Chapter 14

Lateral (Parasagittal) Fluid Percussion Model of Traumatic Brain Injury

Ken C. Van and Bruce G. Lyeth

Abstract

Fluid percussion was first conceptualized in the 1940s and has evolved into one of the leading laboratory methods for studying experimental traumatic brain injury (TBI). Over the decades, fluid percussion has been used in numerous species and today is predominantly applied to the rat. The fluid percussion technique rapidly injects a small volume of fluid, such as isotonic saline, through a circular craniotomy onto the intact dura overlying the brain cortex. In brief, the methods involve surgical production of a circular craniotomy, attachment of a fluid-filled conduit between the dura overlying the cortex and the outlet port of the fluid percussion device. A fluid pulse is then generated by the free-fall of a pendulum striking a piston on the fluid-filled cylinder of the device. The fluid enters the cranium, producing a compression and displacement of the brain parenchyma resulting in a sharp, high magnitude elevation of intracranial pressure that is propagated diffusely through the brain. This results in an immediate and transient period of traumatic unconsciousness as well as a combination of focal and diffuse damage to the brain, which is evident upon histological and behavioral analysis. Numerous studies have demonstrated that the rat fluid percussion model reproduces a wide range of pathological features associated with human TBI.

Key words Fluid percussion, Craniectomy, Endotracheal intubation, Trephination, Mechanical ventilation

1 Introduction

Several excellent reviews have been written documenting the pathophysiology of fluid percussion traumatic brain injury (TBI) in laboratory animals and the high degree of relevance to human TBI [1]. The fluid percussion technique has been applied to a number of species including mouse [2, 3], cat [4], rabbit [5], dog, sheep [6], and pig [7], with the overwhelming majority of applications to the rat (for review *see* [1]). This chapter provides a brief historical background of the fluid percussion model from its inception in larger animals to the current use predominantly in the rat. A major goal is to provide the new user with a practical guide for successful application of the model to the laboratory rat. A secondary goal is to share

Firas Kobeissy et al. (eds.), *Injury Models of the Central Nervous System: Methods and Protocols*, Methods in Molecular Biology, vol. 1462, DOI 10.1007/978-1-4939-3816-2_14, © Springer Science+Business Media New York 2016

tips and nuances that the authors have accumulated over several decades of use that may be helpful even for the seasoned user.

The earliest fluid percussion model procedures involved striking a fluid-filled column secured to the exposed dura of an animal. Denny-Brown and Russell produced a localized pressure pulse to the exposed dura of cats in order to produce a generalized loading to the brain rather than a focal disturbance [8]. They achieved this by rapidly applying extradural injections of fluid and termed this a "percussion concussion" to distinguish the injury from an acceleration concussion.

Gurdjian used a similar technique in dogs, but used compressed air (rather than a fluid) to rapidly and transiently raise intracranial pressure to produce a concussion [9]. Later studies produced concussive injuries by rapidly injecting fluid into a column of water attached to the rabbit vertex [10] or by dropping a weight onto a column of water attached directly to the cerebral cortex of dogs [11].

The evolution of the fluid percussion procedure continued with modifications to control for different amplitudes and durations of the fluid pulse to the brain of rabbits by Stalhammar and colleagues in Sweden [12–14]. The Richmond group, led by Povlishock and Becker, modified the Stalhammar device and applied fluid percussion to the midline of the cat [15]. The next major advance was Dixon's characterization of midline fluid percussion in the rat [16] followed by McIntosh's characterization of the lateral (parasagittal) orientation [17]. The transition to the rat model greatly expanded the applications for cognitive and motor behavioral analysis and testing of pharmacological interventions. The lateral (parasagittal) approach is currently the most commonly used orientation and is the focus of the chapter.

2 Material

2.1 Fluid Percussion Device Setup (Fig. 1)

1. Fluid percussion device (Custom Design and Fabrication model 01-B, Richmond, VA).
2. Extracranial transducer (Sensym ICT model SPTmV0100PG5W02, Milpitas, CA).
3. Digital storage oscilloscope (Tektronix Inc. model TDS 1002, Beaverton, OR).
4. Pressure transducer amplifier (Custom Design and Fabrication, Richmond, VA).

2.2 Animals

1. Male Sprague–Dawley rats (300–325 g; Harlan Laboratories, Hayward, CA).

2.3 Anesthesia Induction and Maintenance

1. Plexiglas® anesthesia induction chamber.
2. Isoflurane, USP.

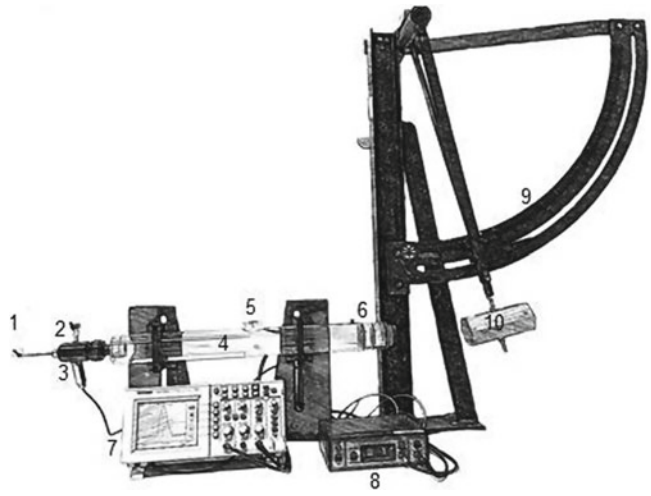

Fig. 1 Schematic diagram of fluid percussion injury device (Model 01-B Custom Design & Fabrication). Luer-lock outlet port (*1*), Luer-lock bubble removal port (*2*), transducer housing (*3*), fluid cylinder (*4*), fill port (*5*), Piston and O-rings (*6*), oscilloscope (*7*), transducer amplifier (*8*), protractor (*9*), and impact hammer (*10*)

3. Isoflurane vaporizer (Highland Medical Equipment, Temecula, CA).
4. Laryngoscope with fiber optics handles (American Diagnostics Corporation).
5. Plexiglas® frame for intubation (Fig. 3).
6. Y-tube anesthesia setup (Fig. 4b).
7. Endotracheal tube (Fig. 4c).
8. Rodent volume ventilator (Harvard Apparatus model 683, Holliston, MA).
9. Blunt end forceps.
10. Polyethylene tubing-PE50 (Becton Dickinson, Franklin Lakes, NJ).

2.4 Animal Preparation and Temperature Measurements

1. Lubricating ophthalmic ointment.
2. Hair clippers.
3. Heating lamp.
4. Temperature controller pad (CWE model TC-1000, Ardmore, PA).
5. Thermalert monitoring thermometer (Physitemp model TH-5, Clifton, NJ).
6. Needle temperature probe (Physitemp model MT-29/2, Clifton, NJ).

2.5 Surgery

1. Stereotaxic frame (Kopf Instruments, Tujunga, CA, USA).
2. Alcohol and betadine swabs.

3. Bupivacaine HCl 0.5% (5 mg/mL) (Hospira Inc., Lake Forest, IL).
4. Scalpel blade holder and blade No. 10 (Henry Schein Inc., Melville, NY).
5. Cotton-tipped applicators.
6. Gauze sponge, non-woven.
7. Lactated Ringer's solution or Saline.
8. Syringes (1, 6, 12 mL) and needles (18–20 G, 22–23 G).
9. Jewelers forceps.
10. Trephine (Miltex model 26-140, York, PA) (Fig. 8a, b).
11. Bone curette (Miltex model 21-322, York, PA) (Fig. 8c).
12. Pin vice with drill bit (No. 47) (Fig. 8d).
13. TBI conduit (Fig. 9).
14. Fluid percussion injury connector tube (Fig. 11b).
15. Anchor screws (round-head machine 2-56, 2.1 mm diameter, 6.0 mm length, Grainger, West Sacramento).
16. Super glue gel (Loctite, West Lake, Ohio).
17. Crosslinked flash acrylic liquid (The Motloid Company, Chicago, IL).
18. Crosslinked flash acrylic powder (Yates & Bird, Chicago, IL).
19. Suture needles and 4.0 braided silk sutures.
20. Needle holder with suture cutters.

