead to neuroinflammation, neurodegeneration, and atrophy [11]. The biochemical mechanisms of injury in these various animal models include oxidative stress, inflammation [30], apoptosis [31], necrosis [32], diffuse axonal injury [33], neurodegeneration, and cognitive deficits [34] at the impact sites and remote sites.

Diffuse Injury Model—Shock Tube Model

In this review, we describe the mechanisms of diffuse injury represented by a rodent model of shock-wave primary blast injury. Primary blast injury has attracted much

Table 1 Animal models of traumatic brain injury discussed in this review

Model	Injury	Clinical relevance	Strength	Weakness
Fluid percussion Midline lateral	Mixed Mixed	Direct brain deformation. Cerebral concussion, focal contusion. Example: Sports-related TBI: boxing, football	Well characterized; severity of injury can be easily adjusted; highly reproducible across laboratories	Requires craniotomy; no immediate post-injury neuroscoring; high mortality rate due to apnea
Controlled cortical impact Open skull Closed skull	Mainly focal	Direct brain deformation. Cerebral contusion with subarachnoid hemorrhage. Example: Sports-related TBI: boxing, football	Severity of injury can be controlled; highly reproducible among laboratories	Lack of brain stem deformation, resulting in minimal mortality; no immediate post-injury neuroscoring
Weight drop Feeney Shohami Marmarou	Mainly focal Mainly focal Mainly diffuse	Hemorrhage and diffuse axonal injury. Example: falling down, motor vehicle accidents	Mechanism is similar to human TBI, severity of injury can be adjusted; well-characterized neuroscoring post-injury; inexpensive, easy and convenient	High mortality rate due to apnea and skull fractures, possibility of rebound injury, chance of inaccuracy
Blast wave model Shock/blast tube	Diffuse	Blast-wave-induced TBI (battlefield)	Similar to soldiers in battlefield	Standardization is needed

attention in recent time because of the serious injury happening in the battlefield [35]. Primary blast-wave injury is a pure form of diffuse injury in which the damage is caused by non-penetrating supersonic blast-wave loading impulse. Shock tube blast wave is generated in the laboratory conditions by using compressed air or by field detonation of explosives. The commonly used animal species in these various blast-wave models include rodents [36], swine [37–39], and primates [40]. The shock tube model is more reproducible than field explosion due to precise control [39, 41]. Thus, laboratory design of shock tube models uses the linear propagation of primary blast-wave energy inside the cylindrical tube, unlike the exponential decay of blast wave that is commonly observed in free-field explosions [37, 42, 43]. The shock tube is made by a cylindrical metal tube consisting of a gas compressor chamber and a blast-wave energy propagation tube with a catch tang metal diaphragm at the end of the tube [41]. Figure 1 shows the laboratory design of shock tube model for exposing the animals in the middle location, at Center for Injury Bio Mechanics, Materials and Medicine, New Jersey Institute of Technology.

In shock tube models, anesthetized animals are restrained in a specimen holder inside, at the exit or outside the propagation tube. The animals are fixed with a Kevlar thoracic protective vest to protect the thorax and part of the abdomen. The pressure wave is generated by releasing the compressed air from the compressor chamber along the closed cylindrical metal tube. The mechanical parameters of the shock wave include the peak pressure, duration, and the shape of the pulse. The mechanism of blast TBI as a closed skull injury is related to the movement of the brain inside the skull caused by rapid rotation of the head [44]. Tissue distortion and shearing produced by inertial forces is believed to cause diffuse axonal injury, diffuse brain swelling, and ischemic brain injury [33, 36, 42, 45, 46].

Locations and Orientation of Test Animal in Shock Tube

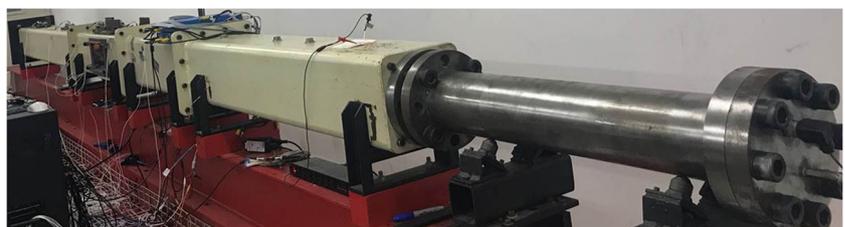
The location of specimen inside, near the exit or outside the shock tube, produces different severity of injury and mortality due to varying mechanical loading [47]. Dynamic loads on animals at different location along the shock-wave tube are

