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

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TITLE:

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

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KEYWORDS:

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SUMMARY:

Here we describe a protocol for the induction of murine traumatic brain injury via an open-head controlled cortical impact.

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.

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[3-5]. 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)[6-10]. Even subclinical concussive events—those

impacts that do not result in 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's[13]. 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 rodents[15]. 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 assays[18]. 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[19-21]. While each of these models have their own distinct advantages their main drawbacks are mixed injury, high mortality and lack of standardization, respectively[22]. 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

1.1. Anesthetize the mouse with ketamine (125 mg/kg) and xylazine (10 mg/kg) injected intraperitoneally.

2. Vital signs monitoring every 15 min

2.1. 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

3.1. Weigh all the mice on the day prior to injury induction.

3.2. Sterilize one set of surgical instruments by autoclaving for each experimental subject.

3.3. 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.

3.4. Set up the operating theater within a sterilized laminar flow hood.

3.4.1. Position the stereotaxic operating frame.

3.4.2. Attach the impacting device to the stereotaxic frame.

3.4.3. 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.

3.5. Don new personal protective equipment and sterile gloves.

3.6. Shave the fur from the operative site using electric clippers.

3.7. Apply protective ointment to the eyes of the mouse to prevent corneal injury and drying.

3.8. Place the mouse into the operating theater.

3.9. Prep the skin with an iodine based surgical scrub alternated with alcohol three times.

4. Application of controlled cortical impact

4.1. Incise the scalp 1 cm in the midline with a scalpel exposing the skull.

4.2. 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.

4.3. 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.

4.4 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.

4.5. Perform a craniectomy using a drill with a trephine drill bit.

4.5.1. 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.

4.5.2. 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.

4.6. Use forceps and a small gauge hypodermic needle to remove the bone flap, fully exposing the underlying dura mater.

4.7. 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.

4.8. 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.

4.9. Apply the injury by activating impactor on the actuating device.

4.10. Rotate the impact device out of the field and remove the animal from the stereotaxic frame.

5. Surgical site closure

5.1. Control bleeding from the skull and injured cortical surface with direct pressure from a sterile cotton tipped applicator.

5.2. Dry the skull with a sterile cotton tipped applicator.

5.3. 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

6.1. Administer post-operative analgesia (e.g., sustained release buprenorphine 0.1–0.5 mg/kg administered subcutaneously providing 72 h of sustained analgesia).

6.2. Place the animal in the lateral decubitus recovery position in a clean pre-warmed cage.

6.3. Observe the animals until awake and mobile, then return each mouse to its home cage.

6.4. Ensure free access to food and water. Normal food and water intake typically resume within one to two hours after injury.

6.5. 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 coordination[18]. 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].

FIGURE AND TABLE LEGENDS:

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].

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 models[15]. 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 question[22]. 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 analysis[24]. Additionally, although CCI produces a reliable and consistent injury, most TBI in human patients are diffuse and occur through multiple simultaneous mechanisms[25]. 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 experiment[24].

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.

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DISCLOSURES:

The authors have no financial conflicts of interest.

REFERENCES:

1 Faul, M. *Traumatic Brain Injury in the United States: Emergency Department Visits, Hospitalizations and Deaths 2002-2006*. Centers for Disease Control and Prevention, National Center for Injury Prevention and Control, Atlanta (GA), (2010).

2 Roozenbeek, B., Maas, A. I., Menon, D. K. Changing patterns in the epidemiology of traumatic brain injury. *Nature Reviews Neurology* . **9** (4), 231-236, (2013).

3 Corso, P., Finkelstein, E., Miller, T., Fiebelkorn, I., Zaloshnja, E. Incidence and lifetime costs of injuries in the United States. *Injury Prevention.* **12** (4), 212-218, (2006).

4 Pearson, W. S., Sugerman, D. E., McGuire, L. C., Coronado, V. G. Emergency department visits for traumatic brain injury in older adults in the United States: 2006-08. *Western Journal of Emergency Medicine.* **13** (3), 289-293, (2012).

5 Whitlock, J. A., Jr., Hamilton, B. B. Functional outcome after rehabilitation for severe traumatic brain injury. *Archives of Physical Medicine and Rehabilitation.* **76** (12), 1103-1112, (1995).

6 Schwarzbold, M., et al. Psychiatric disorders and traumatic brain injury. *Neuropsychiatric Disease and Treatment.* **4** (4), 797-816, (2008).

