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Controlled Cortical Impact in the Rat

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Abstract

Traumatic brain injury (TBI) is a major cause of death and disability world-wide. Following initial injury, TBI patients can face long-term disability as cognitive, physical, and psychological deficits depending on the severity and location of injury. This results in an economic burden in the United States estimated to be \$60 billion due to health-care costs and loss of productivity. TBI is a significant area of active research interests for both military and civilian medicine. Numerous pre-clinical animal models of TBI are used to characterize the anatomical and physiological pathways involved and to evaluate therapeutic interventions. Due to its flexibility and scalability, CCI is one of the most commonly used preclinical TBI models. This unit provides a basic CCI protocol performed in the rat.

Keywords

cortical controlled impact; traumatic brain injury; rat

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- Osier, N. D., & Dixon, C. E. (2016). The Controlled Cortical Impact Model: Applications, Considerations for Researchers, and Future Directions. *Front Neurol*, 7, 134. doi:10.3389/fneur.2016.00134 *Complete protocol, historical perspective, key features, research applications, and factors that can influence outcome and data quality are highlights included in this review article.*
- Romine, J., Gao, X., & Chen, J. (2014). Controlled cortical impact model for traumatic brain injury. *J Vis Exp*(90), e51781. doi: 10.3791 *Includes complete protocol for mouse CCI, images of craniotomy, impactor alignment, gross and histologic brain images, and a video is posted online.*
- Xiong, Y., Mahmood, A., & Chopp, M. (2013). Animal models of traumatic brain injury. *Nat Rev Neurosci*, 14(2), 128–142. doi: 10.1038/nrn3407 *Describes animal models of TBI, lists limitations of models, suggests approaches to improve translation of results, provides detailed diagram of models and tables that summarize the models and their pathologic features.*

INTRODUCTION

With the incidence of TBI deaths accounting for 2.2% of all deaths within the United States in 2013 (Taylor, Bell, Breiding, & Xu, 2017) and the economic burden from TBI reaching \$60 billion (Finkelstein, Corso, & Miller, 2006) in the U.S. alone. TBI is a complex pathology that is the result of various mechanical and physiological mechanisms depending on the type and source of impact (Werner & Engelhard, 2007). The primary injury is the mechanical injury that occurs after initial trauma. It cannot be remedied through therapeutic intervention, it can only be prevented through safety measures, such as helmets. The secondary injury is the result of physiological mechanisms that occur days, weeks, and month after the primary injury and may be attenuated by therapeutic intervention. Animal models of TBI are routinely enlisted to generate the primary pathology associated with these complex injuries and to test neuroprotective and therapeutic agents to ameliorate secondary pathology. Although no single animal model can fully replicate the complexities of human TBI encountered in the clinical setting, CCI is one of the most common animal models of TBI due to its flexibility in degree and location of injury as well as its scalability to different species of animals. Additionally, CCI is an important model for demonstrating contusion injuries rather than closed-head or penetrating brain injuries. Other physiologic parameters of brain injury that are modeled in CCI include tissue loss, axonal injury, inflammation, interference in blood brain barrier as well as blood flow, and edema (Osier, Korpon, & Dixon, 2015).

This protocol describes a basic method to perform cortical controlled impact (CCI) with an electromagnetic impactor device in the rat. This specific protocol details the parameters necessary to injure the left motor cortex because for our research we required an observable sign of acute focal neurological injury (motor deficits). However, the stereotactic coordinates for the site of injury can be altered to accommodate the study of multiple brain areas/regions, such as memory and learning. Specifically, this model describes a moderate-to-severe injury based on depth of impact. The depth of impact may also be modified to produce magnitudes of injury ranging from mild to severe. Although there are multiple steps in CCI, this protocol can be accomplished within 15 minutes once surgical proficiency is achieved.

BASIC PROTOCOL. Moderate-to-Severe CCI of the Rat's Left Motor Cortex

The CCI model described in this protocol uses an electromagnetic impactor device to produce a mechanical deformation of the cortex while leaving the dura mater intact (Brody, MacDonald, Kessens, Yuede, Parsadarian, Spinner, Kim, Schwetye, Holtzman, & Bayly, 2007). The result is a focal brain contusion injury. This injury can be modified by changing the depth, velocity, and dwell time of the impactor to accommodate research needs. The use of a stereotactic frame allows the location of the injury to be controlled as well. The ability to adjust all of these factors is a hallmark of CCI that imparts a high level of precision when producing brain injuries within a research group as well as across different investigators.

