

Chapter 2

Clinical Traumatic Brain Injury in the Preclinical Setting

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Abstract

Traumatic brain injury (TBI) is the leading cause of death and disability for people under 45 years of age. Clinical TBI is often the result of disparate forces resulting in heterogeneous injuries. Preclinical modeling of TBI is a vital tool for studying the complex cascade of metabolic, cellular, and molecular post-TBI events collectively termed secondary injury. Preclinical models also provide an important platform for studying therapeutic interventions. However, modeling TBI in the preclinical setting is challenging, and most models replicate only certain aspects of clinical TBI. This chapter details the most widely used models of preclinical TBI, including the controlled cortical impact, fluid percussion, blast, and closed head models. Each of these models replicates particular critical aspects of clinical TBI. Prior to selecting a preclinical TBI model, it is important to address what aspect of human TBI is being sought to evaluate.

Key words Traumatic brain injury, Preclinical models, Controlled cortical impact, Closed head injury, Fluid percussion, Blast injury

1 Introduction

Traumatic brain injury (TBI) is the leading cause of death and disability for people under 45 years of age [1]. Worldwide, in excess of 10 million deaths or hospitalizations are attributable to TBI each year, and more than 57 million people currently are living with the sequelae of TBI [1]. While recent estimates suggest that the disease burden of TBI continues to increase, few targeted therapeutic interventions have proven effective for this common injury. Most therapeutic interventions in TBI are tested in the preclinical setting, using animal models to simulate the pathophysiology of human TBI. Understanding the issues and challenges related to utilizing animal models to study human TBI pathophysiology is a vital first step in translating these models to the clinical setting. This chapter aims to provide a broad overview of some of the most commonly employed animal models of TBI, to identify practical as well as translational issues in both the execution and application of these models.

1.1 Pathophysiology of TBI

TBI is defined as damage to the brain resulting from an external mechanical force, often leading to temporary or permanent impairment of cognitive, physical, and psychosocial functions. The pathophysiology of TBI can be divided into two distinct processes: primary injury and secondary injury. The primary injury is the result of the immediate mechanical force, which can include diverse mechanisms such as blast wave, crush, impact, penetration, or rapid acceleration or deceleration. These diverse mechanisms can manifest in a wide array of primary injuries including contusion, hemorrhage, and axonal shearing. It is important to note that clinical TBI is often the result of a heterogeneous mixture of mechanical forces leading to mixed primary injuries. Interventions targeting primary injury are essentially preventative, and only effective if they preclude or mitigate primary injury. Examples of interventions targeting primary injury would include measures such as mandatory bike helmet laws or improved airbag technology.

In contrast, interventions targeting secondary injury may have a prolonged window in which to act. After the immediate primary injury is sustained, secondary injury develops over minutes to months to possibly years. Secondary injury reflects a complex cascade of metabolic, cellular, and molecular events including glutamate excitotoxicity, disordered cellular calcium homeostasis, mitochondrial dysfunction, inflammation, apoptosis/necroptosis, diffuse axonal injury (DAI), increased free radical generation and lipid peroxidation. In the extreme, secondary injury can lead to cell death, diffuse inflammation, and brain atrophy.

Despite the long window in which mitigation of secondary injury could occur, promising therapeutic interventions in the preclinical setting have failed to translate to clinical trials, with more than 30 failed TBI clinical trials based on successful preclinical studies [2]. While many reasons for failed TBI trials have been cited, including poor central nervous system drug penetration, delayed treatment initiation, heterogeneity across treatment sites, and insensitive outcomes measures [3], one important factor is the heterogeneity of clinical TBI compared to preclinical TBI. Indeed, clinical TBI often reflects a mixture of primary injuries as well as host-specific differences in the secondary injury response. In contrast, preclinical TBI studies rarely account for confounders including location, nature and severity of the primary injury, preexisting medical conditions, genetic background, age, gender, and illicit and prescribed drug use (to name a few). Thus, a promising therapeutic intervention in the preclinical setting may fail in the face of all the clinical confounders for which it was not tested.

