Murine Model of Controlled Cortical Impact for the Induction of Traumatic Brain Injury

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Abstract

The Centers for Disease Control and Injury Prevention estimate that almost 2 million people sustain a traumatic brain injury (TBI) every year in the United States. In fact, TBI is a contributing factor to over a third of all injury-related mortality. Nonetheless, the cellular and molecular mechanisms underlying the pathophysiology of TBI are poorly understood. Thus, preclinical models of TBI capable of replicating the injury mechanisms pertinent to TBI in human patients are a critical research need. The controlled cortical impact (CCI) model of TBI utilizes a mechanical device to directly impact the exposed cortex. While no model can full recapitulate the disparate injury patterns and heterogeneous nature of TBI in human patients, CCI is capable of inducing a wide range of clinically applicable TBI. Furthermore, CCI is easily standardized allowing investigators to compare results across experiments as well as across investigative groups. The following protocol is a detailed description of applying a severe CCI with a commercially available impacting device in a murine model of TBI.

Keywords

Neuroscience; Issue 150; Traumatic brain injury; craniectomy; dura mater; controlled cortical impact; intraparenchymal hemorrhage; subarachnoid hemorrhage; subdural Hemorrhage; stereotaxic

Introduction

The Centers for Disease Control and Injury Prevention estimate that approximately 2 million Americans sustain a traumatic brain injury (TBI) every year^{1,2}. In fact, TBI contributes to over 30% of all injury related deaths in the United States with healthcare costs nearing \$80 billion annually and almost \$4 million per person per year surviving a severe TBI^{3,4,5}. The impact of TBI is highlighted by the significant long- term neurocognitive and neuropsychiatric complications suffered by its survivors with the insidious onset of behavioral, cognitive, and motor impairments termed Chronic Traumatic Encephalopathy (CTE)^{6,7,8,9,10}. Even subclinical concussive events—those impacts that do not result in

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Disclosures

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clinical symptoms—can lead to long-term neurologic dysfunction^{11,12}. Animal models for the study of TBI have been employed since the late 1800's¹³. In the 1980s, a pneumatic impactor for the purpose of modeling TBI was developed. This method is now referred to as controlled cortical impact (CCI)¹⁴. The control and reproducibility of CCI led researchers to adapt the model for use in rodents¹⁵. Our laboratory uses this model to induce TBI via a commercially available impactor and electronic actuating device^{16,17}. This model is capable of producing a wide range of clinically applicable TBI states depending on the biomechanical parameters used. Histologic evaluation of TBI brains after a severe injury induced in our laboratory demonstrates significant ipsilateral cortical and hippocampal loss as well as contralateral edema and distortion. Additionally, CCI produces a consistent impairment in motor and cognitive function as measured by behavioral assays¹⁸. Limitations to CCI include the need for craniotomy and the expense of acquiring the impactor and actuating device.

Several additional models of TBI exist and are well established in the literature including the lateral fluid percussion model, weight drop model, and blast injury model^{19,20,21}. While each of these models have their own distinct advantages their main drawbacks are mixed injury, high mortality and lack of standardization, respectively²². Furthermore, none of these models offer the accuracy, precision, and reproducibility of CCI. By adjusting the biomechanical parameters input into the actuating device, the CCI model allows the investigator precise control over size of the injury, depth of the injury, and kinetic energy applied to the brain. This gives investigators the ability to apply the entire spectrum of TBI to specific areas of the brain. It also permits the greatest reproducibility from experiment to experiment.

Protocol

All procedures were approved by the Northwestern University Institutional Animal Care and Use Committee. C57BL/6 mice were purchased from the Jackson Laboratory and group housed at a barrier facility at the Center for Comparative Medicine at Northwestern University (Chicago, IL). All animals were housed in 12/12 h light/dark cycle with free access to food and water.

1. Induce anesthesia
 1. Anesthetize the mouse with ketamine (125 mg/kg) and xylazine (10 mg/kg) injected intraperitoneally.
2. Vital signs monitoring every 15 min
 1. Monitor temperature, respiratory rate, and skin color. The mouse should feel warm to the touch. The skin should appear pink and well perfused. Respiratory rate should range 50–70 breaths per minute.
3. Pre-surgical procedures
 1. Weigh all the mice on the day prior to injury induction.
 2. Sterilize one set of surgical instruments by autoclaving for each experimental subject. Sterilize the impacting device before use.