associated with static and time-dependent overpressure pulses. When the animal is placed inside the tube, it is insulted by a load from the pure blast wave comparable to the shock wave generated in open-field conditions. On the other hand, the animal at the exit point undergoes a decay of the initial shock front due to expansion of pressure wave, which is caused by expansion wave from the exit of the shock tube eliminating the exponentially decaying blast wave [47]. This phenomenon causes significant reduction of the positive blast impulse and converts most of the blast energy from supersonic blast wave to subsonic jet wind. This was demonstrated in a blast TBI model by placing the rat 50 mm outside of the shock tube, in which the bulk of the blast impulse was found to be subsonic jet wind. However, the injury was caused by the combination of subsonic jet wind and blast wave, compared with the animal inside the tube [48]. Recent studies using human and animal surrogates at different locations in shock-wave tube model concluded that there are significant deviations when surrogate specimens are placed inside, near exit, or outside the shock tube [49]. The orientation of specimen in prone or supine position inside the shock tube was also found to produce different severity of injury and mortality [47]. The placing of animals in prone or supine position is tailored to mimic differential positions of soldiers in the battlefield. The most common position of animal model in shock tube is placed in prone position with head oriented towards the direction of blast-wave propagation [50, 51].

Assessment of Mild, Moderate, and Severe Injury in Blast TBI

Clinical assessment method such as the Glasgow Coma Scale is not possible to define the severity of brain injuries in animal models because the severity of TBI in human is assessed in verbal response to the state of consciousness immediately at post-injury. It is also challenging to assess animals at such state of consciousness without considering the effects of anesthesia at post-injury. The NSS is commonly used in animal model to assess the severity of injury. This may not be the appropriate factors to define mild, moderate, and severe types of TBI in pure primary blast injury, since the determination of NSS was derived from Shohami model

Fig. 1 Shock-wave tube model. An overview of the 9-in. square cross-section shock tube instrument with pressure sensors designed by Dr. Namas Chandra's group



of focal injury [52, 53]. However, the NSS index score of lower than 20 at 1 h post-injury could be a useful indicator for survival rate, assuming that the neurological severity score within zero to few minutes post-injury is 24 total points. As per NSS, the animal has loss of movement, walking straight, righting reflex, eye reflexes, limb reflexes, beam walking and balancing, and seeking behavior, and there is presence of hemiplegia at this maximum point of 24. These clinical functions will improve during recovery period, which means NSS points will decrease from the total point of 24, and if the NSS points of certain animals remain above 20 at 1 h post-injury, those animals will be non-survival. It is stated that the change in NSS from 1.0 to 24 h post-injury reflects the recovery improvement, which is used for assessment of potential neuroprotective agents but not necessarily defining the classification of mild, moderate, and severe types of TBI.

Cernak et al. (2011) investigated the pathophysiological consequences and functional deficits of graded blast TBI by a developed multi-chamber shock-wave tube model. They tested both supine and prone position of mice in the shock tube, and generated mild, moderate, and severe injury at 103 kPa, 124 kPa, and 190 kPa, respectively. Mortality rate according to mild, moderate, and severe level is 5%, 37%, and 53% at supine position and 0%, 11%, and 33% in prone position. Prone position leads to less injury severity in mortality rate and pathology scoring system compared with supine position [54]. We made an attempt to standardize the scale of mild, moderate, and severe injury in animal model of TBI using the shock tube primary blast [51]. The injury scale determination was based on the percent of rat survival in response to blast overpressure (BOP) dose-dependent from 60 to 450 kPa within 2–8 ms wave propagation. The mortality rate and severity of brain injury increases with increments in BOP and impulse dose-dependently. Moreover, blood-brain barrier (BBB) damage is most commonly observed in BOP-induced traumatic brain injury; thus, the severity of brain injury can also be assessed by the extent of the BBB oxidative damage [51, 55]. A sigmoidal survival over BOP dose-response curve determined the mortality rate. Using this survival-BOP dose-response curve, the upper threshold of mild TBI (145 kPa), moderate TBI (146–220 kPa), and severe TBI (221–290 kPa) were determined based on the logistic regression [55]. As per the logistic regression model, the predicted mortality rates were < 5%, 35%, and 70% respectively for upper mild TBI threshold, moderate, and severe TBI. Although behavior or cognitive function assessment is absent, classification of TBI into mild, moderate, and severe injury on the basis of mortality rate is reliable in animal model because such changes in mortality rate are further validated by neuropathological markers and mechanical injury parameters.

Focal Injury Model: Controlled Cortical Impact Injury Model

The direct cause of focal brain injury such as ballistic injury, contusion, or boxing injury is best reflected in laboratory experimental condition by animal model of CCI as shown in Fig. 2. The model was first developed in ferret [56] and then expanded to rats [57], mice [58], swine [59], and monkeys [60] as experimental subjects. Figure 2 shows the classical rodent model of controlled cortical impact. The brain impacted through a surgically exposed dura by rapidly accelerated rod in which computer-guided software controls the depth of the impact. The size and geometry of the rod can be varied for different species, and the rod is under the control of a pneumatic piston or electromagnetic actuator [57]. The rod hits directly on the exposed brain tissue, which causes blood vessel breakage (blood-brain barrier damage), cortical tissue loss, acute hematoma, and brain cellular injury. Thus, CCI model induces significant cortical contusion, deep brain injury, and neurodegeneration around the impact site mostly at ipsilateral cortex [61]. In recent years, CCI model is expanded to closed skull injury for investigating mild and repetitive TBI [61, 62]. With this improved device, the model appears to take advantage of generating controlled tissue deformation, controlled time, velocity, and depth of impact without causing the risks of rebound injury, which otherwise is commonly observed in gravity-driven weight drop injury (WDI) model. This improved CCI model is believed to offer better understanding of the underlying cellular, biochemical, and molecular mechanisms of brain injury than other models since it has a well-controlled mechanical parameters and depth of brain tissue damage [63].