Another important caveat to utilizing animal models of TBI is the lack of common terminology around injury severity. In human TBI trials, the Glasgow coma scale (GCS) is the primary means for assessing initial injury severity and the Glasgow outcome scale (GOS), or its extended version (GOSe), is the primary method for assessing outcomes. Whether or not these scales provide the optimal

assessment tools is a source of controversy, but irrespective of this controversy, they do provide a common language to describe injury severity and outcomes. No such common language exists in preclinical trials. With no widely adopted common scoring system for injury severity in animal models of TBI, histological changes and functional tests provide the most reliable estimates of severity of TBI. However, subtle changes in injury mechanisms or devices as well as differences in techniques between labs, can sometimes make the comparison of various animal TBI models precarious at best.

Despite these limitations, animal models of TBI are extremely important tools to study the biomechanical, cellular and molecular events after TBI, many of which cannot be feasibly addressed in the clinical setting. The purpose of this chapter is to review some of the most commonly used methods of preclinical TBI both in terms of clinical relevance and potential technical pitfalls. Understanding these models are important prior to developing new models that better recapitulate the spectrum of human TBI.

2 General Experimental Model Approach

Prior to selecting a preclinical TBI model, it is important to address what aspect of human TBI is being sought to evaluate. For example, an investigator studying sports-related TBI would be wise to avoid a model with high-associated mortality, intraparenchymal hemorrhages, and skull fractures. New model development can take months to years, and translating established models to a new laboratory setting may also require significant effort and time. Understanding sources of variability in the model and establishing baseline functional and histological outcomes are particularly important prior to testing therapeutic interventions. In an attempt to help standardize preclinical TBI studies, the National Institutes of Health (NIH) has recommended the use of common data elements for preclinical TBI models (Table 1).

2.1 Controlled Cortical Impact Injury Model

2.1.1 Introduction

The controlled cortical impact (CCI) model has been utilized mostly to model moderate or severe human TBI. The classic CCI device employs a pneumatic or electromagnetic mechanism to drive a rigid impactor onto the exposed, intact dura, resulting in acute subdural hematoma, axonal injury, blood–brain barrier (BBB) dysfunction, and cortical tissue loss [4, 5]. CCI has been applied to species including mice, rats, ferrets, swine, and monkeys. One of the key advantages of CCI is that injury severity can be carefully calibrated by altering mechanical factors such as time, velocity, and depth of impact. In contrast to weight drop models, CCI lacks a rebound injury and produces a more focal injury compared to FPI. Another advantage of CCI is its use in developing novel therapeutic treatments for brain injury [6–11].

Table 1**Common Data Elements (CDEs) for preclinical TBI research from the NIH**

Animal characteristics	Animal history	Assessments and outcomes
Species	Pre-injury subject housing	Outcome timing
Birthdate	Pre-injury conditions	Assessment date and time
Age	Pre-injury surgical procedures	Acute neurological assessment Apnea indicator
Age group	Injury group	Apnea duration
Sex	Injury date and time	Righting response time
Animal vendor	Anesthetic type	Toe pinch response
Strain/genetic modifications	Anesthetic route	Acute physiological assessments Brain imaging type
Weight measurement	Anesthesia duration	Chronic physiologic assessments
		Memory/retention tests
		Learning/acquisition tests
		Sensory/motor tests
<i>Injury model characteristics</i>	Analgesia type	Anxiety tests
External cause modeled	Injury severity	Social interaction tests
Injury model	Number of injury exposures	Body weight change
Device manufacturer	Interval between injuries	Histopathology
Device manufacturer other text Animal stabilization method	Post-injury surgical procedures	
Impact location side	Post-injury conditions	
Impact location cortical region Impact location coordinates	Post-injury subject housing	
	Treatment group	
	Treatment onset	
	Drug treatment route	
	Treatment or therapy type	
	Treatment control	
	Treatment dose	
	Survival time	
	Euthanasia date and time	
	Euthanasia type	

2.1.2 Methods

In rodent CCI models the device is hooked up to an air tank, injury controller, and mounted on a crossbar where the impactor can be adjusted to a range of desired angles of injury. Animals are induced using isoflurane or a comparable anesthetic before the head is secured in a stereotactic frame. A midline incision is made on the top of the head and the periosteum removed to prevent interference with the drill. Depending on desired area of injury, a unilateral [12] or bilateral [13] craniotomy is performed, most commonly, between the bregma and lambda. In both models the diameter of the craniotomy ranges from 4 to 6 mm. Injured mice can receive a mild–severe injury depending on the depth of brain deformation caused by the piston (0.2–1.2 mm) while sham animals receive only a craniotomy. The impact velocity can be adjusted from 0.5 to 10 m/s and impact duration ranges from 25 to 250 ms.