7 Whelan-Goodinson, R., Ponsford, J., Johnston, L., Grant, F. Psychiatric disorders following traumatic brain injury: their nature and frequency. *Journal of Head Trauma Rehabilitation.* **24** (5), 324-332, (2009).

8 Peskind, E. R., Brody, D., Cernak, I., McKee, A., Ruff, R. L. Military- and sports-related mild traumatic brain injury: clinical presentation, management, and long-term consequences. *Journal of Clinical Psychiatry.* **74** (2), 180-188, (2013).

9 Martin, L. A., Neighbors, H. W., Griffith, D. M. The experience of symptoms of depression in men vs women: analysis of the National Comorbidity Survey Replication. *JAMA Psychiatry.* **70** (10), 1100-1106, (2013).

10 Makinde, H. M., Just, T. B., Cuda, C. M., Perlman, H., Schwulst, S. J. The Role of Microglia in the Etiology and Evolution of Chronic Traumatic Encephalopathy. *Shock.* **48** (3), 276-283, (2017).

11 Belanger, H. G., Vanderploeg, R. D., McAllister, T. Subconcussive Blows to the Head: A Formative Review of Short-term Clinical Outcomes. *Journal of Head Trauma Rehabilitation.* **31** (3), 159-166, (2016).

12 Carman, A. J., et al. Expert consensus document: Mind the gaps-advancing research into short-term and long-term neuropsychological outcomes of youth sports-related concussions. *Nature Reviews Neurology* . **11** (4), 230-244, (2015).

13 Kramer, S. P. VI. A Contribution to the Theory of Cerebral Concussion. *Annals of Surgery.* **23** (2), 163-173, (1896).

14 Lighthall, J. W. Controlled cortical impact: a new experimental brain injury model. *Journal of Neurotrauma.* **5** (1), 1-15, (1988).

15 Dixon, C. E., Clifton, G. L., Lighthall, J. W., Yaghmai, A. A., Hayes, R. L. A controlled cortical impact model of traumatic brain injury in the rat. *Journal of Neuroscience Methods.* **39** (3), 253-262, (1991).
16 Schwulst, S. J., Trahanas, D. M., Saber, R., Perlman, H. Traumatic brain injury-induced alterations in peripheral immunity. *Journal of Trauma and Acute Care Surgery.* **75** (5), 780-788, (2013).
17 Trahanas, D. M., Cuda, C. M., Perlman, H., Schwulst, S. J. Differential Activation of Infiltrating Monocyte-Derived Cells After Mild and Severe Traumatic Brain Injury. *Shock.* **43** (3), 255-260, (2015).
18 Makinde, H. M., Cuda, C. M., Just, T. B., Perlman, H. R., Schwulst, S. J. Nonclassical Monocytes Mediate Secondary Injury, Neurocognitive Outcome, and Neutrophil Infiltration after Traumatic Brain Injury. *Journal of Immunology.* **199** (10), 3583-3591, (2017).
19 Thompson, H. J., et al. Lateral fluid percussion brain injury: a 15-year review and evaluation. *Journal of Neurotrauma.* **22** (1), 42-75, (2005).
20 Marmarou, A., et al. A new model of diffuse brain injury in rats. Part I: Pathophysiology and biomechanics. *Journal of Neurosurgery.* **80** (2), 291-300, (1994).
21 Reneer, D. V., et al. A multi-mode shock tube for investigation of blast-induced traumatic brain injury. *Journal of Neurotrauma.* **28** (1), 95-104, (2011).
22 Ma, X., Aravind, A., Pfister, B. J., Chandra, N., Haorah, J. Animal Models of Traumatic Brain Injury and Assessment of Injury Severity. *Molecular Neurobiology.* 10.1007/s12035-018-1454-5, (2019).
23 Makinde, H. M., et al. Monocyte depletion attenuates the development of posttraumatic hydrocephalus and preserves white matter integrity after traumatic brain injury. *PLoS One.* **13** (11), e0202722, (2018).
24 Osier, N. D., Dixon, C. E. The Controlled Cortical Impact Model: Applications, Considerations for Researchers, and Future Directions. *Frontiers in Neurology.* **7** 134, (2016).
25 Iaccarino, C., Carretta, A., Nicolosi, F., Morselli, C. Epidemiology of severe traumatic brain injury. *Journal of Neurosurgical Sciences.* **62** (5), 535-541, (2018).