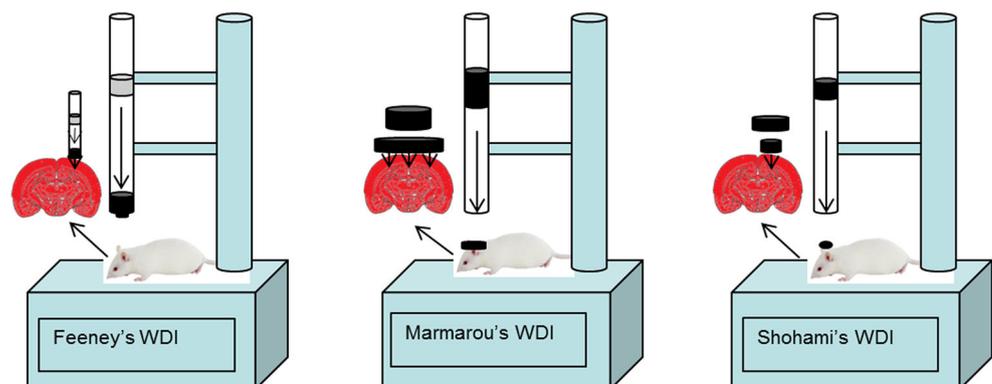


Fig. 2 Controlled cortical impact device fabricated by Amscien Instruments. This model uses pressed air or an electromagnetic-driven piston to penetrate the brain at a known velocity and distance

Assessment of Mild, Moderate, and Severe Injury in CCI Model

The CCI model of TBI has produced a wide range of neurohistopathology and behavioral outcomes as a result of mild, moderate, and severe TBI. There is no gold-standard definition of mild, moderate, and severe injury in CCI model. The depth of the tissue injury and the velocity of the rod that impacts the head injury determine the type of mild, moderate, and severe injury in open-skull CCI model. The depth of injury and velocity of the rod differ from one lab to the other for generating mild, moderate, and severe injury depending on the calibration of the CCI device. For example, Yu et al. induced brain injury in rat at the depth of 0.5 mm, 1.0 mm, or 2.0 mm with a velocity of 6.0 m/s to generate mild, moderate, and severe TBI respectively [2, 64], while Washington et al. generated brain injury in mice at the depth of 1.5 mm, 2.0 mm, and 2.5 mm with a velocity of 5.25 m/s for mild, moderate, and severe TBI [65]. Interestingly, there is a similar pattern of brain damage from these independent findings as observed in the visualization of metabolically active brain tissue from dead tissue. In this regard, there was about 10%, 25%, and 40% tissue damage in rat and about 5%, 15%, and 30% tissue damage in mice that corresponded to mild, moderate, and severe respectively [65]. However, the mechanical parameters differ across different laboratories, for example, in a recent study, Wang et al. induced mild, moderate, and severe CCI by depth of 0.2 mm, 1.0 mm, and 1.2 mm at a velocity of 3.5 m/s [66]. These different definitions of injury severity make it challenging to clarify specific sequelae and treatment development according to severity levels. Recently, Siebold et al. conducted a PubMed search on articles that used CCI mice models in the past 5 years. After thorough evaluation and analysis, they proposed guiding principles by recommending the use of depth < 1.0 mm and velocity < 4.0 m/s for mild TBI; the use of depth 1.0–1.5 mm and velocity 4.0–5.0 m/s for moderate TBI; and the use of depth > 2.0 mm at velocity 5.0–6.0 m/s for severe level [67].

Fig. 3 Weight drop injury models. In the Feeney's weight drop model, the weight is delivered directly onto the exposed dura through craniotomy. In the Marmarou's weight drop model, a metal disk is placed over the skull to protect bone fractures and mimic diffuse TBI. In the Shohami's model, the weight is dropped on one side of the unprotected skull, to duplicate focal injury



Weight Drop Injury Model

Focal Injury WDI model has been used in rodents for several decades to promote understanding of pathophysiology of TBI [68]. As shown in Fig. 3, it utilizes the gravitational forces of a free-falling weight to generate a focal [69] or diffuse brain injury [70]. Initially, Feeney's WDI model was developed to deliver the impact to the intact dura through a craniotomy that leads to cortical contusion [71, 72], hemorrhage lesions [73], BBB damage [74, 75], infiltration of immune cells, and activation of glial cells according to the severity of injury [76, 77]. Later on, the closed head WDI rodent model of Shapira et al. (1988) and Chen et al. (1996) introduced the fixing of head on a hard platform to avoid impact of acceleration diffuse injury in rat and mouse [69, 78]. Then modified models of shorter fall heights were developed to generate concussion, cell death, short-term edema, and long-term cognitive deficits [73]. In general, such weight drop injury models in mild WDI are related to a diffuse injury pattern while the more severe injury generates a focal contusion [79].