Once the skull flap is removed, using the injury controller the piston is set in the down position and the exposed brain is raised so that it is just touching the piston. The piston is then set back up, the apparatus is unlocked and the dial adjusted to the desired depth. After relocking, the piston is fired onto the brain using the controller. After completion of the injury, the incision is sutured, antibiotic cream is applied, and the animal is placed in a separate cage to wake up (*see* **Notes 1–4**). Common data elements for CCI studies are found in Table 2.

2.1.3 Clinical Relevance

Animals subjected to CCI have a wide range of functional deficits, which are highly related to both the depth of deformation and the velocity of the impact [12, 14]. Functional deficits after CCI include but are not limited to sensorimotor deficits (wire grip, rotarod, wire grip) memory deficits (Morris Water Maze (MWM) performance) and increased depression, anxiety, and impulsivity (forced swim,

Table 2
Controlled cortical injury (CCI) relevant data elements

Invasive surgery
Craniotomy size
Impactor angle
Impactor angle measurement
Impactor tip/projectile shape
Impactor tip rigidity
Impactor depth setting
Impactor dwell time
Impactor velocity
Surface material

elevated-plus maze, acoustic startle) [14–18]. Some functional deficits can be persistent up to 1 year post-CCI injury. The most marked histopathological change after CCI is the development of a cavitory lesion. However, CCI can result in a spectrum of anatomic injury including diffuse axonal injury (DAI) [5, 19]. Beta-amyloid precursor protein (APP) positive cells are increased in both the contused cortex and ipsilateral hippocampus as early as 6 h. The highest immunoreactivity levels occur on days 1–3 after injury and the positive cells can be seen for weeks [20]. Neuronal degeneration is also observed in early and late stages after injury [21, 22]. Due of the contusive nature of this injury, CCI causes inflammation and a dramatic increase in cytokines and chemokines [15, 20, 23]. Microglia activation is also accumulated in the lesion [15, 24].

2.1.4 Caveats

Most clinical cases of contusive injuries have a concussive injury as well. However, the CCI model lacks a concussive impact because the animals head is fixed in a stereotactic frame. To overcome this the experimental animals can be subjected to a concussion immediately following the contusive injury [25]. The spectrum of functional deficits after CCI is dependent on the localization of injury. Injuries located in the posterior cortex result in transient motor deficits as well as impaired memory and sensory function [15].

2.2 Fluid Percussion Injury

2.2.1 Introduction

Although it was originally created for use in larger animals, Fluid Percussion Injury (FPI) has become of the most commonly used methods of creating a non-penetrating head injury in rodents [26]. In FPI models, TBI is the result of a fluid pressure pulse transmitted to the intact dura through a craniotomy. The FPI device consists of a cylindrical Plexiglas reservoir of sterile saline with one end attached to a transducer which, through a tube, connects to the cap cemented to the skull. The injury is generated by a pendulum striking the piston, which creates a pressure pulse that travels through the transducer and onto the plastic cap causing a deformation of the intact dura. The fluid pulse produces brief displacement and deformation of brain tissue, and the severity of injury depends on the strength of the pressure pulse. In its most severe forms, FPI can result in intracranial hemorrhage, edema and progressive grey matter damage. FPI models can be categorized by the location of the craniotomy, into midline (centered on the sagittal suture), parasagittal (<3.5 mm lateral to midline), and lateral models (>3.5 mm lateral to midline) [26–29]. Midline and parasagittal FPIs cause bilateral cortical damage, while lateral FPI (LFPI) inflicts primarily unilateral cortical damage, rarely involving the contralateral cortices and brainstem.

2.2.2 Methods

Animals are anesthetized using 3% halothane via a vaporizer and a vacuum trap or a comparable anesthetic, placed in a prone position on a warming pad and secured in a stereotactic frame. The head is shaved, eye lubricant is applied, and the area of incision is prepped

using three alternating treatments of Betadine and ethanol. A 1.5 cm sagittal incision is made at the midline between the ears and extended toward the nose. The periosteum is removed with a cotton swab, then, using a 5 mm drill bit, a craniotomy is performed either centrally over the midline (FPI) or laterally (LFPI) between the bregma and lambda. A cannula is set in the cranial opening so that it is touching the intact dura and fixed it to the skull using glue. The cannula is filled with saline and any bubbles are removed from the apparatus.