3 Methods

3.1 Fluid Percussion Device

The first fluid percussion device was originally developed over several decades ago by Sullivan et al. studying brain trauma in cats [15]. The design and manufacture of the percussion device was later adapted for TBI in rodents and it has become a mainstay in the field of neurotrauma research. There are several manufacturers of the percussion devices, including AmScien Instruments, Dragonfly Inc., and Custom Design and Fabrication (formerly VCU Biomedical Engineering). The Custom Design and Fabrication (Model 01-B) consists of a Plexiglass® cylindrical chamber filled with deionized distilled water or normal saline (Fig. 1). The fluid chamber has a solid Plexiglass® piston with O-rings on one end and a pressure transducer housing with male Luer-lock at the other end. An impact hammer attached to a pendulum can be adjusted on a fixed protractor to allow for varying heights of free-fall to produce different severities of the injury. To induce TBI, the female Luer injury conduit on the acrylic assembly on the rat is connected to the male Luer output port of the pressure transducer housing. Once the experimenter releases the pendulum, the impact hammer strikes

the piston, producing a transient pressure that is transmitted through the fluid-filled cylinder to the Luer-lock outlet at the opposite end of the device. The fluid pressure comes into contact with the dura surface of the animal.

The changes in the transient pressure are detected by the pressure transducer (model SPTmV0100PG5W02; Sensym ICT, Milpitas, CA), relayed to the transducer amplifier and recorded on the digital storage oscilloscope (model TDS 1002; Tektronix Inc., Beaverton, OR). The voltage changes are converted into atmospheres of pressure according to a calibration equation. It is important to accurately calibrate the fluid percussion transducer/oscilloscope system output voltage to a range of precise known pressures. This will ensure accuracy and consistency of pressure recorded during the injury process. Extreme care should be taken to remove all air bubbles throughout the entire fluid system, as air bubbles will greatly alter the pressure dynamics during injury. If air bubbles are present in the system, they will be detected on the oscilloscope tracing as erratic peaks or multiple peaks (be sure to disengage any smoothing filters on the transducer amplifier) (Fig. 2) (*see* **Note 1**). Refer to the manufacturer's website for additional detailed information on the assembly, operations, and troubleshooting of the fluid percussion device.

3.2 Anesthetics

There has been a long-standing debate over the potential neuroprotective effects of anesthetics after brain injury. Since the 1960s, specific properties of certain volatile anesthetics and sedative agents have been recognized and reported as potential neuroprotective agents [18–23]. Hence, the effect of anesthesia in experimental TBI is noteworthy and should require a careful evaluation. Two broad categories of anesthetics are inhalable anesthetics and injectable anesthetics. Halothane and isoflurane are examples of inhalable anesthetics while barbiturates and dissociative agents are examples

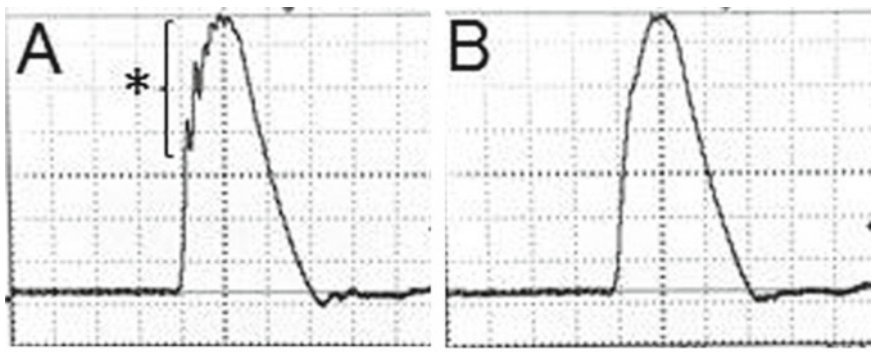

Fig. 2 Fluid percussion pressure traces on the digital oscilloscope for a moderate TBI (3.18 V = 2.16 ATM). The multiple peaks on the pressure curve (see * bracket) are due to the air bubbles inside the fluid cylinder (**a**). The pressure curve is smooth without the air bubbles (**b**)

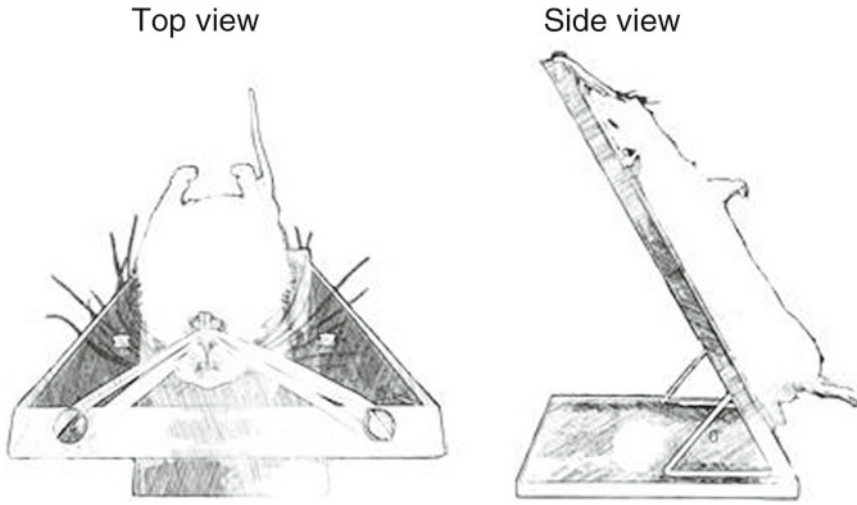

Fig. 3 Endotracheal intubation in an animal. After anesthesia induction, the animal is positioned on the Plexiglas® frame tilted at 60°

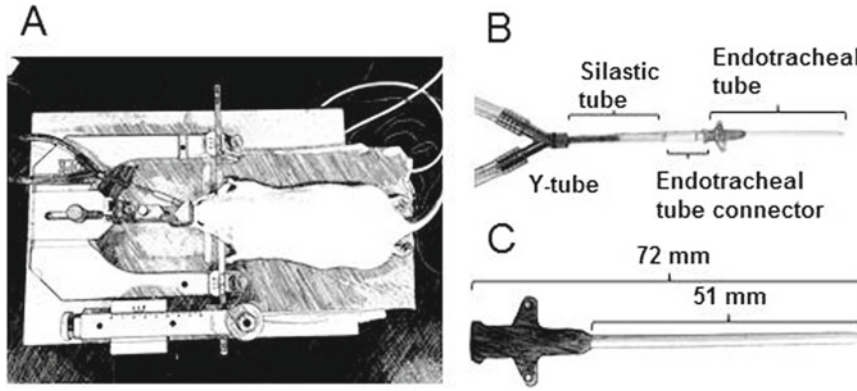

Fig. 4 Stereotaxic setup of an animal under isoflurane anesthesia prior to the craniectomy surgery (**a**), Y-tube and endotracheal intubation unit (**b**), and an example of an endotracheal intubation tube (**c**)

of injectable anesthetics. Injectable anesthetics are commonly used in animal studies, however, there are two disadvantages associated with them: (1) the need for repeated administration if the surgical procedure is longer than anticipated and (2) delayed emergence from anesthesia if the surgical procedure is completed early. These disadvantages make injectable anesthetics difficult for application in TBI studies. In contrast, characteristics of inhalable anesthetics that make them ideal for TBI studies include: (1) rapid induction, (2) control over depth and duration of general anesthesia by the experimenter, and (3) rapid emergence and recovery time.