The protocol consists of the following sections: equipment set-up, pre-surgical preparation of animal, craniotomy, injury, closure, and post-surgical recovery and monitoring. In

equipment set-up, the CCI device is discussed as well as other pertinent equipment. The pre-surgical preparation of the animal describes the boundaries of fur removal and the importance of properly aligning and securing the animal within the stereotactic frame. The craniotomy section describes stereotactic coordinates that define injury location and details the removal of the bone flap needed to expose the cortex while maintaining an intact dura mater. The injury section begins with instructions on how to align the impactor with the craniotomy hole, includes details about setting the Z-coordinate, conveys the appropriate manipulations of the CCI impactor necessary to induce the brain injury, and offers guidance about keeping surgical notes. The closure of the injury site discusses preparation of dental acrylic, replacement of bone flap, and suture of skin. Post-surgical recovery and monitoring is brief, but it is a critical component of any surgical procedure.

Throughout the performance of this protocol, it is essential that the researcher(s) adhere to sterile surgical techniques and closely monitors animals for level of sedation through reflexes and rate of respiration.

Materials for CCI surgery

It is recommended that a rolling container be used to organize all materials for transport and storage. This makes it easier to keep items in one location, protected from dust, well-stocked, and available at all times. An open cart can be used to contain larger equipment if the surgical suite is a shared space that demands mobility of items. Simply cover equipment with plastic to prevent dust accumulation.

Animals

Male and female Sprague-Dawley rats (**RRID:RGD_737891**) 8–10 weeks old (This age range models young adult rats. Other age ranges and strains may be used depending upon research goals.)

Equipment

Anesthesia induction chamber (11" L × 5.75" W × 7" H)

Anesthesia machine/vaporizer

2 heating pads (one for surgical area, one for post-surgical recovery area. For our protocols, we used water-based heating pads that were connected to circulating water baths adjusted to maintain a temperature of approximately 37 degrees Celsius)

Animal temperature monitor/probe

CCI electromagnetic impactor (we used the Leica/myNeuroLab.com Benchmark model, which was an earlier model of the Leica Biosystems Impact One Stereotaxic Impactor for CCI, #39463920)

Stereotactic frame

Digital stereotactic driver

Clear recovery chamber

Electric surgical drill with drill bits

Glass bead sterilizer

Extra light source for surgery area

Instruments

Agricola micro dissecting retractor

Disposable scalpel (#10)

Drill bits (0.6 mm)

Fur clippers

Impactor tip (size, shape depends on study parameters)

Micro-dissecting forceps

Micro-dissecting scissors

Olsen-Hegar needle holder scissor combination

Personal Protective Equipment

Surgical face masks

Hair bonnets

Shoe covers

Protective eyewear (critical during surgery drilling and impact)

Sterile surgical gloves

Sterile surgical gown

Dressing

Sterile surgical drapes to cover animals

Sterile drapes for surgical field

Sterile dry gauze

Sterile surgical drape for base of stereotaxic frame

Miscellaneous Items and Reagents

Sutures (4-0, Nylon, Monofilament, FS-2, Reverse Cutting Needle, 18'')

18 gauge needles for saline administration

50 mL conical tubes to hold betadine; 70% alcohol; sterile cotton tipped applicators

50 mL tube rack

12mL syringes for saline rinse

70% isopropyl alcohol in spray bottle

70% isopropyl alcohol stock

Adhesive tape

Container of gauze in 70% isopropyl alcohol

Betadine

Dental acrylic (methyl methacrylate skull fixture adhesive) (alternative is using bone wax)

Isoflurane (or other anesthetic as advised by site veterinarian/Animal Care and Use Committee)

Sterile ophthalmic ointment

Oster clip disinfectant/lubricant spray

Sterile cotton tip applicators

Sterile saline in IV bag

Sterile Surgifoam absorbable gelatin sponges (2 cm × 6 cm × 7mm; Ethicon #1972)