Diffuse Injury The Shohami, Feeney, or Shapira weight drop models of focal injury are different from Marmarou WDI model of diffuse TBI. Marmarou model is resulted from "whole head" accelerated motion that represents human diffuse TBI from falls or car accidents [80]. The whole head acceleration and rotational acceleration models are capable of producing hemorrhage, cell loss, diffuse axonal injury, and astrogliosis from mild to severe range [81]. This injury model was designed to mimic motor vehicles accidental injury caused by rotational acceleration following linear acceleration injury [33]. The accuracy of brain tissue deformation and injury severity is highly variable in these models because of the involvement of rebound impact and variation of impact velocity by the falling impactor. Marmarou WDI model of diffuse TBI was further modified as Maryland model to cause frontal acceleration impact [82] or lateral impact on a helmet-protected head to mimic concussive head injury in football players [83]. Weight drop models of Shohami's, Feeney's, or

Shapira's are also utilized in repeated mild concussive TBI studies, as mixed type of injury models [69, 71, 78].

Assessment of Injury Severity of Weight Drop Injury

Unlike the classification of other animal models as mild, moderate, and severe levels from pathological and functional changes, WDI severity level is less investigated in the past. In WDI, injury severity is related to the gravity force, which is associated by the different mass of the weight and the height it falls. NSS is used to evaluate the neurological impairment for the assessment of injury severity in WDI models. Khalin et al. (2016) used a modified mouse closed head Shohami model, to investigate cognitive deficient assessments [84]. They induced severe weight drop injury using 250 g falling weight from 2 cm height, while 200 g weight falling from 2 cm height generated mild closed head injury in mice, depending on the cognitive deficiency. By using Marmarou's impact acceleration model, Hsieh et al. (2017) claim that the WDI model can generate graded brain injury as well as neurobehavioral deficits [85]. Mild, moderate, and severe WDI was generated by using a 450 g weight falling from 1.0, 1.5, and 2.0 m height, and the mortality rates were 0%, 12.5%, and 50%, respectively. They observed progressive degrees of injury severity in the neurological score and balance function, which is associated with graded mechanical impact levels. Overall, WDI models provide a simple way to mimic clinical conditions ranging from focal to diffuse injuries, and it is inexpensive and capable to reproduce graded DAI. However, the risk of rebound impact and variation of impact velocity by the falling impactor may lead

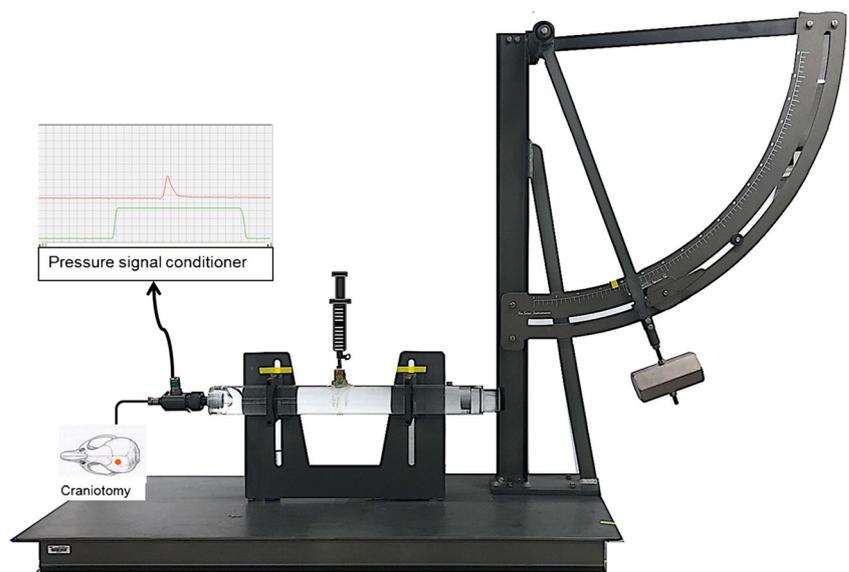
to failure of accurately describe the brain deformation, and the injury severity is relatively high variable.