Halothane and isoflurane are the most common inhalation anesthetics for laboratory animal use. However, evidence

showing positive effects of inhalable anesthetics, especially in neuroprotection studies, has prompted researchers to weigh their options carefully and design their experiments accordingly. TBI often produces alterations of cerebral blood flow, cerebrovascular autoregulation dysfunction, cerebral metabolic impairment, and elevated intracranial pressure [24–28]. Halothane is infrequently used in cerebral ischemia and TBI studies because it is a very potent cerebral vasodilator. Even at a low concentration, halothane significantly increases cerebral blood flow [29] and raises intracranial pressure when mixed with nitrous oxide [30–32].

Anesthetics that alter the cerebral blood flow and intracranial pressure can have a significant effect on experimental outcomes [33]. As a result, isoflurane has become the preferred anesthetic for veterinary medicine and experimental TBI studies. The appropriate isoflurane concentration used for induction and surgical maintenance is 4 % and 2 %, respectively. Nitrous oxide (NO) is generally used as a carrier gas to reduce the dose of isoflurane required during anesthesia maintenance. NO is mixed with oxygen at 70 % and 30 %, respectively. The following steps should be taken to minimize total anesthesia time and reduce experimental variability: (1) Anesthesia should be completely switched off immediately after injury. Isoflurane is only required during the TBI surgery and is no longer needed post-injury. Usually, moderate to severe TBI would render the animal unconscious for longer than 10 min so additional anesthesia is not necessary when closing the surgical incision. (2) Total time spent under anesthesia should be the same for each animal. For sham-injured animals, there is no TBI so the animal should remain on anesthesia until the surgical incision is closed.

3.3 Mechanical Ventilation and Endotracheal Intubation

Severe clinical TBI patients often require intubation and mechanical ventilation to control respiration and prevent further insults to the brain. The use of a mechanical ventilator is becoming commonplace in animal research [34], especially in experimental animal TBI studies. TBI impact is usually followed immediately by apnea which, if prolonged causes hypoxia, hypercapnia, and cerebral edema leading to poorer outcomes [35]. Secondary insults, such as hypotension and hypoxia, markedly exacerbate sensorimotor dysfunction, cognitive deficits, neuronal degeneration [36–38], and increase mortality and morbidity [39]. In experimental TBI, important considerations for the proper settings of a mechanical ventilator should be addressed to prevent hypoventilation or hyperventilation of the animal. Adequate sedation and oxygenation are usually maintained at the tidal volume of 2.0–2.5 mL and 75–85 strokes per min for adult rats (~300 g). Ventilator settings to achieve normal blood gases should be confirmed by arterial blood gases analysis.

When used in conjunction with a mechanical ventilator, endotracheal intubation can be used for the precise delivery of different concentrations of anesthetics. It also keeps the airway patent after

injury, an important factor for reducing complications after TBI. Inhalable anesthetics are also often delivered via a nose cone but there are limitations to this method in TBI studies. The nose cone anesthesia setup does not allow the experimenter to have control over the animal's respiration and apnea post-TBI. In contrast, endotracheal intubation with mechanical ventilation enables the experimenter to do the following: (1) maintain precision control over the rate of respiration and (2) reduce the incidences of hyperventilation or hypoventilation. It is important to maintain normoventilation to minimize complications associated with TBI and reduce experimental variability.

Endotracheal intubation involves insertion of a plastic cannula between the vocal chords into the trachea. There are several variations on how to perform an endotracheal intubation [40, 41]. The technique is challenging and requires considerable practice. Intravenous over-the-needle catheters (size 14) are ideal for use as an endotracheal intubation tube for rats (Fig. 4) (*see* **Note 2**).

After anesthesia induction, the unconscious animal is suspended by its superior incisors on a 60° tilted Plexiglas® frame in a supine position (Fig. 3). Blunt end forceps are used to gently pull the tongue to the side and then a small size laryngoscope—with a light source attached to the retractor—is used to hold the tongue against the base of the mouth to visualize the vocal folds and glottis. A blunt-end polyethylene tube (PE50) is used as a stylet to guide the endotracheal tube (*see* **Note 3**). A needle stylet should not be used because of the high risk of bleeding caused by the sharp, rigid metal piece. To facilitate the insertion of the endotracheal tube into the trachea, insert the tip of the polyethylene tube stylet between the vocal chords during inspiration. The endotracheal tube is then advanced into the larynx. The guide stylet is withdrawn while securing the endotracheal tube. Any observations of gurgling and bubbling sounds should be addressed immediately by withdrawing the fluids with a suctioning catheter.

After anesthesia induction, a successful intubation should take place in less than 30 s to avoid the need to re-anesthetize the animal. The number of intubation attempts should be limited. Continue to perform the intubation attempts only if there are no signs of tracheal bleeding or oropharyngeal swelling or damage. Verify the proper placement of the endotracheal tube before proceeding to the next step of the surgery (*see* **Note 4**).

3.4 Body Weight Measurements

The appropriate weight range for the specific study should be maintained within a narrow range to ensure experimental consistency. On the day of surgery, pre-injury body weight of the animals is recorded as a baseline measurement. Fasting before surgery is optimal, but generally not necessary. It is important that the animal's body weight be recorded daily from the baseline date until the experimental endpoint. Generally, TBI will cause appetite loss

and fatigue during the first few days post-injury. Frequently, changes in the animal body weight are due primarily to dehydration, therefore, fluid management is critical for the animal's well-being and to avoid introducing variability into the experimental design. Severe dehydration can also cause other complications that can confound the experimental results.

Room temperature lactated Ringer's or normal saline (6 mL) can be administered daily (subcutaneous) for balanced fluid replacement. The variation in body weight loss post-injury and the interval when the animal begins to regain weight are usually dependent on the injury severity. As generally observed in the author's experiments, the steady rebound to baseline body weight for mild, moderate, and severe TBI-injured rats is between 1 and 3 days, 5–7 days, and greater than 10 days, respectively. Animals that lose up to 20 % of their body weight should be euthanized and excluded from the study. For sham-TBI animals, the craniectomy and surgical anesthesia should have no significant effect on their grooming and appetite. Sham-injured animals should regain baseline bodyweight within 1 day post-surgery.

3.5 Temperature

Surgical anesthesia as well as TBI can alter the animal's ability to regulate and maintain normal body and brain temperature [42, 43]. Raised body and brain temperature are common occurrences in both the clinical and experimental TBI studies [44–46] and even a 1–2 °C elevation in body and brain temperature is detrimental to recovery [44]. On the other end of the spectrum, post-traumatic brain hypothermia can improve outcomes in both clinical [47–49] and experimental TBI [50–53]. For animal TBI studies, a temperature-controlled heating pad (CWE model TC-1000, Ardmore, PA) and heat lamp are useful to help maintain the body temperature within the normal range during surgery and post-injury. The brain temperature can be monitored with a 29-gauge needle temperature probe (Physitemp unit TH-5, probe MT-29/2, Clifton, NJ) placed in the temporalis muscle and is a dependable indirect measure of brain temperature [43, 54]. It is important to monitor and maintain appropriate body and temporalis muscle temperatures at 37 ± 0.5 °C and 36 ± 0.5 °C, respectively.

3.6 The Lateral Fluid Percussion Surgery

3.6.1 Anesthesia Induction

The survival surgery procedures described herein should be performed in accordance with aseptic rodent surgery guidelines. For the purpose of endotracheal intubation, the animal is placed in an induction chamber and lightly anesthetized with 4 % isoflurane in 100 % air for 4–5 min. Afterwards, the head is shaved prior to intubation. The muscle relaxation effect of isoflurane should allow a relatively easy access for oral endotracheal intubation. After successfully performing the intubation, the animal is connected to a mechanical ventilator (Harvard Apparatus model 683, Holliston, MA) and isoflurane vaporizer (Highland Medical Equipment) setup

that allows inspiration and expiration via a y-shaped tube (Fig. 4) (*see* **Note 5**). The surgical level of anesthesia is maintained with 2% isoflurane in a 70% nitrous oxide and 30% oxygen gas mixture.