Figure 1

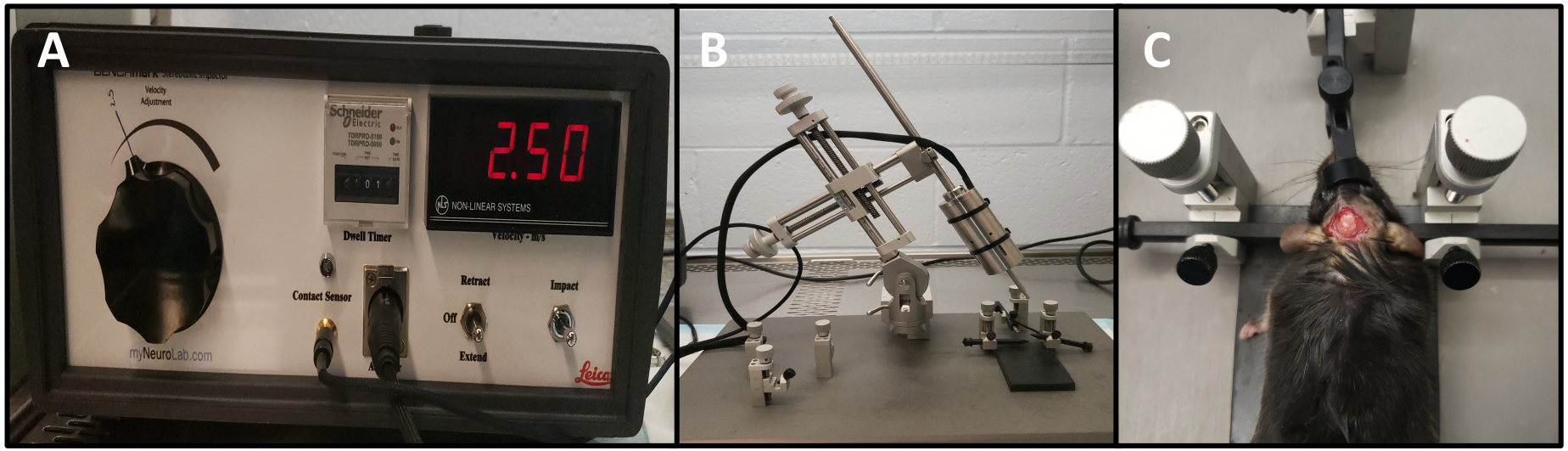

Figure 2

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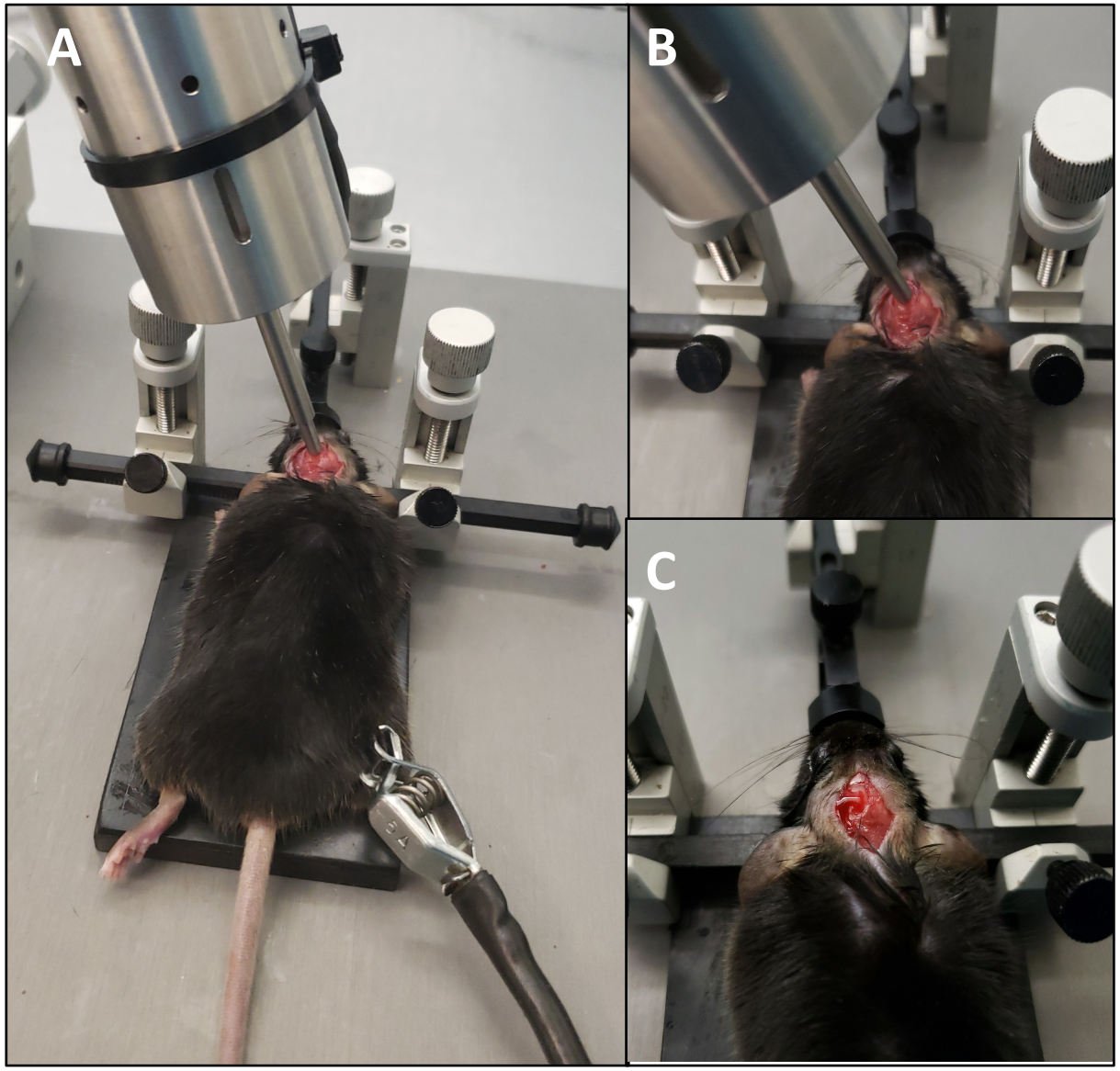