CCI Protocol

Before beginning any animal work, all protocols using live animals must be reviewed and approved by an Institutional Animal Care and Usage Committee (IACUC) and conform to local, institutional, and governmental regulations regarding the care and use of laboratory animals. All researchers who seek to perform the following CCI procedure must contact their institution's Department of Comparative Medicine (animal facility and veterinarians) and IACUC for development and approval of an animal protocol for the proposed research in accordance with current guidelines. This is also critical to ensure that all participating researchers have appropriate training to perform these studies. As part of protocol development, researchers are also advised to consult with a biostatistician to properly power the study design. Ideally, male and female animals should be equally represented to keep in accordance with recent National Institutes of Health guidelines to improve translational relevance of preclinical studies. This is especially vital to any research that involves the use of a novel therapeutic.

The following procedure was approved by the National Institutes of Health Clinical Center ACUC under protocols LDRR09-03 and LDRR12-02.

Surgical logs (see Figure 1 for an example) for each animal are recommended. This allows investigators to note any surgical issues, such as excessive bleeding, dural tear, etc. during procedure and track post-surgical monitoring, such as body weight, suture removal, etc.

- 1 Equipment set-up
 - a. Check components of surgical anesthesia
 - b. Place heating pads under animal induction area, surgical area, and recovery area

- c. Set up surgery lights if necessary
 - d. Turn on:
 - 1. Power strips
 - 2. Isoflurane Vaporizers and scavenging units
 - 3. Digital lab stereotactic system
 - 4. Temperature controller
 - 5. Bead sterilizer (used to sterilize surgical instruments between animals)
 - 6. Electromagnetic stereotactic impactor
 - 7. other (electric razor, drill control)
 - e. Set up for stereotactic impactor for CCI
 - 1. For moderate-to-severe injury:
 - a. Velocity: 5 m/s
 - b. Dwell time: 100 ms (lowest unit on dial is 1/10 of sec)
 - 2. Contact sensor – Attaches to ear bar to ground impactor
 - 3. Insert 5 mm flat impactor tip into housing (other sizes and shapes are available)
 - a. Only handle with sterile gloves
 - b. Wipe with 70% isopropanol
 - c. Inspect tip before each use to ensure there are no nicks or burs on surface that can add variability to injury or damage dura
 - 4. Set impactor tip at 10° tilt (Dial is in 10° units); angle should match curvature of skull
 - f. Get out sterile gloves, alcohol, tools, dental acrylic, 4.0 suture and needles, 50mL conical tubes with isopropyl alcohol and Betadine, sterile cotton swabs, sterile gauze, sterile pads, masks, sterile normal saline, sterile syringes, sterile needles, etc.
 - g. Place paper towel in anesthesia chamber
 - h. Ensure that heating pad is functioning and in place atop base of stereotactic platform.
- 2 Animal pre-surgical preparation:
- a. Anesthetize rat with 2–4% isoflurane in 100% oxygen within induction chamber

1. Specific flow rates may vary depending on equipment and anesthetic used. Work with local veterinarian and equipment manufacturer to optimize for site
 - b. Shave rat's head from behind ears to between eyes to ensure clean surgical field
 - c. Replace rat into induction chamber if needed to re-anesthetize after shaving
 - d. Lower anesthesia to maintenance flow rate (1–2% isoflurane in 100% oxygen)
 1. Check depth of anesthesia with toe pinch (if there is a withdrawal response, animal is not deep enough) and watch respiration (70–115 beats/minute is normal; it should slow during anesthesia. If there is an increase in respiration, animal is not deep enough.
 - e. Transfer rat to nose cone on stereotactic frame
 - f. Place rat in stereotactic frame; head must be level and secure; no movement
 1. Hold rat gently with one hand around ribcage
 2. Place teeth over bar using stick of cotton swab, not fingers
 3. Let rat rest on its forearms with head parallel to stereotactic stage
 4. Insert ear bars until each aligns at roughly 0 (both sides) and head is immobile
 5. Attach contact sensor from impactor controller to one of the ear bars to ground instrument
 - g. Ensure temperature monitor is appropriately attached to rat and monitor throughout procedure.
 - h. Apply sterile ophthalmic lubricant to both eyes to prevent corneal abrasions
 - i. Place sterile surgical drape over animal; only shaved skull is exposed
- 3 Craniotomy:
- a. Surgically prep skin of head with sterile cotton swabs
 1. 70% isopropanol, wipe in circular motion from center to outer edge of surgical field
 2. Betadine, wipe in circular motion from center to outer edge of surgical field