Always check and ensure that the animal is anesthetized before proceeding to the next step of the procedure. Use the hindpaw withdrawal reflex pinch to monitor for adequate depth of anesthesia. Also, ensure there is adequate oxygenation by observing skin color of paws and ears. Adjust the anesthesia level accordingly if the animal is responsive to a toe pinch. If necessary, temporarily and slightly increase the anesthesia to sedate the animal; however, remember to reduce to an appropriate level to avoid anesthesia overdosing. As part of standard preoperating procedures, the following steps should be taken prior to any incision: (1) Apply an ophthalmic ointment to the animal's eyes to prevent dryness of the corneas. (2) Apply and clean the surgical site with alcohol and betadine swabs.

3.6.2 Skull Exposure

The application of a local anesthetic is recommended to minimize any potential residual postoperative pain and discomfort from the surgical procedures. Prior to an incision, a nonsteroidal local analgesic, bupivacaine (0.025% diluted in saline), is applied to the subcutaneous space along the incision site. A 2-cm midline scalp incision extending from the eyes to the tip of ear is made with the scalpel (#10 blade) to expose the bone for trephining the craniectomy (*see* Fig. 5 for a step-by-step illustration of the craniectomy surgery). The incision should not be made past the ears to avoid major bleeding of the neck muscles. Reflect and retract the skin laterally. Use sterile cotton-tipped swabs to scrape the periosteal connective tissue and fascia from the skull. There will be an increase in bleeding from the

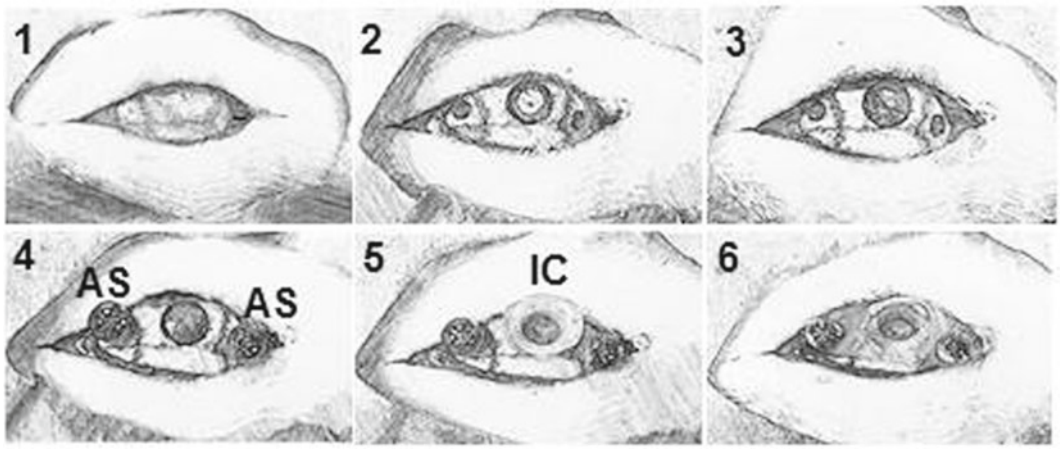

Fig. 5 Step-by-step procedure of the craniectomy surgery. Midline scalp incision (step 1). The craniectomy is created by trephination of the skull over the right parietal bone, two burr holes for anchor screws (step 2). The bone flap is removed with the dura intact (step 3). The two steel anchor screws (AS) are secured in place (step 4). The injury conduit (IC) is positioned over the intact dura (step 5). Dental acrylic TBI injury assembly (step 6)

skull surface if using a scalpel to scrape away the periosteum. A syringe containing room temperature Ringer's solution or normal saline is used to rinse the skull when cleaning out the fascia.

3.6.3 Craniectomy: Size and Location

The animal's head is mounted and secured in a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA) with the head level at the interaural axis. The lateral fluid percussion injury involves creating a right-side craniectomy to access the dura overlying the brain parenchyma by removal of a specific size bone flap from the parietal cortex. The bone flap will not be replaced after the injury. The authors perform a 4.8 mm diameter craniectomy. However, other variations in the size of the craniectomy are reported in the literature [55–57]. For adult rats, the distance from bregma to lambda is 9 mm. Bregma is the intersection of the sagittal and coronal sutures. Lambda is the invisible point where the lambdoid and the sagittal suture meet. The animal's head should be aligned to a skull flat position so that bregma and lambda are in the same horizontal plane (Fig. 6). Adjust the incisor bar on the stereotaxic instrument so that the dorsal–ventral readings on the Vernier scale are similar between the bregma and lambda landmarks. Studies using animals of the same age do not require re-leveling of the incisor bar between animals and experiments. Placement of the center of the craniectomy is midpoint between bregma and lambda (−4.5 mm A-P) and midpoint between the sagittal suture and the lateral ridge (+3.0 mm M-L) (Fig. 7). The craniectomy's position should be exact to reduce experimental variability between animals. This is especially important in TBI behavioral and pathological studies because small shifts can affect behavioral outcome and differential lesion development [58, 59].

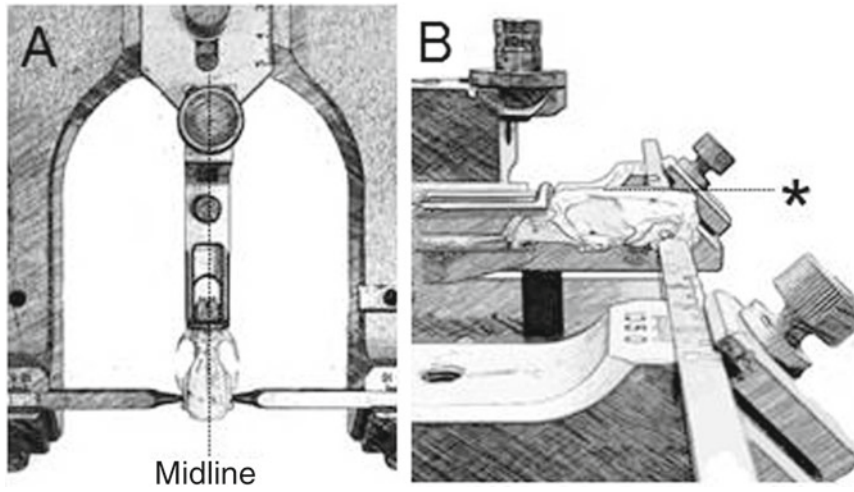

Fig. 6 Stereotaxic headholder (Kopf instruments) with a rat's skull (Sprague–Dawley) immobilized by the ear bars (**a**). The incisor bar is adjusted so that bregma and lambda lie in the same horizontal plane (*) for the accurate positioning of the craniectomy (**b**)

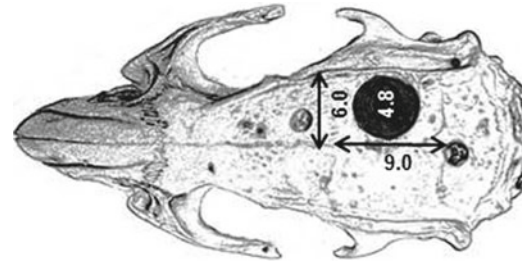

Fig. 7 Stereotaxic coordinates for the craniectomy and anchor screws. The center of the 4.8 mm craniectomy is positioned at 4.5 mm A-P and 3.0 mm M-L from bregma. Burr holes are made approximately 2 mm anterior to bregma and 1 mm posterior to lambda. Dimensions are in millimeters