Figure 3

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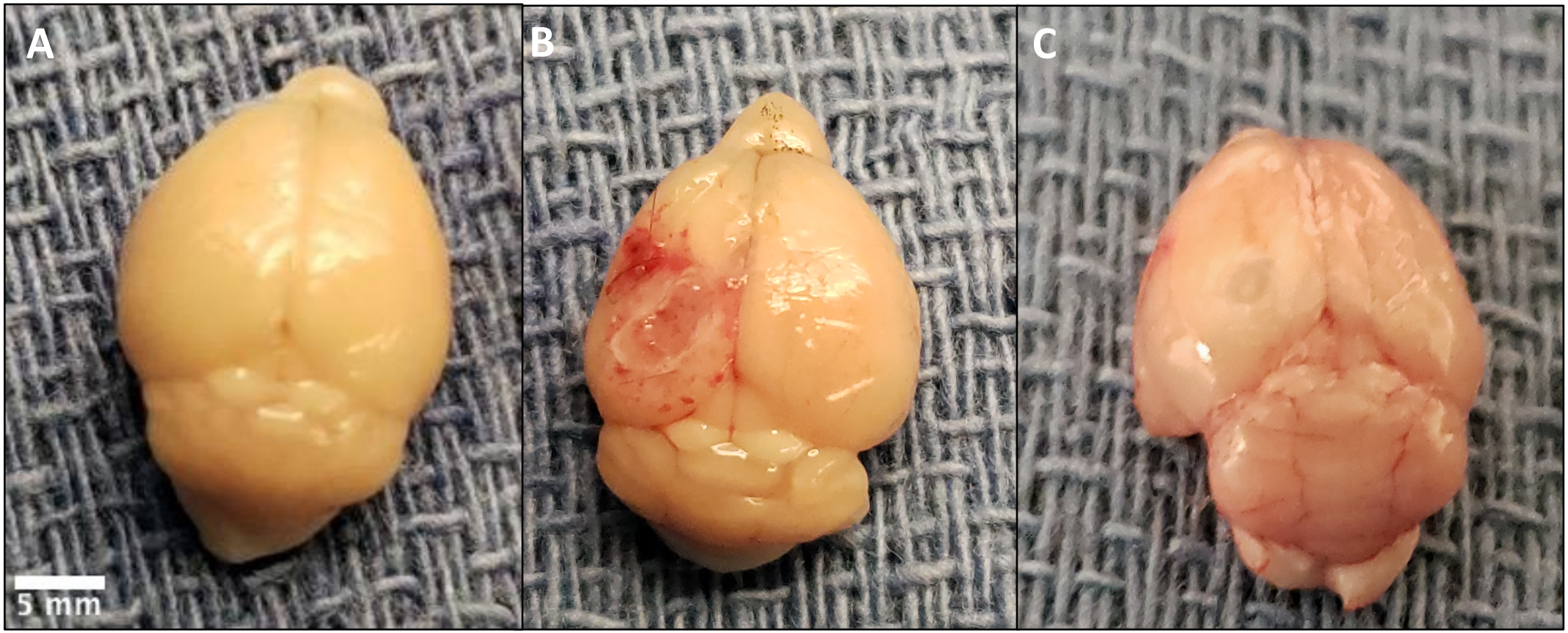