To determine the resting position, set the pendulum at 90° perpendicular to the ground and make sure it is touching the piston. Double check and remove any bubbles from all tubing and connections and ensure that the tubing from FPI device is filled with water. Using forceps to hold the cannula and tubing, attach them together by twisting in opposing directions. This prevents the application of excessive torque, which would cause the head cannula to detach from the rat's skull; Thereby causing leakage and an indeterminable injury. Set the pendulum to the desired height, clear the path from the pendulum to the piston and release the pendulum, allowing it to strike the piston. Severity of the injury depends on the intensity of the pressure pulse, which is controlled by the height of the pendulum drop. There are three levels of injury, mild, moderate, and severe. Mild injury occurs following a percussion of 0.1–1 atm (10.13–101.29 kPa), moderate after 1.5–2.0 atm, and severe after 2.5–3 atm. After the piston strikes, check for any leaking of saline and make sure to catch the pendulum to prevent any inadvertent injury. Remove the cannula, fill the burr hole with bone wax, suture the incision and place the animal on a heating pad to wake up (*see* **Notes 5** and **6**). Common data elements for FPI studies are found in **Table 3**.

Table 3

Fluid percussion injury (FP) relevant data elements

Craniotomy size
Connector angle
Connector tube
Connector tube length
Connector tube material
Port distal diameter
Cement
Transducer manufacturer
Cap characteristics
Peak pressure pulse
Pressure wave duration

2.2.3 *Clinical Relevance*

LFPI, the most commonly utilized FPI procedure in rats, produces a combination of focal cortical contusion and diffuse subcortical neuronal injury (including injury in the hippocampus and thalamus). As in CCI, the contused cortex beneath the injury site becomes a cavitory lesion surrounded by reactive gliosis. Over days to months, progressive degenerative changes may be found in ipsilateral hippocampus, thalamus, medial septum, striatum and amygdala. Widespread β -APP expression, comparable to human DAI, may be seen in more severe FPI models [30–32]. Functional deficits in motor and memory outcomes are similar to those seen after CCI, and can persist for up to 1 year after injury.

2.2.4 *Caveats*

FPI models are associated with high mortality, probably due to brainstem-mediated apnea. Post-injury seizures are common. The FPI model can be difficult to calibrate, since the height of pendulum is the only adjustable mechanical parameter. Reproducibility has been a challenge of FPI models until recently, when a microprocessor-controlled, pneumatically driven instrument has been developed. Though LFPI is the most commonly employed FPI model, there has been recent interest in midline FPI models, which are believed to better represent sports-related and military TBI.

2.3 *Penetrating/ Direct Impact TBI*

2.3.1 *Introduction*

A wide variety of experimental penetrating TBI models have been designed to generate cerebral deformation including microinjection [33], cryolesion [34], mechanical suction [35], vacuum pulse [36, 37], and inflation of a balloon [38, 39] and others [40]. However, because of their clinical relevance, the most commonly used are penetrating ballistic like blast injury (PBBI) and fragment penetration models. In PBBI models, the insult is caused by transmission of a high energy projectile that produces a cavitory lesion much larger than the projectile. There may be marked intracerebral hemorrhage, but less diffuse axonal injury than other models. Outcomes are directly related to the anatomical path and energy transfer of the projectile.

2.3.2 *Methods*

In Carey et al.'s [41–43] model anesthetized animals are placed in a stereotactic frame and the sloping outer wall of the right frontal sinus is removed; thereby allowing the missile like object to penetrate the intact and vertically disposed sinus wall. A 2-mm, 31-mg steel sphere fired from 80 cm at 220 or 280 m/s penetrates the right frontal bone and traverses the right cerebral hemisphere from anterior to posterior. The energy from the projectile can range from 0.9 to 1.4 J. Finnie's [44] model utilizes a 0.22 caliber fire-arm to inflict a head wound to restrained sheep. The bullet is fired at the temporal region of the skull, causing a right to left transverse injury to the temporal lobes. Recently several new PBBI models

have been developed in rodents [45, 46] and fragment blast models are becoming more common as well [47]. Recently a novel, non fatal, low velocity model for PBBI has been established in rats [48] and is similar to CCI in that a cylindrical carbon fiber rod is accelerated and enters the brain creating a cavitory lesion. However, the pin (2 mm in diameter) is attached to a secondary projectile that is accelerated by a pellet fired from a modified rifle. The secondary projectile and pin, guided by a tube, penetrates the brain at a speed of 90 m/s. The base of the projectile is surrounded by a compressible ring (ferrule) that controls the depth of the penetration. Although the depth is usually set at 5 mm, depth, speed and shape of the pin can be altered to obtain different pathological outcomes. This model has also been modified for use in mice [49].