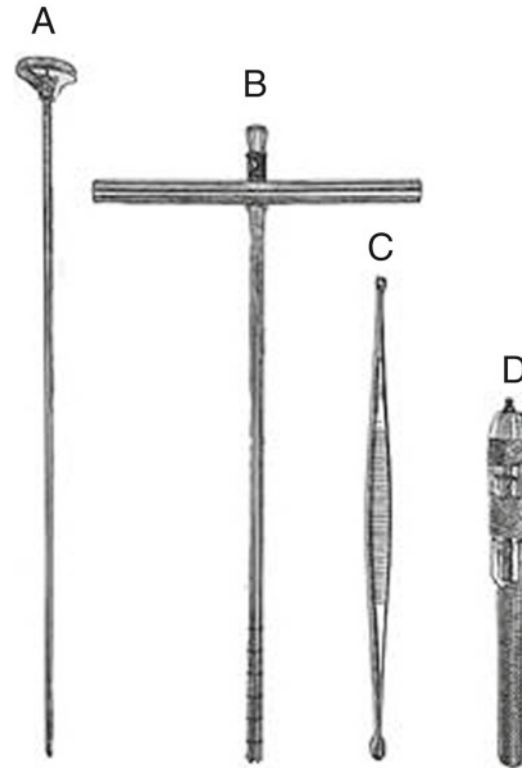

Fig. 8 Surgical instruments used for lateral fluid percussion. Trephine guide stylet (**a**), T-Trephine (**b**), bone curette (**c**), pin vise with drill bit (**d**)

3.6.4 Trephination

A craniectomy can be created by using an electric dental drill or a handheld T-style trephine (Miltex 26-140) (Fig. 8). The use of an electric drill may cause heat-induced cellular death in the cortex directly near the drilling site that is not due to the TBI impact [unpublished data]. Hence, the small T-style handheld trephine is the recommended drilling instrument (*see* **Note 6**). Unlike the

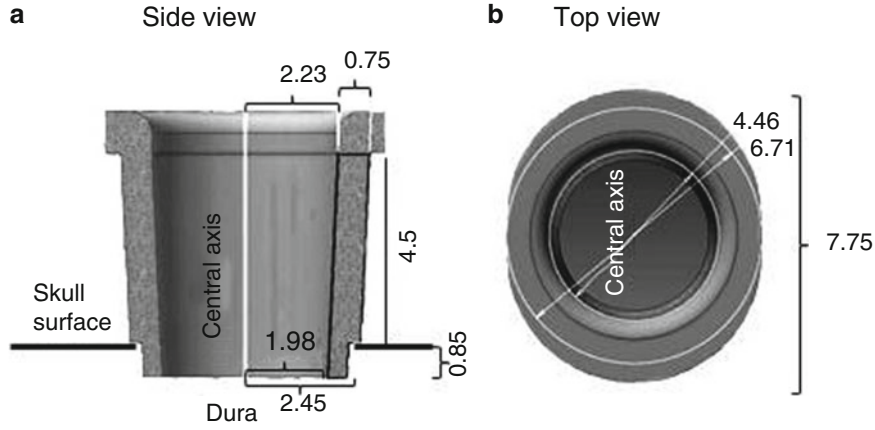

Fig. 9 The TBI injury conduit from a side view and top view rotated about the central axis. The injury conduit is made with the exact dimensions to fit the craniectomy and connector tube. The inside dimensions are the female Luer-lock taper. Dimensions are in millimeters

electric drill technique, the T-style handheld trephine can drill and generate a perfect circular craniectomy that is usually free of broken bone chips (*see* **Note 7**). The most common surgical mistake when drilling a craniectomy is the use of excessive force on the trephine. It is easy to mistakenly break through the dura and damage the brain. It is both necessary and important to use the surgical microscope to monitor the drilling process. Bleeding should not occur from underneath the craniectomy site since no major blood vessels are located in that area. Fine forceps can be used to gently tap on the bone flap for movement. The drilling should come to a halt once the skull bone becomes loosened. Carefully and gently slide the forceps underneath the skull bone flap to lift and remove it while at the same time avoiding any contact with the dura. A bone curette is used to remove any bone fragments. By keeping the dura intact, the brain tissues will not be in direct contact with the fluid bolus during TBI. Any animals showing signs of damage to the dura should be excluded from the study.

3.6.5 Anchor Screws

The components of a TBI acrylic assembly consist of two anchoring screws, an injury conduit and a hardened dental cement, to secure the conduit to the craniotomy. The placement of the anchor screws (round-head machine 2-56, 2.1 mm diameter, 6.0 mm length) is essential to prevent the dislodging of the acrylic assembly during the impact. Two small burr holes for the anchor screws are positioned away from the site of impact approximately 2 mm rostral to Bregma and 1 mm caudal to Lambda. Gently drill at an angle using drill bit (size 47) in a pin vise tool to create a burr hole that is shallow enough to allow the screws to catch without creating a deep penetration into the skull (*see* **Note 8**).

3.6.6 Injury Conduit and TBI Acrylic Assembly

The rigid injury conduit (Fig. 9) is made with a 3D printer from UV curing resin (Stratasys) to specific dimensions which allow the conduit to securely fit into the 4.8 mm craniectomy. An alternative option is to modify a Luer-Loc needle conduit (22 G) with similar dimensions (*see* **Note 9**). In preparation for TBI, the conduit is placed into the craniectomy over the exposed intact dura (*see* **Notes 10** and **11**). A perfectly secured conduit will tilt at an angle from the skull surface (Fig. 10). Cyanoacrylate adhesive acrylic (Plastics One, Roanoke, VA) is applied to secure the conduit and anchoring screws (*see* **Note 12**). The acrylic drying process usually takes 5 min depending on the dilution of the acrylic mixture. Once the drying is complete, attach the FPI connector tube to the conduit (Fig. 11). The connector tube should be completely filled with normal sterile saline to prevent any bubbles from forming in the connector tube. This volume of the sterile saline will also prevent the non-sterile fluid in the fluid percussion device cylinder from entering into the cranium during the TBI impact. The connector tube is attached to the fluid percussion device for delivery of the fluid pressure to the dura (Fig. 12).

3.6.7 Lateral Fluid Percussion TBI

Ensure that the percussion device is working properly and adjusted for the desired magnitude of injury before inducing the TBI. The isoflurane anesthesia is turned off just prior to injury. The animal is

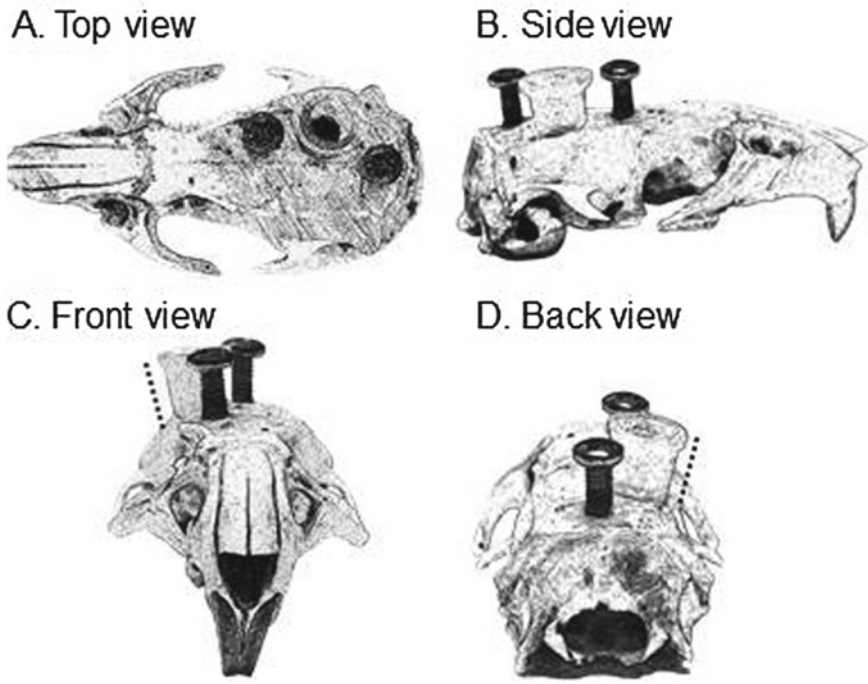

Fig. 10 Multiple views of the animal's skull with the anchor screws and injury conduit attached to the craniectomy. When properly fit, the injury conduit is slightly tilted to the side due to the natural slope of the skull surface (*dashed lines*)