3. 70% isopropanol, wipe in circular motion from center to outer edge of surgical field
- b. Make midline incision with scalpel from above eyes to just behind ears
- c. Spread skin with forceps or hemostat
 1. Lengthwise, expose from above Bregma to behind Lambda (behind eyes to between ears)
 2. Laterally, expose from midline to left lateral ridge
- d. Blot blood with sterile gauze and sterile cotton swabs
- e. Clear skull of periosteum/soft tissues (Figure 2A)
 1. Drag scalpel flat across periosteum to scrape (not slice) it once; this will loosen it from skull
 2. Push remaining soft tissues of skull to sides with dry swabs
 3. Blot blood with sterile gauze and swabs as necessary
 4. Must remove these tissues or they will clog drill bit
- f. Get skull as dry as possible for best visualization of Bregma and Lambda
- g. Insert microdissecting retractor to spread skin and expose skull
- h. Locate Bregma
- i. Lower impactor tip to just above skull at Bregma
- j. Check angle of impactor with cortex (10°)
 1. Impactor should be perpendicular to cortex
 2. View impactor and skull from side; space between the two should be equal around tip
 3. Due to anatomy of animal, angle may need adjustment
- k. Dial stereotactic coordinates to proper location for injury; for our protocol we injured left motor cortex as follows:
 1. Zero out x, y, z coordinates before proceeding
 2. Set X-coordinate: Dial 2.5 Lateral to midline (to injure left motor cortex)
 3. Set Y-coordinate: Dial 1.0 Anterior to Bregma
 4. Set Z-coordinate: Later in protocol
- l. Mark area of desired craniotomy hole

1. If using drill to mark size of craniotomy hole, be careful not to touch impactor or it can produce a sharp bur that can injure dura
 2. Permanent marker is safer to designate craniotomy location; skull must be dry
 3. Craniotomy hole should be 1mm larger than impactor tip size (6mm hole for 5mm impactor tip)
 4. If hole is too small, impactor can fracture skull
- m.** Raise impactor tip and swing out of the way for now
- n.** Drill out craniotomy hole 0.5mm from midline (right side) and to lateral ridge (left side) (Figure 2B)
1. Begin drilling slowly and keep drill perpendicular to surface of skull
 - a.** Using drill at an angle can cause breach of skull between its layers and damage brain tissue
 2. If midline is breached, excessive bleeding will occur via superior sagittal sinus
 - a.** If excessive bleeding occurs, use small piece of Surgifoam to clot or animal can exsanguinate
 3. Wipe away any blood with gauze to ensure visibility of landmarks
 4. As drilling proceeds, introduce sterile saline into the field often to cool skull; blot with sterile gauze
 5. Periodically, apply light, gentle pressure to center of craniotomy with sterile forceps to evaluate progress; bone flap will begin to flex/move as drilling nears completion
 6. Prior to craniotomy completion, flush field with sterile saline to remove all debris (Figure 2C)
 7. Once bone flap flexes when pressed, it can be peeled away from skull (Figure 2D)
 - a.** Use microdissection forceps to grab area of flap that has been separated from skull
 - b.** Use cotton swab in other hand to apply gentle constant pressure to intact skull for leverage
 - c.** Lift bone flap gently but firmly straight off of skull
 - d.** Do not twist or contort bone flap; do not allow it to contact brain during removal