Mixed Injury Model: Lateral Fluid Percussion Injury Model

Lateral fluid percussion injury (LFPI) is one of the best characterized and mostly used animal models of TBI. The injury in FPI is induced by a pendulum striking the piston at the end of a tube filled with fluid, shown in Fig. 4. The fluid pressure impulse then hits the exposed dura directly through the Luer-Lock that has been implanted surgically known as craniotomy [86]. Briefly, a Luer-Lock is implanted into a 3.0-mm diameter of an opened skull circular window, which is connected to FPI device fluid impulse outlet and fluid pressure detector. In this way, the fluid pressure delivered on the exposed dura is accurately measured by the detector at the time of injury. The location of the craniotomy is the site of Luer-Lock implantation and the site of impact, and it defines the types of injury in FPI models. Thus, when craniotomy is performed at the center of the sagittal suture, it is known as Midline FPI [87]. In Lateral FPI, craniotomy is performed at 3.5 mm lateral to midline [88], and for parasagittal FPI, the Luer-Lock is implanted less than 3.5 mm lateral to midline [89, 90]. Brain tissue deformation, displacement, and severity of the injury in these types of FPI are resulted from fluid pressure pulse [88]. The pathophysiological changes in FPI are correlated with the pressure transients, in which upper mild, moderate, or severe type of pressure impulse causes focal and diffuse injury, while the lower threshold of mild pressure impulse produces diffuse injury [68].

The application of FPI model is used for replicating the human TBI without skull fracture that generates edema,

Fig. 4 Fluid percussion injury device with pressure signal conditioner. The fluid pulse impacts at the exposed dura by craniotomy



hemorrhage, and gray matter damage [86]. The primary type of injury in fluid pressure impulse includes contusion, shearing/stretching of tissue, subdural hematoma, and hemorrhage as seen in concussive sports injury [91]. Such primary injury is followed by secondary mechanisms of molecular, biochemical, and cellular changes arising from focal and diffuse injury. The vascular disruption like hemorrhage and on-site necrotic cell death are likely an outcome of primary injury, while activation of inflammatory glial cells and neuronal cell death could be resulted predominantly from secondary mechanisms of injury. The LFPI model has the advantage of comparing the extent of damage in ipsilateral (injured side) and contralateral (non-injured side) of the brain hemisphere. LFPI produces focal cortical contusion and diffuse subcortical neuronal injury in the ipsilateral side of the brain [92], which is also believed to be minimally expanded in contralateral hemisphere. A post-injury cavity line is generated around glial cells at the impact site, which appears to result from continuous chronic cell death [93]. This progressive neurodegeneration that begins from seconds to year post-injury at the ipsilateral side of brain region appears to occur predominantly at the cortex, hippocampus, thalamus, striatum, and amygdala [94].

Such differential regional neuronal loss in LFPI appears to produce neurobehavioral impairments that are observed in TBI patients, such as reflex suppression, vestibulomotor function, and cognitive function [73, 95]. The impairments are believed to continue for years after severe LFPI [96]. Reliability and reproducibility of FPI-induced neurodegeneration, neurological impairments, and mortality rate are dependent on the location of craniotomy. This is because a small shift from the midline FPI (MFPI) can cause significant changes in brain infarct volume and neurological outcomes. The mortality of MFPI is higher than other models, which is due to brainstem-compromised apnea [26]. The LFPI is the improved model of FPI that has reliability and reproducibility to human head injury in terms of neurohistopathology, behavioral outcomes, and physiological function like blood pressure or electroencephalogram. In addition, unraveling of the cellular, molecular mechanisms of injury and subsequent development of experimental therapy is best studied in LFPI model. The evolution of recent computer-controlled LFPI device that can monitor the fluid percussion waveforms with adjustable rise times, peak pressures, and durations is an advanced reliable improvement to the model [97].

Assessment of Mild, Moderate, and Severe Injury in FPI Model

Perhaps the definition of mild, moderate, and severe injury is the best established in animal model of FPI. The strength of the injury classification lies in the combination of mortality rate, the pressure exposure range, the righting reflexes, and

reliably reproducibility of the injury model. For example, Alder et al. (2011) performed mild to moderate injury in mice model of LFPI on the basis of mortality rate, pressure range, and righting reflexes [91]. Injury pressures are delivered in the range of 0.9–2.1 atmospheric (atm) pressure that produces mild injury with 0–5% mortality rate and a righting reflex time of 2–4 min or moderate injury with a mortality rate of 10–20% and a righting reflex time of 6–10 min.