2.3.3 Clinical Relevance

This model mimics gun-related brain injuries caused by a high-velocity penetrating object. The injury can be induced by multiple mechanisms including the high pressures in front of an object; longitudinal shock wave and pressure waves from kinetic energy transfer [42]. Injury phenotypes are dependent on the injured part of brain and the most common consequences after injury are intracerebral hemorrhages, edema, elevated intracranial pressure, decreased cerebral blood flow, significant neuroinflammation, and notable lesion [38, 50]. Additionally, both apoptosis and necrosis are observed in the lesion [50, 51]. Motor function and cognitive impairments are observed in this model [51–53], including deficits in motor (balance beam and rotarod tasks) and memory (MWM test) that correlate with the degree of injury severity as well as clinical moderate-severe TBI [54, 55].

2.3.4 Caveats

The original model was established by Carey et al. using mongrel cats, however, it is difficult to run behavioral tasks on larger animals, thus rodent models have become increasingly popular [48, 49, 56, 57]. However, rodent PBBI models are relatively new and less standardized than other TBI models. Even though they are underdeveloped, rodent PBBI models provide opportunities to use genetic modification to investigate the mechanisms of injury and therapeutic targets. Due to the high velocity of objects entering the brain, heat damage of the tissue is a potential factor, though this may be also relevant to clinical penetrating TBI.

2.4 Weight Drop Models

2.4.1 Introduction

In weight-drop models, a free falling, guided weight is allowed to impact the skull (with or without a craniotomy). One of the major advantages of weight drop models is their relative inexpensiveness. Injury severity in these models can be altered by adjusting the mass of the weight and the height from which the weight falls. In addition, several modifications of the weight-drop model have been made, which markedly alter both functional and histopathological outcomes.

Models such as Feeney's weight-drop, are delivered to the exposed dura which results in a cortical contusion [58] and progresses to a cavitory lesion similar to those found in CCI. Shohami's group later introduced a modified rodent weight drop model, delivering closed head injury (CHI) to the intact but unprotected skull [59–63]. Of *Note*, in both the Feeney and Shohami methods, there is minimal rotational acceleration of the fixed head. In attempt to add rotational acceleration, a prominent feature in the primary injury of human TBI, Marmarou's impact acceleration model was developed to recapitulate this important mechanism. In the Marmarou method, a sectioned brass weight is allowed to fall freely from a designated height through a Plexiglas tube, onto the exposed skull of rats placed on a foam bed. Marmarou's model, which often results in a high mortality rate in the absence of mechanical ventilation, is characterized by diffuse axonal injury (DAI) and neuronal injury, as well as functional deficits in beam walking and memory [64, 65]. Subsequent modifications of the Marmarou model have been designed to reproduce the frontal impact characteristic of motor vehicle and sports-related injuries [66, 67].

In general, these models do not result in mortality, prolonged apnea, skull fractures or cortical contusions, and are thought to be more representative of the vast majority of human TBI, which is classified as mild. Common data elements for weight drop studies are found in Table 4.

2.4.2 Methods

In a recent model of mild TBI [66], animals are induced for 45 s with 4% isoflurane or alternative anesthetic. Immediately following, a timer is started to initiate the loss of consciousness (LOC) and the animal is placed supine on a Kimwipes. The animal's tail and each end of the Kimwipes are secured by hand and the animal's head is placed directly beneath a hollow tube and positioned