8. SAVE BONE FLAP ON STERILE GAUZE for replacement during closure

3 Injury:

- a. Blot any blood from around craniotomy hole with sterile gauze or cotton swabs; do not contact brain tissue
- b. Extend impactor tip with switch on instrument
- c. Center impactor tip over hole; this represents your X and Y coordinate
- d. Lower tip carefully with stereotactic dial until tip barely contacts dura; contact alarm will sound
- e. Zero out Z; it is critical to properly set zero at surface of brain for consistency of injuries
- f. Retract impactor tip with switch on instrument (Figure 2E)
- g. Set Z-coordinate: Dial to -2.5 for our moderate to severe injury
- h. Turn off anesthesia briefly during impact to prevent apnea after injury; this should be a quick (seconds only) cessation of anesthetic to allow the rat to take a breath of pure oxygen prior to injury)
- i. Push impact switch on instrument; impactor will slam down and then retract (Figure 2F)
- j. Resume anesthesia immediately
- k. Flip retractor switch to Off (middle) position
 1. Monitor briefly for any evidence of hemorrhage; if excessive bleeding, control with Surgifoam
 2. Note dural tear, subdural hematoma formation, excessive swelling etc. in surgical notes
 3. Clean impactor tip with alcohol and tighten between each animal

4 Closure:

- a. Mix dental acrylic per manufacturer's instructions (bone wax may also be used as an alternative to dental acrylic)
- b. Gently place bone flap over craniotomy hole (Figure 2G)
- c. Dry skull around craniotomy hole with sterile swab or dental acrylic will not stick to it
- d. Place drops of dental acrylic on center of skull flap
- e. Allow time for acrylic to spread and begin to harden (Figure 2H)
- f. Remove retractor from skin

- g. Suture scalp closed with interrupted 4.0 nylon sutures (Figure 2I)
- 5 Post-surgical recovery and monitoring:
- a. Turn off anesthesia
 - b. Remove rat from stereotactic frame and place in warm cage
 - c. Carefully monitor rat during recovery. Document time of injury as well as time that rat is able to move and right itself. Note any complications on surgical log.
 - d. Do not return rat to home cage until fully mobile and alert
 - e. Administer appropriate post-operative analgesic as stated in IACUC protocol (acetaminophen 100 mg/kg by mouth every four hours as needed and/or buprenorphine 0.25 mg/kg subcutaneously every eight hours as needed were used for our protocols; please confer with your site's veterinarian for current institutional recommendations)
 - f. Monitor animal daily (including body weight) and provide analgesics if necessary for next 72 hours
 - g. At approximately 9 days post-surgery, remove sutures

Different anesthetic agents can be used. Isoflurane is most common anesthetic used by researchers, however some do use xylazine/ketamine or other agents. It has been noted in the literature (Luh et al., 2011; Statler et al., 2006) that many of the anesthetics used by TBI researchers generate a neuroprotective effect. However, volatile gases such as isoflurane are still the most commonly used agents as they are short-acting anesthetics that allow the investigator to recover animals from surgery more quickly. Details regarding usage and dosage of appropriate anesthetics and post-operative analgesics should be discussed with local veterinarians and established in the protocol approved by the institutional ACUC.

Another caveat of protocol alteration involves whether the researcher does a craniotomy (Cole et al., 2011) or a craniectomy (Glover et al., 2012). This protocol performs a craniotomy, meaning that the bone flap is returned to the skull after injury completion. Others discard the bone flap, which is a craniectomy. There are two sides to the debate. Some researchers think replacing the flap is a better representation of closed head injuries since removal of the bone flap provides a mechanism to relieve intracranial pressure and can confound results. Researchers that discard the bone flap think that replacing it could provide another source of injury to the brain, increasing the likelihood of injury variability. It is left to the individual investigator to decide which option is best-suited to their research goals.

COMMENTARY

Background Information

TBI encountered in the clinical population is a heterogeneous disease that is complex and multifaceted. Within preclinical research, there are various models of TBI available to investigators since no single model can accurately mimic all of the features of human TBI.

The majority of brain injury models can be assigned to one of the following categories: fluid percussion injury (FP), penetrating ballistic-like brain injury (PBBi), weight drop injury (WDI), blast injury, and CCI (Xiong, Mahmood, & Chopp, 2013). These TBI models either utilize an open skull and produce a focal local injury (CCI, FPI, some WDI models, and PBBi) or utilize a closed skull and produce a diffuse global injury (some WDI models, blast, and repeat injury models). The CCI model has been most widely used due to the flexibility and reproducibility of its injury parameters as well as the ability to calibrate the injury to accommodate various animal species from mice (Smith et al., 1995) to pigs (Manley et al., 2006).