Kabadi et al. (2010) described that mild, moderate, and severe injury occurs at 1.8–2.2 atm (26–32 psi), 2.4–2.8 atm (35–41 psi), and pressures greater than 2.8 atm (41 psi), respectively in standard lateral FPI model (Amscien Instruments) [98]. They also established these classifications in developed rat model of microprocessor-controlled, pneumatically driven instrument, micro-FP device. With micro-FPI, pressures of 2.2–2.4 atm (32–35 psi), 2.6–2.8 atm (38–41 psi), and > 3.0 atm (44 psi) correspond to mild, moderate, and severe magnitudes of injury, respectively. They observed that mortality rates were 0% at 2.4 atm, 12% at 2.6 atm, 20% at 2.8 atm, 33% at 3.0 atm, and > 65% above 3.2 atm. The cognitive performance, lesion volume, and neuronal loss were significantly different between mild and moderate level. Similarly, Prins et al. (1996) classified the types of injuries in rat model of FPI through a comparative study on mortality rate, morphology, intracranial pressure, and mean arterial blood pressure [99]. They investigated mild pressure range from 1.35 to 1.45 atm (20–21 psi), moderate injury pressure range from 2.65 to 2.75 atm (39–40 psi), and severe injury pressure range from 3.65 to 3.75 atm (54–55 psi). The mortality rate corresponding to mild, moderate, and severe injury pressure range was respectively 27%, 36%, and 100% for postnatal 17 days, and 0%, 20%, and 55% mortality for adult rats [99]. They reported that the duration of apnea for adult rat was 10.4 s, 20.3 s, and 24.9 s and duration of unconsciousness was 97.8 s, 183.0 s, and 291.2 s for mild, moderate, and severe, respectively. The increment of intracranial pressure and mean arterial blood pressure appeared to contribute the mortality rate in the corresponding mild, moderate, and severe injuries. Moreover, weight loss post-injury is also correlated with injury severity. Mild, moderate, and severe injury was found to result in 0–10%, 15–20%, and 25–30% weight loss, respectively [25]. Here, we summarized the injury severity range from all these FPI studies where a mild TBI is defined at the pressure range of 0.9–1.5 atm (13–22 psi) [30, 100, 101], moderate TBI range is at 1.6–2.5 atm (23–37 psi) [102–104], and severe injury is defined above 2.5 atm (37 psi) [105–107], as given in Table 2.

The suppression time of reflexes such as pinna, cornea, and righting has been established as a standard indicator of neurological evaluation in animal model immediately after FPI. The pinna reflex is defined as a head shake or twitching of ear

Table 2 Parameters to assess injury severity levels for each type of animal models

Injury model	Injury severity	Parameters to assess injury severity				References
		Pressure range (atm)	Mortality rate	Righting reflex time (min)	Apnea (s)	
Fluid percussion injury (lateral FPI)	Mild	0.9–1.5	0–5%	2–4	~ 10.4	[91, 99–107]
	Moderate	1.6–2.5	10–25%	6–10	~ 20.3	
	Severe	> 2.5	> 33%	> 10	~ 24.9	
Weight drop injury (Marmarou model)	Injury severity	Weight (g)	Height (m)	Mortality rate		[85]
	Mild	450	1.0	0%		
	Moderate		1.5	12.5%		
Controlled cortical injury (open skull)	Injury severity	Depth (mm)	Velocity (m/s)	Infarct volume		[65–67]
	Mild	< 1.0	< 4.0	5–10%		
	Moderate	1.0–1.5	4.0–5.0	15–25%		
Blast TBI shock-wave tube	Injury severity	Blast overpressure (kPa)	Mortality rate			[51]
	Mild	80–145	< 5%			
	Moderate	146–220	35%			
	Severe	221–290	70%			

when touching the inside of the pinna with a cotton-tip applicator. The corneal reflex is when there is a blink response after touching the cornea gently with a saline-moistened wisp of cotton. The righting reflex is examined by recording the time for the animal to roll over on their feet when they are placed on their back, for three times [108]. The non-invasive righting reflex appears to correlate with injury severity; thus, it is used commonly as an indicator of injury severity [95, 109]. Apart from righting, apnea, and pinna reflexes, the types of injury severity are also assessed by physiological functions such as arterial blood gases, vital signs, intracranial pressure, and electrophysiology.

It is true that there is no common standard for classification of mild, moderate, and severe injury in animal models, like the Glasgow Coma Scale in human TBI patients. The classification of mild, moderate, and severe injury in animal models differs from model devices, and from one laboratory to another. In FPI, some groups do not record the apnea duration since immediate ventilation support is given post-injury to alleviate apnea-induced effects. The use of different anesthesia methods may also influence the outcomes of injury, including apnea and righting reflex. In CCI model, the velocity of punch varies across laboratories, leading to different depth of injury. In addition to the depth of injury, brain infarct volume is used as a standard to assess mild, moderate, and severe TBI in CCI models. Although challenging, a reliable classification of mild, moderate, and severe TBI needs the combined knowledge of injury mechanics, mortality rate, neurological reflexes, physiological function, and histological outcomes.

Neurobehavioral Assessment in TBI

The neurobehavioral deficits that are commonly assessed in fluid percussion-induced traumatic brain injury include the functional outcomes of motor, cognition, and depression or anxiety-like behaviors. Motor tests measure the motor co-ordination, grip, and balance of the animal, while cognitive tests examine the working memory and learning in rats with the help of visual cues. Tests that measure anxiety and depression often study the explorative behavior of animals and correlate anxiety to decreased exploration in a new environment. Such behavioral deficiencies originating from fluid percussive TBI are mostly observed at the early time points of post-injury with gradual improvement over time depending on the severity of the FPI [110, 111], although the outcome measures after few weeks post-injury may not match sham animals at the same time-point [112]. A similar pattern of improvement in cognitive deficits over time has also been observed in cases of human TBI, which was implicated to compensatory strategies of the neurons or new learning [113]. The post-injury motor deficits in rats have been assessed by beam balance, beam walking test, ladder rung walk test [114], inclined plane test, rotating pole test, rope grip, and rota-rod test [115–118]. The rotating rod of constant or accelerating speed in rota-rod tests of the vestibular motor co-ordination appears to be the most sensitive measure in midline FPI model [119]. The rota-rod test is believed to be capable of detecting deficits in mild midline TBI-inflicted animals unlike the beam balance or the beam walk test.