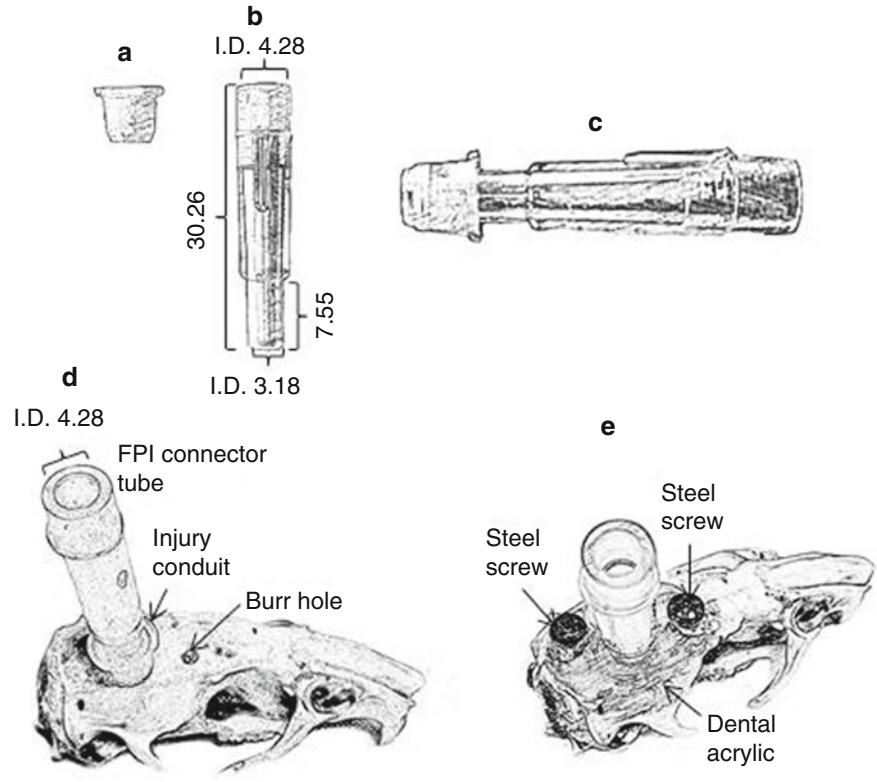

Fig. 11 The specific components of a TBI injury assembly. The TBI injury conduit (**a**) and the fluid percussion device connector tube (**b**) are connected together (**c**) and are attached to the craniectomy (**d**). The dental acrylic and anchor screws secured the injury conduit and connector tube (**e**). Dimensions are in millimeters

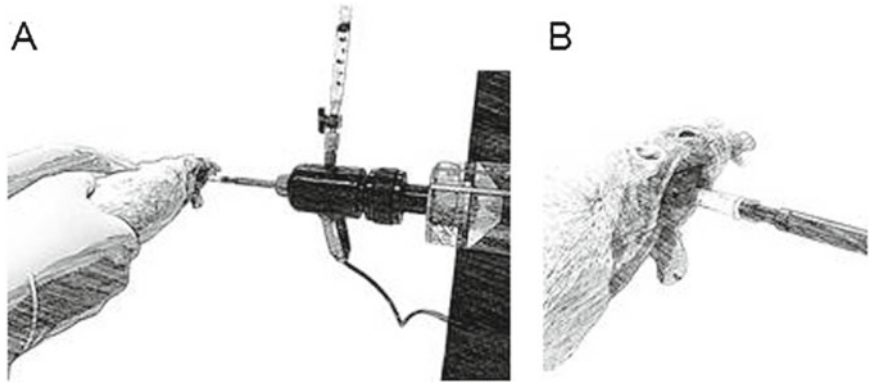

Fig. 12 Lateral orientation of the animal for delivery of a pressurized fluid pulse to the exposed intact dura. The animal is connected to the fluid percussion device by an injury connector tube that is prefilled with sterile saline

disconnected from the mechanical ventilator and immediately connected to the fluid percussion device. The LFP injury is a quick process that takes place in less than 10 s while the animal is still sedated. It is important to not introduce air bubbles into the FPI device connector tube to avoid dampening of the pressure pulse. A weakened pressure pulse will diminish the anticipated TBI pressure resulting in less injury to the brain.

It is better to have two people carry out the TBI stage of the experiment. The first experimenter holds the animal on its side and attaches the connector tube to the device. Injury is produced with the lateral orientation. The second experimenter releases the pendulum to induce the TBI. After the impact, one can determine that a successful TBI has occurred based on the following observations: (1) No fluid leaks from the acrylic assembly, (2) the column of fluid in the connector tube remains full, (3) the pressure pulse shown on the oscilloscope is within the target range and the pressure tracing is smooth with a single peak (Fig. 2).

3.6.8 Sham-TBI

When designing experimental TBI studies, it is essential to have a sham-TBI control group to properly assess the effects of surgical anesthesia or the craniectomy on the animals. Sham-TBI animals are not subjected to TBI induction but they do undergo the same surgical procedures as the TBI animals.

3.6.9 Postoperative Care

After inducing TBI, disconnect the injury tube from the device and promptly reconnect the animal to the mechanical ventilator. The animal is ventilated with a 2:1 nitrous oxide/oxygen mixture in the absence of isoflurane until its spontaneous breathing resumes. A spatula is used to remove the acrylic cap from the skull. Warm lactated Ringer's or normal saline is used to rinse and clean the incision. Perform the hindpaw withdrawal reflex pinch to ensure that the animal is unresponsive before suturing the scalp incision. Once the incision is closed and the animal is able to spontaneously breathe, place the animal in a heated recovery cage for postoperative monitoring. The animal is extubated and the righting reflex is assessed. Extubation of the animal is done only after it has been observed to exhibit no signs of respiratory distress. Gently withdraw the intubation tube from the animal to avoid introducing any trauma to its trachea. The animal is placed in a supine position at regular intervals (~20 s) to test its ability to spontaneously revert back to a prone position. The duration of the suppressed righting reflex is used as an additional indicator of injury severity. The experimenter should observe the animal until it becomes ambulatory. If the animal does not regain its consciousness within 30 min of the TBI induction, it should be euthanized. This is typically an indication of hemorrhage around the brainstem. Any residual effects of isoflurane anesthesia typically do not alter consciousness for longer than 2 min. TBI animals remain unconscious for 10–15 min due to injured brain functions rather than anesthetic effects.

3.7 TBI: Complications, Morbidity, and Mortality

The lateral fluid percussion model reproduces morbidity and mortality that are often comparable to those of humans. Similar to clinical patients, TBI-injured animals also exhibited brain injury hallmarks such as cerebral contusions, subdural hematomas, and intracranial hemorrhages [60]. The complications and mortality associated with experimental TBI are usually indicative of the injury severity. Common complications included prolonged apnea, respiratory distress [61, 62], acute pulmonary edema [63, 64], and dysautonomia [65] (decorticate and decerebrate postures). Mortality after moderate to severe TBI usually occurs within the first 30 min after injury and is associated with excessive intracranial hemorrhage. The authors have observed that mild, moderate, and severe LFP injuries produce a mortality rate of approximately 0, 25 %, and greater than 50 %, respectively.

4 Notes

1. Removal of air bubbles from the fluid percussion device: Filling the apparatus with degassed water or saline will reduce the formation of air bubbles. Degassing can be achieved prior to filling the apparatus by applying a vacuum to the distilled water in an Erlenmeyer flask (using a magnetic stir bar aids in the process).
2. An endotracheal tube can be constructed with PE 200 polyethylene tube (I.D. 1.40 mm; O.D. 1.9 mm).
3. A soft material guide stylet should be utilized to minimize trauma during endotracheal intubation. The polyethylene guide stylet (PE50) is inserted inside and approximately 4 mm past the distal end of the endotracheal tube.
4. Since direct visualization of the tracheal intubation is not possible in the animal, the correct placement of the tracheal tube position is confirmed by observing the exhaled breath on a smooth black surface (e.g., lab bench).
5. *See* Fig. 4b for the different parts used to connect the anesthesia Y-tube to the endotracheal tube. A short piece of Silastic tube is attached to the Y-tube and to the endotracheal tube. The Silastic tubing can be connected directly to the PE 200 endotracheal tube. If using the IV over-the-needle endotracheal tube, the Silastic tubing needs to be secured to a hard plastic connector with a male Luer outlet for insertion into the IV over-the-needle endotracheal tube. It is important to perform routine internal cleaning of the anesthesia Y-tube unit and endotracheal tubing. A clogged tube will restrict an adequate amount of anesthesia and oxygen to the animal. If not closely monitored, the animal will become hypoxic and possibly die during surgery.