Current CCI models were first developed in ferrets and used pneumatic impactors to better control injury parameters and characterize the results of varying velocity, depth, and dwell time (Lighthall, 1988). By 1991, researchers adapted the parameters of the CCI model to accommodate rats (Dixon, Clifton, Lighthall, Yaghmai, & Hayes, 1991).

Since that time, CCI models have been scaled to accommodate various animal species. However, brain injuries in larger species such as pigs or nonhuman primates can require extensive post-operative care like encountered in human medicine. Additionally, behavioral and neurological tests like those used to evaluate lab rodents are not well-established in higher animal species (Manley et al., 2006). Most recently, mice have become the focus of CCI models as transgenic mice have given investigators the opportunity to evaluate their hypotheses and validate treatment options in mice expressing disease-relative genetics (Longhi et al., 2001; Zhang et al., 2014).

The model of TBI should be decided upon based on purpose of research and patient population the researcher wants to target. Since no single TBI model can replicate the many facets of human brain injuries it is important that informed decisions be made by each investigator as to which TBI model is best-suited for their research aims.

Critical Parameters

After any step that manipulates skull (removing soft tissues, drilling, etc.), ensure that ear bars are still firmly holding head of rat parallel to stereotactic stage. Any movement or irregular position of head during impact will decrease consistency of injuries between animals.

Four critical parameters can reduce consistency of injury:

1. Head movement during impact due to ear bars being improperly aligned/tightened.
2. Head not being parallel to stereotactic stage (level).
3. Failure to properly zero impactor tip at surface of brain before setting injury depth (Z).
4. Not matching angle of impactor tip to curvature of skull.

Anticipated Results

Overall, animals tolerate injury well. In our experience, animals are alert and mobile within 20 minutes of removal from anesthesia and animals begin eating and drinking within 2 hours of being placed in the animal's home cage. However, post-surgical monitoring of animals is important as complications can arise. Additionally, analgesics should be provided as directed per institutional policy.

When CCI is performed over the left motor cortex as in this protocol, on the day of injury rats will typically display an abnormal gait when first awakening. These abnormalities become more subtle over subsequent days post-injury. Abnormalities can be assessed by a variety of neurological examinations, depending upon the target area of injury (Gold, Su, López-Velázquez, Haus, Perez, Lacuesta, Anderson, & Cummings, 2013), including foot fault analysis to assess motor function (Kamens & Crabbe, 2007). An example of typical findings from longitudinal foot fault analysis after performance of CCI over the left motor cortex is shown in Figure 3. Signs of neurological injury from CCI in rats are typically most severe within the first 24 hours after injury, with recovery to close to normal levels by 10 days post-injury.

Contact attending veterinarian immediately

1. If incision becomes red, swollen, or has discharge
2. If animal has pain that cannot be controlled with analgesic
3. If animal opens incision, exposing skull
4. If animal loses significant weight (10%) in first 72 hours
5. If animal has seizure lasting over 2 minutes, or recurrent seizures

Animals that have constant pain, seizures, and significant weight loss usually indicate an outlier whose injury is more severe than other animals or one that has less tolerance for the injury. These animals usually require euthanasia, but their occurrence is rare (less than 2%). Open incisions can easily be cleaned and closed. Peri-incisional infections are rare and can easily be treated. The presence of infection may be a confounding factor depending on research endpoints and might require animal euthanasia, consult your attending veterinarian.

Examples of the typical evolution of the CCI lesion on *in vivo* magnetic resonance imaging (MRI) over the month post-injury are shown in Figure 4. Additional examples of both *in vivo* MRI and corresponding *ex vivo* MRI and histology findings can be found in the literature (Turtzo, Budde, Gold, Lewis, Janes, Yarnell, Grunberg, Watson, & Frank, 2013) (Turtzo, Lescher, Janes, Dean, Budde, & Frank, 2014) (Turtzo, Budde, Dean, Gold, Lewis, Janes, Lescher, Coppola, Yarnell, Grunberg, & Frank, 2015).