3. Prepare a recovery cage by placing a clean cage over an electric heating pad set to “low” setting and positioned in a manner such that the mice can move away from the heat once ambulatory.
4. Set up the operating theater within a sterilized laminar flow hood.
 1. Position the stereotaxic operating frame.
 2. Attach the impacting device to the stereotaxic frame.
 3. Set the actuating device with the desired biomechanical parameters for velocity and dwell time.

NOTE: In this protocol a severe brain injury is described utilizing a 3 mm diameter impact tip via a 5 mm diameter craniectomy with the velocity set at 2.5 m/s and a dwell time of 0.1 s. A wide range of biomechanical parameters may be used to induce the full spectrum of TBI.

5. Don new personal protective equipment and sterile gloves.
6. Shave the fur from the operative site using electric clippers.
7. Apply protective ophthalmic ointment to the eyes of the mouse to prevent corneal injury and drying.
8. Place the mouse into the operating theater.
9. Prep the skin with an iodine based surgical scrub alternated with alcohol three times.
4. Application of controlled cortical impact
 1. Incise the scalp 1 cm in the midline with a scalpel exposing the skull.
 2. Position the mouse within a stereotaxic operating frame by securing the bilateral temporal bones between miniature ear bars and locking the incisors within an incisor clamp creating a stable three-point-hold on the mouse head.
 3. Retract the scalp away from the operative site with a hemostat or locking forceps to ensure the scalp does not come in contact with the drill bit during craniectomy.
 4. Identify the sagittal and coronal sutures on the exposed skull.

NOTE: This protocol centers the craniectomy 2 mm left of the sagittal suture and 2 mm rostral to the coronal suture.

 5. Perform a craniectomy using a drill with a trephine drill bit.
 1. To perform the craniectomy, first activate the drill at maximum speed and then apply the trephine drill bit perpendicular to the skull at the site of craniectomy.

2. Apply gentle, even pressure to the drill once contact is made with the skull. A slight “give” will be felt once the drill penetrates through the skull. Do not penetrate the underlying dura.

NOTE: This protocol utilizes a 5 mm trephine drill bit to perform the craniectomy.

6. Use forceps and a small gauge hypodermic needle to remove the bone flap, fully exposing the underlying dura mater.
7. Rotate the impactor tip into the operative field and lower it until it makes contact with the exposed dura mater. Once contact is made the instrument’s contact sensor will make an audible tone to alert the surgeon that contact has been made. This will mark the zero point from which the deformation depth is set.

NOTE: This protocol utilizes a 3 mm impacting tip to generate a severe injury. Tips as small as 1 mm may be used to apply more localized injury.

8. Retract the impacting tip and set the desired impact depth by lowering the impactor position on the stereotaxic frame. NOTE: In this protocol we describe a severe injury by setting the deformation depth to 2 mm.
9. Apply the injury by activating impactor on the actuating device.
10. Rotate the impact device out of the field and remove the animal from the stereotaxic frame.

5. Surgical site closure

1. Control bleeding from the skull and injured cortical surface with direct pressure from a sterile cotton tipped applicator.
2. Dry the skull with a sterile cotton tipped applicator.
3. Close the scalp over the craniectomy using a commercially available surgical adhesive or monofilament suture.

NOTE: In this protocol a veterinary surgical adhesive is used to close the scalp. The bone flap is not replaced and is discarded.

6. Post-operative care and monitoring

1. Administer post-operative analgesia (e.g., sustained release buprenorphine 0.1–0.5 mg/kg administered subcutaneously providing 72 h of sustained analgesia).
2. Place the animal in the lateral decubitus recovery position in a clean pre-warmed cage.
3. Observe the animals until awake and mobile, then return each mouse to its home cage.

4. Ensure free access to food and water. Normal food and water intake typically resume within one to two hours after injury.
5. Measure body weight every three days throughout the experiment.