The post-injury cognitive deficiencies in rats are commonly evaluated by using the Morris water maze test, radial arm

maze, and novel object recognition tests [120–122]. Both the lateral and midline FPI models are known to affect the hippocampus leading to deficits in learning and memory, which can last for days or weeks post-injury [25]. In a study comparing LFPI to weight drop impact-acceleration model with 10 min of secondary hypoxia, it was identified that the lateral FPI produced more cognitive impairments. The cognitive deficits remained higher in the LFPI model even when injury severity, measured by the righting reflex, was matched in the two models [123]. These findings suggest that cognitive deficits differ between an opened head injury and closed head injury model in spite of maintaining the same physiological condition. Although the controlled cortical impact (CCI) produces significantly more sensory deficits than LFPI, there was no significant difference found in the motor co-ordination and cognitive deficits between these two TBI models [124]. The sensory deficit was assessed by the bilateral tactile adhesive removal task. In FPI models, the location of craniotomy plays a key role in determining the extent of cognitive deficits. A small shift of 1–2 mm craniotomy location from the midline FPI can significantly affect the cognitive outcome, as such midline FPI is known to produce more severe cognitive impairments than the LFPI [89, 125].

A short-term exhibition of behavioral changes is anxiety-like symptom in rats at post-injury in LFPI model. It has been shown that there is a significant increase in plus maze time spent in the open arms at 24 h post-injury even at mild 1–1.5 atm pressure compared with sham control animals. This effect seems to decline gradually with time that the effect diminishes at 4 weeks post-injury [100]. Such short-term observation of plus maze elevation was true for moderate LFPI at 2.2 atm pressure; however, a depression-like behavior was exhibited by rats during novelty suppressed feeding, forced swim, and social interaction tests [126]. These findings indicate that anxiety-like behavior changes occur only in first few days at post-TBI. Thus, blunt injury like LFPI is increasingly associated with motor deficits, cognitive deficits, and depression/anxiety behavior that can last for a few days to weeks post-injury. Cognitive deficits in rodent blunt TBI model have been consistently reported by the Morris water maze test, while vestibular and motor co-ordination deficits have been demonstrated using beam balance, beam walk, and rota-rod tests post FPI [90, 111, 127]; these tests are the most investigated and well-established in behavioral studies of animal models. The severity of injury, the location of injury, and the types of open/closed head injury can affect the behavioral and cognitive function.

Future Scope

Although, animal models of head injury have been developed more than half a century ago, it is still an evolving field of

research. It is so because of the complexity of head injury itself and development of innovative technology devices for better understanding of the injury mechanisms. With such progress in head injury targeted devices, the scientific community is able to define the different types of injury mechanisms in focal, diffuse, or mixed injury that represent certain traumatic brain injury in human. One of the advancements made in such animal models is fluid percussive methods, which reproduces concussive clinical syndrome in human. The orientation of lateral fluid percussion injury has emerged as the most reliable, reproducible, and utilized models in experimental TBI. Bruce Lyeth (2016) comprehensively described the advantages of LFPI in his article “Historical Review of the Fluid-Percussion on TBI Model” [128]. The advantages of LFPI model include the comparison of injury vulnerability between contralateral (non-injured side) and ipsilateral (injured side) of brain hemisphere, and being able to distinguish focal injury and diffuse injury in the ipsilateral. Craniotomy involved in LFPI diminishes the rupture of sagittal sinus, blood loss, and reduction of venous blood outflow that was commonly observed in midline FPI model. The LFPI model appears to produce consistent cortical and hippocampal neuronal degeneration that recapitulate concussive TBI in human. The improvement of LFPI also includes the reduction of direct compression on the brain stem and respiratory center, which allows application of higher fluid percussive pressure with lower mortality rate than midline FPI.