Time Considerations

Although the CCI procedure can be accomplished within 15 minutes, once surgical proficiency is achieved, there are issues that can arise at various steps and slow progress. Anatomical differences between animals can produce challenges to protocol. Be aware that these differences are possible and confront them in the following ways.

Head alignment—Achieving proper ear bar alignment and head position can be a challenge depending on anatomy of your animal. However, care and time must be spent to ensure that the head of your animal is completely immobile and is parallel to the surgical stage or consistency of injuries cannot be achieved.

Excessive bleeding—Additionally, excessive bleeding can happen during craniotomy, during injury, or during both procedures and impede progress. If the midline suture is breeched while drilling the craniotomy hole, excessive bleeding can ensue and lead to animal death. Thus, maintaining at least a 0.5 mm distance from the midline suture is recommended. Likewise, some animals can bleed excessively upon impact. Usually this is due to a dural tear, but it can happen without an obvious tear in the dura. To avoid dural tears, always verify the integrity of the impactor tip (deformation free) prior to use. If either of these excessive bleeding scenarios happens, the Surgifoam pads listed in materials section can be used to expedite blood clotting. If any complications or irregularities occur, make a note upon the animal's surgical log.

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Significance Statement

In 2013, the United States Center for Disease Control estimates that there were 2.5 million traumatic brain injury (TBI)-related emergency room visits, 282,000 patients with TBI-related hospital admissions, and 56,000 deaths attributed to TBI. Thus, in 2013, 2.2% of all deaths within the United States could be attributed to TBI. This unit will demonstrate the instruments, procedures, and methods necessary to perform the cortical controlled impact (CCI) model of traumatic brain injury (TBI) in the rat. As one of the most commonly used animal models of TBI, CCI can be modified to produce distinct degrees of injury, at multiple locations in order to optimize this model to fulfill a wide variety of research goals.

Emergency POC: (List Name and Phone Number of Emergency Point-of-Contact here)

This is an example of a surgical log used to record peri-operative details as well as post-surgical recovery.

Figure 2.

Representative images from critical portions of the controlled cortical impact procedure are shown. The rat's head is positioned in the anesthetic nose cone and stereotactic apparatus so that the nose is towards the top of the page. A) Initial incision after clean up to expose skull; B) Initial scoring of skull for target area; C) Bone flap immediately prior to removal; D) Bone flap removed to expose intact cortex prior to impact; E) Impactor positioned and impactor tip retracted above target area of cortex; F) Cortex after impact. Note the different in color between (D) and this post-impact image, representing contusion below the intact dura. If the dura had ruptured, free blood would be hemorrhaging from the cortex, rather than be contained beneath the dura; G) Bone flap replaced; H) Bone flap sealed with dental acrylic; I) Interrupted sutures to close scalp incision.

Figure 3.

Example of results from motor function assessments using longitudinal foot fault analysis after controlled cortical impact (CCI). For this experiment, three cohorts were followed, a CCI plus treatment group (CCI+Tx), a CCI plus treatment vehicle group (CCI+Vehicle), and an uninjured control group. Each cohort for this experiment contained 6 rats. The day indicates the time post-CCI. At early time points there are more foot faults per distance traveled observed in rats after CCI than in uninjured rats, and no statistically significant differences between the two treatment groups. Post-CCI and control rats are more similar one week after injury. For the example shown, statistical analysis was performed by one-way analysis of variance (ANOVA) with post-hoc Tukey analysis.

Figure 4.

Examples from two rats (A–C, D–F) depicting of the evolution of the controlled cortical impact (CCI) lesion on *in vivo* MRI. The left side of the rat is towards the left-hand side of the page, in contrast to how human clinical MRI images are portrayed. (A,D) At two days post-injury, there is hyperintensity surrounding the area of impact and extending into the corpus callosum, indicative of tissue damage and edema. (B,E) At nine days post-injury, this edema has resolved but the area of injury is still visible. (C,F) By thirty days post-injury, there is some degree of tissue atrophy on the side of injury, as indicated by thinning of the cortex and enlargement of the lateral ventricle. The protocol was performed identically in both rats, but the rat depicted in A–C manifested less atrophy by 30 days than the rat shown in D–F. Even under the most controlled of circumstances, there can be variability in the appearance and response of individual rats to the CCI injury.