Representative Results

The impactor mounts directly on the stereotaxic frame allowing for as much as 10 μm resolution for control of the point of impact, depth and penetration. The electromagnetic forces employed can impart impact velocities ranging 1.5–6 m/s. This allows for unparalleled precision and reproducibility over the entire range of clinically relevant TBI. Investigators can run pilot experiments changing the injury parameters such as impactor tip size, impact velocity, and impact depth to determine the parameters that best produce the desired degree of injury. This protocol describes a severe TBI to the left parietotemporal region by performing a 5 mm craniectomy 2 mm left of the sagittal suture and 2 mm rostral to the coronal suture (Figure 1A). A controlled cortical impact is delivered with a 3 mm impacting tip at 2.5 m/s and a deformation depth of 2 mm (Figure 2). Injury consists of subdural, intraparenchymal, and subarachnoid hemorrhage (Figure 3). Neurocognitive testing one month after this injury demonstrates persistent deficits in working memory, skill acquisition, and motor coordination¹⁸. Histologic evaluation of TBI brains after a severe injury induced in our laboratory demonstrates significant ipsilateral cortical and hippocampal loss as well as contralateral edema and distortion. MRI examination of severely injured brains using this model demonstrates progressive tissue loss and replacement by cerebrospinal fluid (Figure 4)²³. Lastly, flow cytometric analysis of injured and sham brains demonstrates a marked difference in infiltrating inflammatory cells throughout the course of injury^{17,18}.

Discussion

There are several steps that are critical for applying a reliable and consistent injury. First, the mouse must reach a deep plane of surgical anesthesia ensuring no movement during the performance of the craniectomy. While numerous anesthetic regimens may be used to induce general anesthesia in rodents, anesthetics that induce respiratory depression such as inhalational anesthetics may result in respiratory arrest when combined with a severe TBI. This protocol utilizes ketamine (125 mg/kg) and xylazine (10 mg/kg) injected intraperitoneally. This combination of drugs produces a surgical plane of anesthesia within 5 min of administration for a duration of approximately 30–45 min. Furthermore, this combination of drugs does not result in respiratory depression. The next critical step is the performance of the craniectomy. The craniectomy should always be performed with a fresh trephine drill bit at high speed to ensure that minimal heat and vibration are transmitted to the mouse brain. Heat and vibration can result in damage to adjacent brain tissue outside the area of CCI leading to inconsistent size and mechanism of injury between subjects and experiments. Next, the mouse head must be firmly secured within the stereotaxic frame prior to the application of the CCI to ensure the depth and location of injury are consistent between injury applications. Miniature ear bars and an incisor clamp are essential components in properly securing the mouse head within the stereotaxic frame. Lastly, it is

critical to utilize a device with a contact sensor. The sensor will indicate the exact point of contact between the impacting tip and the exposed dura mater. This allows investigator to note the exact zero point from which the depth of injury is can be set with the stereotaxic frame ensuring a precise and reproducible degree of injury.

To ensure that the incised scalp is outside the field at the time of CCI, it is often necessary to use a retractor such as clamp or forceps to pull to scalp away from the site of craniectomy. Should the scalp fall back into the CCI field as the injury is applied, the size and severity of injury will be unreliable. Additionally, although it is imperative to ensure the mouse head is immobilized within the stereotaxic frame, the investigator must ensure that the fixation does not impair respiration. Hypoxia at the time of injury secondary to restricted respiration will introduce a secondary form of injury making the degree, severity, and mechanism of injury unreliable between experimental subjects.

Given the ability to precisely specify multiple biomechanical parameters, CCI is one of the most consistent and reliable methods for inducing traumatic brain injury in rodent models¹⁵. However, there are a number of limitations that the investigator should be aware of when choosing which model of TBI is most appropriate to answer their scientific question²². CCI suffers from the same limitations as all preclinical models of brain injury in that it requires anesthesia and a surgical procedure (craniectomy) prior to the induction of injury. Both anesthesia and craniectomy are capable of generating an inflammatory response and must be considered as potential confounders during data analysis²⁴. Additionally, although CCI produces a reliable and consistent injury, most TBI in human patients are diffuse and occur through multiple simultaneous mechanisms²⁵. This may make direct translation to human TBI patients problematic as CCI produces a focal injury with varying degrees of diffuse effects depending on the severity of injury applied. Lastly, CCI requires the purchase and maintenance of several mechanical components that may prove to be cost prohibitive to some research groups. Without proper maintenance of the mechanical components, there may be substantial drift in the actual biomechanical parameters applied from experiment to experiment²⁴.