Table 4

Weight drop injury (WD) relevant data elements

Invasive surgery	Weight drop height	Impactor retraction
Surface material	Weight drop guidance	WD-specific pre-injury surgical procedures
Craniotomy size	Weight drop characteristics Impactor velocity	WD-specific post-injury surgical procedures
Impactor/projectile mass	Contact surface type	
Impactor/projectile material	Contact surface area	
Impactor tip/projectile shape	Impactor dwell time	
Impactor tip rigidity		

with the tube opening directly posterior to the eyes. A cylindrical metal rod (54 g) is dropped dorsally on the animal's head between the coronal and lambdoid sutures. Upon impact the animal's head readily penetrates the Kimwipes, causing the head and body to fall in a circular trajectory while the tail remains secured. The severity of the injury can be varied by altering the weight of the object and the height that it is dropped from. Mild injuries are 54 g dropped from 28", while severe ones are 54 g at 60". The mouse is then placed on its side and allowed to wake up and the timer is stopped. The (LOC) is recorded as the latency in seconds to spontaneous ambulation. The control group consists of sham animals given anesthesia but no weight drop and all animals are allowed to recover in room air in their cages.

Other commonly used models vary in the level of surgical invasiveness and are aimed to reproduce different clinical aspects of brain injury. The Feeney et al. model involves dropping a weight onto the intact dura through a craniotomy [68], which produces hemorrhages and cell death. In the Marmarou model, which mimics DAI and diffuse TBI, the animal's head rests on a foam pad and a guided brass weight impacts a stainless steel disk mounted on the skull [69]. Shohami's model drops the weight onto one side of an unprotected skull while resting on a hard surface [60] and has been used to investigate BBB disruption. Weight drop models are primarily conducted on mice, rats, and swine (*see* **Notes 7 and 8**).

2.4.3 Clinical Relevance

Each variation of the weight drop model has distinct clinical features that should be evaluated prior to commencing. Model selection can be based on which clinical features of TBI are desired, for example loss of consciousness may be sought after in modeling TBI in military populations, since 4.9% of US soldiers returning from Iraq had loss of consciousness [70]. However, the majority of patients with sports-related concussions do not experience loss of consciousness and a model minimizing this outcome might be desirable in modeling sport related TBI [71]. Many of the current weight drop models feature a rapid acceleration and deceleration of head movement [72]. The drastic change in speed and direction of the head is the main cause of concussions and axonal injury [73–75] in closed head traumas. The concussive injury does not induce immediate cell death; however, it does result in axonal injury and DAI at the extreme end of the spectrum. Tau pathology, an area of much clinical investigation in the field of TBI, may or may not be present. Reactive gliosis may also be a prominent feature of these models. The closed head model has been shown to result in impaired neurological and cognitive outcomes including motor, learning, memory, and anxiety [72, 76, 77].

2.4.4 Caveats

While weight-drop models are generally easy and inexpensive to employ, they can result in relatively high variability in injury severity. Another concern is the possibility of rebound injury. However,

in general, weight-drop procedures are capable of producing graded axonal injury that is highly relevant to the vast majority of human TBI.

2.5 Non Impact Blast

2.5.1 Introduction

Blast models of TBI have been developed to better understand the effects of primary blast waves on the CNS. Some estimates suggest that nearly one in five veterans of the conflicts in Afghanistan and Iraq were exposed to TBI, much of which was blast-associated TBI. Initial efforts at simulating blast associated TBI were challenged by resultant systemic injuries, but the use of thoracic/abdominal protective strategies have minimized these types of associated injuries, allowing investigators to study the effects of blast associated TBI in isolation. Most commonly, blast models deliver primary injury using a compression-driven shock tube to simulate blast effects.

2.5.2 Methods

The most commonly used blast models have been established in rodents [78–82] and swine [83, 84] and utilize explosives [80, 83, 84] or compressed air [82, 85] to create a shock wave. In these models, anesthetized animals are individually fixed in a cylindrical metal tube in such a way that prevents any movement of the body. However, in some cases, the head and neck are able to freely to flex, extend, and rotate during the injury [86]. Additionally Kevlar vests, which encase the thorax and part of the abdomen, have been used to test acute mortality in rats [79]. The blast is generated by a controlled detonation or by the release of compressed air. Blast parameters can be regulated to generate mild to severe injury. Intensity of the blast and subsequent injury is controlled by altering the amount of explosives or pressure of the compressed air; or by changing the animal's distance from the blast point. When using compressed air more complex waveforms can be created by adding additional chambers of compressed air. Although reproducible, the blast tube and other models lack the clinical relevance and unpredictable conditions of real life event. In a recent open field study [87], 12 anesthetized animals are loosely fixed in individual compartments using a plastic net and placed at 4 or 7 m from the detonation point. The net allows for their body to be exposed to the blast without being injured by shrapnel or debris. The platforms are then elevated to a height of 1 m to prevent any interference from blast wave reflection off the ground. Pressure meters are placed at each distance to detect the intensity of the blast. After the detonation of 500 g of TNT, the mice are allowed to wake up and the pressure sensors data are recorded (*see* **Notes 9** and **10**).