6. Producing a sharp point on the tip of the trephine stylet with a file will prevent the trephine from "walking" from the precise skull location during the trephining process.
7. Normal saline or lactated Ringer's solution at room temperature can be used to irrigate the skull to facilitate the drilling process. The trephine can penetrate moistened bone more freely and provide a cleaner cut.
8. The placement of the anchor screws into the burr holes should be done prior to removing the craniectomy bone flap. The intact bone flap keeps the brain protected from accidental trauma from a slip of the surgical screwdriver into the craniectomy during the placement of the screws.
9. The main advantage of producing a conduit with a 3D printer is a superior fit without the uneven edges that can potentially breach the dura and compromise the experiment.
10. A 1 mL syringe can be temporarily attached to the conduit to help guide and facilitate the steady placement of the conduit into the craniectomy. Once the conduit is locked into the craniectomy, gently rotate the syringe in a counterclockwise motion to detach it from the hub. It is difficult to handle and properly guide the small size conduit into the craniectomy with the use of the fingers.
11. Use the wooden end of a cotton-tipped swab to spread a very small amount of superglue around the conduit's stepped-shoulder bottom to ensure a sealed, tighter fit in the craniectomy. It is important to use the glue sparingly to prevent it from contacting the dura surface. Dried glue on top of the dura will form a thick barrier, impeding and diminishing the fluid pulse pressure.
12. It is recommended to use a 1 mL syringe to slowly inject a small amount of the acrylic adhesive to create the acrylic assembly. The acrylic-filled syringe allows the experimenter to have control over the flow rate of the injection, preventing any accidental excessive overflowing. Avoid excessive thickness of the acrylic adhesive since it is exothermic upon curing and could cause a thermal lesion to the brain. Prior to curing, separate the acrylic adhesive from the skin while the acrylic is still soft.

References

1. Thompson HJ, Lifshitz J, Marklund N, Grady MS, Graham DI, Hovda DA, McIntosh TK (2005) Lateral fluid percussion brain injury: a 15-year review and evaluation. J Neurotrauma 22:42–75
2. Alder J, Fujioka W, Lifshitz J, Crockett DP, Thakker-Varia S (2011) Lateral fluid percussion: model of traumatic brain injury in mice. J Vis Exp (54)
3. Carbonell WS, Maris DO, McCall T, Grady MS (1998) Adaptation of the fluid percussion injury model to the mouse. J Neurotrauma 15:217–229
4. Povlishock JT, Becker DP, Cheng CL, Vaughan GW (1983) Axonal change in minor head injury. J Neuropathol Exp Neurol 42:225–242
5. Hartl R, Medary M, Ruge M, Arfors KE, Ghajar J (1997) Blood-brain barrier breakdown

occurs early after traumatic brain injury and is not related to white blood cell adherence. Acta Neurochir Suppl 70:240–242

6. Millen JE, Glauser FL, Fairman RP (1985) A comparison of physiological responses to percussive brain trauma in dogs and sheep. J Neurosurg 62:587–591
7. Pfenninger EG, Reith A, Breitig D, Grunert A, Ahnefeld FW (1989) Early changes of intracranial pressure, perfusion pressure, and blood flow after acute head injury. Part 1: An experimental study of the underlying pathophysiology. J Neurosurg 70:774–779
8. Denny-Brown D, Russell WR (1940) Experimental cerebral concussion. J Physiol 99:153
9. Gurdjian ES, Lissner HR, Webster JE, Latimer FR, Haddad BF (1954) Studies on experimental concussion: relation of physiologic effect to time duration of intracranial pressure increase at impact. Neurology 4:674–681
10. Lindgren S, Rinder L (1966) Experimental studies in head injury. II. Pressure propagation in "percussion concussion". Biophysik 3:174–180
11. Metz B (1971) Acetylcholine and experimental brain injury. J Neurosurg 35:523–528
12. Stalhammar D (1975) Experimental brain damage from fluid pressures due to impact acceleration. 1. Design of experimental procedure. Acta Neurol Scand 52:7–26
13. Stalhammar D (1975) Experimental brain damage from fluid pressures due to impact acceleration. 2. Pathophysiological observations. Acta Neurol Scand 52:27–37
14. Stalhammar D, Olsson Y (1975) Experimental brain damage from fluid pressures due to impact acceleration. 3. Morphological observations. Acta Neurol Scand 52:38–55
15. Sullivan HG, Martinez J, Becker DP, Miller JD, Griffith R, Wist AO (1976) Fluid-percussion model of mechanical brain injury in the cat. J Neurosurg 45:521–534
16. Dixon CE, Lyeth BG, Povlishock JT, Findling RL, Hamm RJ, Marmarou A, Young HF, Hayes RL (1987) A fluid percussion model of experimental brain injury in the rat. J Neurosurg 67:110–119
17. McIntosh TK, Vink R, Noble L, Yamakami I, Fernyak S, Soares H, Faden AL (1989) Traumatic brain injury in the rat: characterization of a lateral fluid-percussion model. Neuroscience 28:233–244
18. Statler KD, Alexander H, Vagni V, Holubkov R, Dixon CE, Clark RS, Jenkins L, Kochanek PM (2006) Isoflurane exerts neuroprotective actions at or near the time of severe traumatic brain injury. Brain Res 1076:216–224
19. Kawaguchi M, Furuya H, Patel PM (2005) Neuroprotective effects of anesthetic agents. J Anesth 19:150–156
20. Koerner IP, Brambrink AM (2006) Brain protection by anesthetic agents. Curr Opin Anaesthesiol 19:481–486
21. Matchett GA, Allard MW, Martin RD, Zhang JH (2009) Neuroprotective effect of volatile anesthetic agents: molecular mechanisms. Neurol Res 31:128–134
22. Statler KD, Alexander H, Vagni V, Dixon CE, Clark RS, Jenkins L, Kochanek PM (2006) Comparison of seven anesthetic agents on outcome after experimental traumatic brain injury in adult, male rats. J Neurotrauma 23:97–108
23. Statler KD, Kochanek PM, Dixon CE, Alexander HL, Warner DS, Clark RS, Wisniewski SR, Graham SH, Jenkins LW, Marion DW, Safar PJ (2000) Isoflurane improves long-term neurologic outcome versus fentanyl after traumatic brain injury in rats. J Neurotrauma 17:1179–1189
24. Udomphorn Y, Armstead WM, Vavilala MS (2008) Cerebral blood flow and autoregulation after pediatric traumatic brain injury. Pediatr Neurol 38:225–234
25. Bouma GJ, Muizelaar JP (1992) Cerebral blood flow, cerebral blood volume, and cerebrovascular reactivity after severe head injury. J Neurotrauma 9(Suppl 1):S333–S348
26. Bouma GJ, Muizelaar JP (1995) Cerebral blood flow in severe clinical head injury. New Horiz 3:384–394
27. Brown JI, Moulton RJ, Konasiewicz SJ, Baker AJ (1998) Cerebral oxidative metabolism and evoked potential deterioration after severe brain injury: new evidence of early posttraumatic ischemia. Neurosurgery 42:1057–1063, discussion 1063-1054
28. Philip S, Udomphorn Y, Kirkham FJ, Vavilala MS (2009) Cerebrovascular pathophysiology in pediatric traumatic brain injury. J Trauma 67:S128–S134
29. Christensen MS, Hoedt-Rasmussen K, Lassen NA (1967) Cerebral vasodilatation by halothane anaesthesia in man and its potentiation by hypotension and hypercapnia. Br J Anaesth 39:927–934
30. Bain JA, Catton DV, Cox JM, Spoerel WE (1967) The effect of general anaesthesia on the tolerance of cerebral ischaemia in rabbits. Can Anaesth Soc J 14:69–78
31. Jennett WB, Barker J, Fitch W, McDowall DG (1969) Effect of anaesthesia on intracranial pressure in patients with space-occupying lesions. Lancet 1:61–64