Identifying appropriate controls for each experiment is critical. Sham-injured mice are an important control in every experiment. The sham injury group should receive anesthesia, scalp incision, placement into the stereotaxic frame, and post-operative analgesia. However, the sham-injury group should not undergo craniectomy. The vibration and heat transfer from craniectomy, even when performed quickly with expert precision, does result in a mild traumatic brain injury. Although this injury is difficult to see grossly, it is readily identified microscopically. Lastly, investigators should consider using a group of age-matched naïve mice to rule out any normal changes that occur within the brain as the mice age.

Despite limitations, CCI remains the most consistent and reproducible model for inducing TBI in rodents. CCI is easy to standardize across subjects and experiments as compared to alternative methods of inducing TBI and allows investigators to apply the entire spectrum of TBI to precisely defined anatomic regions of the brain. The protocol above describes the application of a severe TBI to the left parietotemporal cortex in a mouse. This model utilizes a 5 mm craniectomy performed with a trephine drill bit at high speed. A 3 mm impacting tip

is used with an injury depth of 2 mm at a velocity of 2.5 m/s and a dwell time of 0.1 s. When applied appropriately, and when the experimental subject is properly recovered, a long-term survival rate approaching 100% can be obtained allowing for short, intermediate, and long-term studies of murine TBI to be performed.

Materials List

Materials

Name	Company	Catalog Number	Comments
AnaSed Injection Xylazine Sterile Solution	LLOYD, Inc.	5939911020	
Buprenorphine SR Lab 0.5mg/mL	Zoopharm-Wildlife Pharmaceuticals USA	BSRLAB0.5-182012	
High Speed Rotary Micromotor KiT0	Foredom Electric Company	K.1070	
Imapact one for Stereotaxix CCI	Leica Biosystems Nussloch GmbH	39463920	
Ketathesia Ketamine HCl Injection USP	Henry Schein, Inc	56344	
Mouse Specific Stereotaxic Base	Leica Biosystems Nussloch GmbH	39462980	
Trephines for Micro Drill	Fine Science Tools, Inc	18004-50	

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Figure 1: Equipment setup for the murine model of controlled cortical impact.

(A) The actuating device is set a velocity of 2.5 m/s and a dwell time of 0.1 s. (B) The impactor with a 3 mm impacting tip is secured to the stereotaxic frame. (C) A mouse with 5 mm craniectomy is secured into the stereotaxic operating frame with ear bars and an incisor bar.

Figure 2: Severe TBI via open-head controlled cortical impact.

(A) The grounding cable is clipped to the mouse's hind region and the impacting tip is lowered onto the dura mater until the contact sensor alarms. This is the zero point. (B) The impacting tip is retracted, a 2 mm depth of injury is dialed into the stereotaxic frame, and the impact is applied. (C) After the CCI is applied, the impacting tip is rotated out of the field and the mouse is recovered from the stereotaxic frame.

Figure 3: Gross examination of mouse brains after severe TBI induced by controlled cortical impact.

(A) Brain from a 12-week-old naïve mouse. (B) Brain from a 12-week-old mouse 24 h after sustaining a severe TBI via controlled cortical impact. (C) Brain from a 12-week-old mouse 7 days after sustaining a severe TBI via controlled cortical impact.

Figure 4: Histologic and MRI evaluation of severe TBI after controlled cortical impact. Hematoxylin and eosin (H&E) stained coronal sections and representative coronal T1-weighted MR images. **(A)** Sham injury, consisting of craniotomy only. **(B)** CCI results in a severe TBI with large volume loss of cortex (*Ctx*) at the site of impact as well as loss and distortion of the underlying hippocampal formation (*HF*) and thalamus (*TH*). **(C)** MRI at 1-day post-TBI demonstrates tissue trauma and edema over the left parietotemporal cortex. **(D–E)** Representative images from post-injury days 7 and 14 demonstrate increased areas of hyperattenuation representing progressive replacement of devitalized tissue with cerebrospinal fluid. Figure has been adapted from Makinde, et al.²³.