2.5.3 Clinical Relevance

Non-impact models of blast injury are characterized by cerebral edema, hyperemia and delayed vasospasm, the degree of which corresponds to injury severity. The most prominent histopathological finding in blast models is DAI, which corresponds to changes in

diffuse tractography imaging seen in blast exposed military veterans [88]. Associated with DAI, blast-exposed mice also demonstrate phosphorylated tauopathy, myelinated axonopathy, chronic neuroinflammation and neurodegeneration, consistent with clinical reports of chronic traumatic encephalopathy in military populations. Functional deficits reported after blast models of TBI include deficits in social recognition, spatial memory, and motor coordination [89].

2.5.4 Caveats

Clinically, blast injuries consist of primary, secondary, tertiary, and quaternary mechanisms, of which the blast preclinical models really only recapitulates primary mechanism. In addition, animal placement in relation to the shock tube (that is, inside, outside or near the exit of the tube) can significantly modify injury type and severity. Blast models, while highly clinically relevant, also suffer from the most variability within and between models, making comparisons between studies very difficult. Utilization of common data elements (Table 5) is particularly important in this setting. Additionally shock tube models are incredibly complex and require the use of heavy machinery.

Table 5

Blast-induced neurotrauma (BIN) relevant data elements

Blast-induced delivery device Pressure wave type	Distance between animal and tube	Reflective surfaces
Detonation type	Animal orientation to blast wave	Primary blast effects
Detonation material quantity	Overpressure peak	
Driver gas	Overpressure rise time	Secondary blast effect type
Pressure wave medium	Overpressure wave duration	Secondary blast effect specifications
Distance from detonation	Impulse	Tertiary blast effects
Blast tube or column area	Reflective wave overpressure	Tertiary blast effect specifications
Blast tube length	Blast wind pressure	Quaternary blast effects
Shock tube driven section length	Pressure sensor orientation	Systemic injury
Membrane thickness	Pressure sensor type	Extracranial injuries
Membrane burst method	Pressure sensor sampling frequency	BIN-specific pre-injury surgical procedures
Membrane burst pressure	Incident pressure time history	BIN-specific post-injury surgical procedures
Tube end configuration	Body exposure	
Placement relative to shock tube	Protective shielding location	
	Protective shielding type	

3 Notes

1. Ensuring a snug fit in stereotactic frame is essential before starting as it makes drilling difficult and keeping the head still ensures consistent injuries.
2. Make sure head and stereotactic frame are out of the way when setting the impactor.
3. If the impactor is set at 90 degrees perpendicular to the ground the piston will not be flush with the brain because of the curvature of the brain. Make sure to make the contact between the piston and brain the same between animals.
4. Do not replace skull flap from craniotomy as there will be edema which will cause an increase in ICP.
5. The cannula should fit the burr hole snugly and there should be nothing obscuring visualization of the dura through the center of the cannula. Optionally, a few drops of cresyl violet dye can be added to the saline to facilitate observation of any leaks around the burr hole site during the injury.
6. Bubbles can be removed by disconnecting the distal end of the tubing from the rat and forcing fluid through the reservoir and tubing until all air bubbles have been expelled. The tubing is then reconnected to the rat.
7. When taking mouse and Kimwipes off of the table, hold one end of Kimwipes and tail and place mouse vertical in the air then grab the other end and flatten out. This will stretch the mouse's body out and make placing the head under the tube easier. Hold the Kimwipes tight but not too tight as to rip it.
8. In order to get a more direct hit on top of the head and not over the cerebellum/spinal cord, tilting the fore body upward towards the pipe.
9. When using compressed air model, it is critical that the mylar membrane is mounted correctly. If the sheet gets folded at all, the membrane doubles in thickness and creates inconsistent blasts.
10. Production of mylar sheets is sometimes inconsistent. Therefore, test each new box before running an experiment by generating and measuring the blast.

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