32. McDowall DG, Barker J, Jennett WB (1966) Cerebro-spinal fluid pressure measurements during anaesthesia. Anaesthesia 21:189–201
33. Smith AL, Wollman H (1972) Cerebral blood flow and metabolism: effects of anesthetic drugs and techniques. Anesthesiology 36:378–400
34. Rivard AL, Simura KJ, Mohammed S, Magembe AJ, Pearson HM, Hallman MR, Barnett SJ, Gatlin DL, Gallegos RP, Bianco RW (2006) Rat intubation and ventilation for surgical research. J Invest Surg 19:267–274
35. Chesnut RM, Marshall LF, Klauber MR, Blunt BA, Baldwin N, Eisenberg HM, Jane JA, Marmarou A, Foulkes MA (1993) The role of secondary brain injury in determining outcome from severe head injury. J Trauma 34:216–222
36. Bramlett HM, Dietrich WD, Green EJ (1999) Secondary hypoxia following moderate fluid percussion brain injury in rats exacerbates sensorimotor and cognitive deficits. J Neurotrauma 16:1035–1047
37. Bramlett HM, Green EJ, Dietrich WD (1999) Exacerbation of cortical and hippocampal CA1 damage due to posttraumatic hypoxia following moderate fluid-percussion brain injury in rats. J Neurosurg 91:653–659
38. Feng JF, Zhao X, Gurkoff GG, Van KC, Shahlaie K, Lyeth BG (2012) Post-traumatic hypoxia exacerbates neuronal cell death in the hippocampus. J Neurotrauma 29:1167–1179
39. Werner C, Engelhard K (2007) Pathophysiology of traumatic brain injury. Br J Anaesth 99:4–9
40. Costa DL, Lehmann JR, Harold WM, Drew RT (1986) Transoral tracheal intubation of rodents using a fiberoptic laryngoscope. Lab Anim Sci 36:256–261
41. Thet LA (1983) A simple method of intubating rats under direct vision. Lab Anim Sci 33:368–369
42. Mrozek S, Vardon F, Geeraerts T (2012) Brain temperature: physiology and pathophysiology after brain injury. Anesthesiol Res Pract 2012:989487
43. Jiang JY, Lyeth BG, Clifton GL, Jenkins LW, Hamm RJ, Hayes RL (1991) Relationship between body and brain temperature in traumatically brain-injured rodents. J Neurosurg 74:492–496
44. Childs C (2008) Human brain temperature: regulation, measurement and relationship with cerebral trauma: part 1. Br J Neurosurg 22:486–496
45. Sacho RH, Childs C (2008) The significance of altered temperature after traumatic brain injury: an analysis of investigations in experimental and human studies: part 2. Br J Neurosurg 22:497–507
46. Thompson HJ, Tkacs NC, Saatman KE, Raghupathi R, McIntosh TK (2003) Hyperthermia following traumatic brain injury: a critical evaluation. Neurobiol Dis 12:163–173
47. Marion DW, Penrod LE, Kelsey SF, Obrist WD, Kochanek PM, Palmer AM, Wisniewski SR, DeKosky ST (1997) Treatment of traumatic brain injury with moderate hypothermia. N Engl J Med 336:540–546
48. Clifton GL, Allen S, Barrodale P, Plenger P, Berry J, Koch S, Fletcher J, Hayes RL, Choi SC (1993) A phase II study of moderate hypothermia in severe brain injury. J Neurotrauma 10:263–271, discussion 273
49. Marion DW, Obrist WD, Carlier PM, Penrod LE, Darby JM (1993) The use of moderate therapeutic hypothermia for patients with severe head injuries: a preliminary report. J Neurosurg 79:354–362
50. Dietrich WD, Alonso O, Busto R, Globus MY, Ginsberg MD (1994) Post-traumatic brain hypothermia reduces histopathological damage following concussive brain injury in the rat. Acta Neuropathol 87:250–258
51. Clifton GL, Jiang JY, Lyeth BG, Jenkins LW, Hamm RJ, Hayes RL (1991) Marked protection by moderate hypothermia after experimental traumatic brain injury. J Cereb Blood Flow Metab 11:114–121
52. Jiang JY, Lyeth BG, Kapasi MZ, Jenkins LW, Povlishock JT (1992) Moderate hypothermia reduces blood-brain barrier disruption following traumatic brain injury in the rat. Acta Neuropathol 84:495–500
53. Lyeth BG, Jiang JY, Liu S (1993) Behavioral protection by moderate hypothermia initiated after experimental traumatic brain injury. J Neurotrauma 10:57–64
54. Hasegawa Y, Latour LL, Sotak CH, Dardzinski BJ, Fisher M (1994) Temperature dependent change of apparent diffusion coefficient of water in normal and ischemic brain of rats. J Cereb Blood Flow Metab 14:383–390
55. Rink A, Fung KM, Trojanowski JQ, Lee VM, Neugebauer E, McIntosh TK (1995) Evidence of apoptotic cell death after experimental traumatic brain injury in the rat. Am J Pathol 147:1575–1583
56. Hicks RR, Zhang L, Atkinson A, Stevenon M, Veneracion M, Seroogy KB (2002) Environmental enrichment attenuates cognitive deficits, but does not alter neurotrophin gene expression in the hippocampus following lateral fluid percussion brain injury. Neuroscience 112:631–637
57. Sinson G, Voddi M, McIntosh TK (1995) Nerve growth factor administration attenuates cognitive but not neurobehavioral motor dysfunction or

hippocampal cell loss following fluid-percussion brain injury in rats. J Neurochem 65:2209–2216

58. Vink R, Mullins PG, Temple MD, Bao W, Faden AI (2001) Small shifts in craniotomy position in the lateral fluid percussion injury model are associated with differential lesion development. J Neurotrauma 18:839–847
59. Floyd CL, Golden KM, Black RT, Hamm RJ, Lyeth BG (2002) Craniectomy position affects morris water maze performance and hippocampal cell loss after parasagittal fluid percussion. J Neurotrauma 19:303–316
60. Maas AI, Stocchetti N, Bullock R (2008) Moderate and severe traumatic brain injury in adults. Lancet Neurol 7:728–741
61. Holland MC, Mackersie RC, Morabito D, Campbell AR, Kivett VA, Patel R, Erickson VR, Pittet JF (2003) The development of acute lung injury is associated with worse neurologic outcome in patients with severe traumatic brain injury. J Trauma 55:106–111
62. Rincon F, Ghosh S, Dey S, Maltenfort M, Vibbert M, Urtecho J, McBride W, Moussouttas M, Bell R, Ratliff JK, Jallo J (2012) Impact of acute lung injury and acute respiratory distress syndrome after traumatic brain injury in the United States. Neurosurgery 71:795–803
63. Bahloul M, Chaari AN, Kallel H, Khabir A, Ayadi A, Charfeddine H, Hergafi L, Chaari AD, Chelly HE, Ben Hamida C, Rekik N, Bouaziz M (2006) Neurogenic pulmonary edema due to traumatic brain injury: evidence of cardiac dysfunction. Am J Crit Care 15:462–470
64. Baumann A, Audibert G, McDonnell J, Mertes PM (2007) Neurogenic pulmonary edema. Acta Anaesthesiol Scand 51:447–455
65. Hendricks HT, Heeren AH, Vos PE (2010) Dysautonomia after severe traumatic brain injury. Eur J Neurol 17:1172–1177