It is obvious now that LFPI and CCI models have contributed much better understanding of the cellular and molecular mechanisms of TBI and its subsequent neurocognitive outcomes. The mechanistic nature of opened skull CCI model is purely a focal injury. In FPI, the upper threshold of mild to severe type of pressure impulse produces mostly focal and less of diffuse injury, but the lower threshold of mild pressure impulse causes diffuse injury. Here, we propose the sequence of injury event and the mechanisms of injury for a moderate LFPI pressure pulse exposure in experiment TBI, as shown in Fig. 5. Tissue deformation at the site of impact includes rupturing the subarachnoid and pial vessels that leads to intracranial hemorrhage of the ventricle area on the impact side. The subsequent event of hemorrhage is likely to produce subarachnoid coagulation preventing effective CSF clearance and thrombotic vessel occlusion. Such events of hemorrhage and coagulative thrombosis resulting from primary focal contusive injury are likely to cause necrotic cell death that is different from the secondary mechanisms of injury/cell death as depicted in the two arms of schematic pathways. The critical role of coagulation in trauma patients after TBI has become a reality that majority of TBI patients are treated with novel oral anticoagulants therapy [129]. The objective of anticoagulants therapy trauma patients is to prevent the intracranial hemorrhage after TBI. The same study reported that severity of intracranial hemorrhage, neurosurgical intervention, and

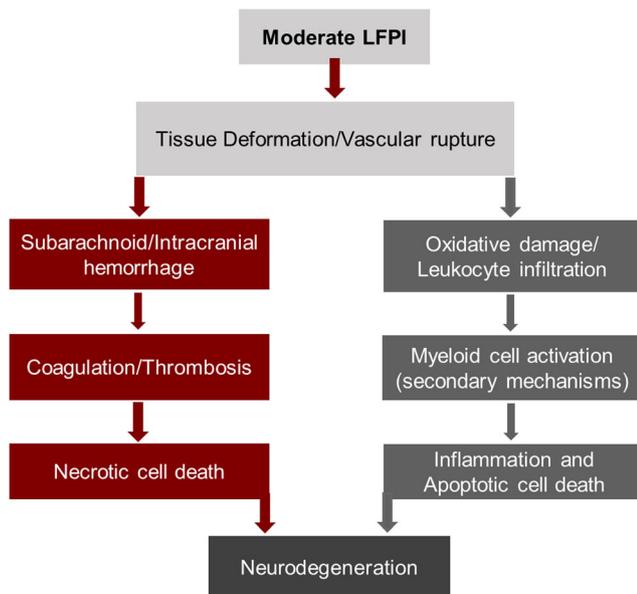


Fig. 5 Schematic drawing showing underlying mechanism of moderate lateral fluid percussion injury

mortality rate gets worsened in trauma patients who are on anticoagulants prior to brain injury. It makes sense because the presence of anticoagulants before or immediately after TBI will prevent the coagulative immune reaction with a possible outcome of bleeding to death, as we have observed in experimental TBI.

The consequence of primary injury leads to the propagation of secondary mechanisms of injury. It involves the alterations of molecular, biochemical, and cellular components that are mostly regulated by a sequence of signaling pathways. For example, there is immediate induction of oxidative stress and infiltration of immune cells at the deformed and vascular rupture area. Myeloid cells like microglia are spontaneously activated in response to ongoing necrotic cell death (right arm) and changes in brain microenvironment (left arm) as depicted in the pathways (Fig. 5). These cascades of inflammatory processes like the oxidative stress, neuroinflammation, and apoptotic cell death comprise the secondary mechanisms of brain injury and neurodegeneration. In LFPI model, such post-TBI neurodegenerative process appears to occur at certain specific brain region of the ipsilateral side. Whether such secondary mechanisms of neurodegenerative process can be classified as diffuse axonal injury resulting from rapid acceleration and deceleration of brain volume during TBI is debatable. Unfortunately, this knowledge gap in TBI arising from diffuse injury models still remains unclear, unlike in CCI/FPI models. A better understanding of the injury mechanisms proposed in the two arms of the pathways is expected to contribute successful regenerative therapy for millions of trauma patients. Such effective neuroprotective therapy is much needed for alarming incidence of 69 million individuals worldwide who sustain TBI each year as per recent epidemiological reports [4].

Conclusion

In order to strategize a proper diagnosis and an effective therapeutic application, we need to define convergent standard criteria for mild, moderate, and severe TBI in these various animal models of TBI. Such preclinical criteria in complex focal, diffuse, and mixed types of brain injury should be *in sync* with clinical outcomes. To this end, first, mortality rate in response to dose-dependent pressure force can be used as common criteria. Here, we postulate mortality rate of 0–5%, 6–25%, and greater than 25% to be classified as mild, moderate, and severe TBI respectively. Such mortality rate can be further correlated to the extent of brain infarct volume and histological validation in all animal models of TBI. Second, the types of injury mechanisms need to be defined in various TBI models for formulating a logical therapeutic application. In that, delineating the distinction between primary and secondary types of injury as described in the “Future Scope” section will be useful for effective therapeutic treatment of TBI. It may be reasonable to state that deficits of cognitive and motor functions are the outcomes of these primary and secondary injuries during the neuropathological progression of TBI. However, such functional outcomes may also be used as reliable pointer for injury severity.

Authors’ Contributions XM carried out the studies of literature research and performed the acquisition of data and writing in manuscript preparation. AA wrote the part of “behavioral assessment” and helped XM in manuscript preparation. BJB and NC proofread the manuscript and gave important comments from their area of expertise. JH supervised the development of work, gave critical revisions, and edited the manuscript. All authors read and approved the final manuscript.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests.

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