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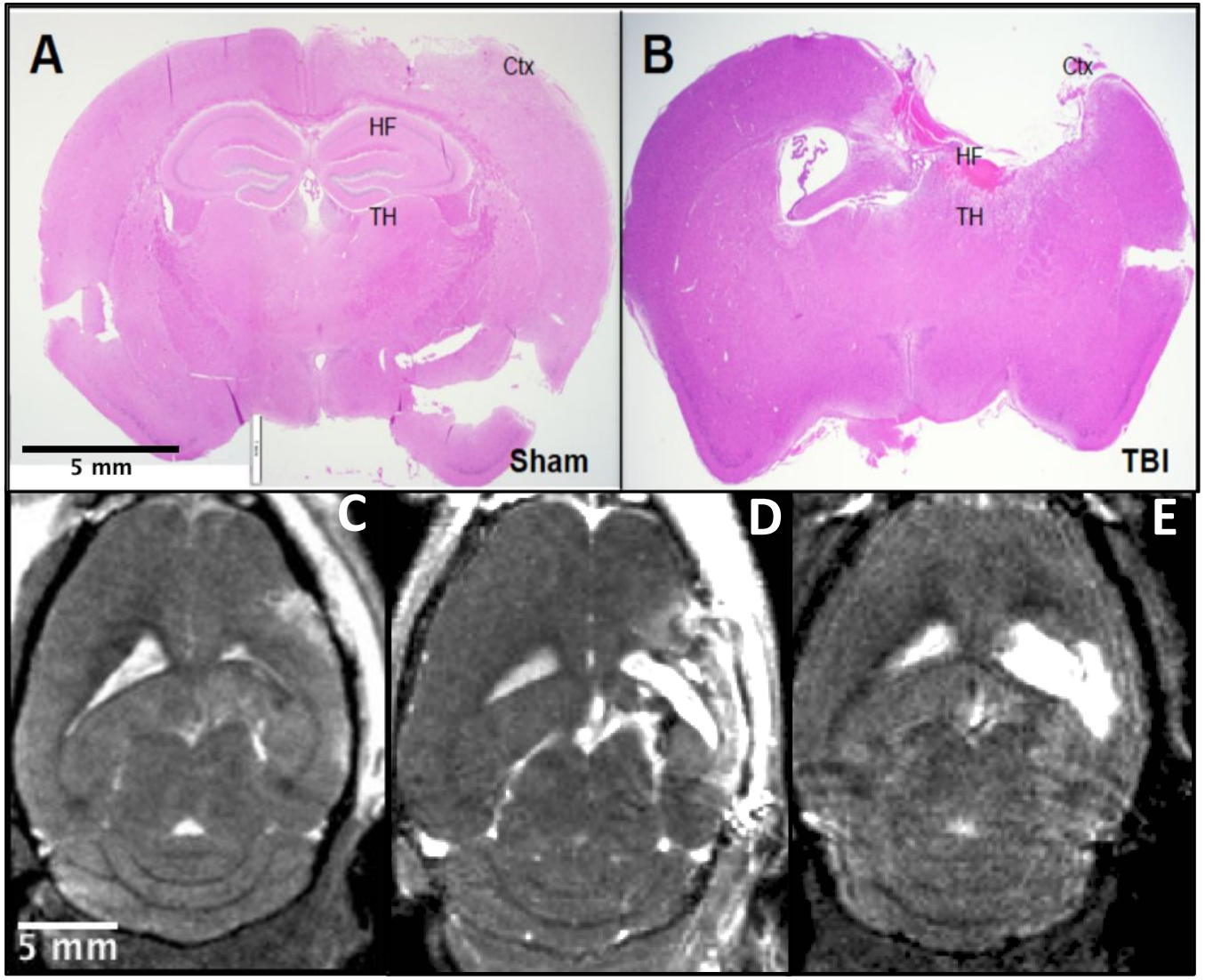

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AnaSed Injection Xylazine Sterile Solution	LLOYD, Inc.
Buprenorphine SR Lab 0.5mg/mL	Zoopharm-Wildlife Pharmaceuticals USA
High Speed Rotary Micromotor KiT0	Foredom Electric Company
Imapact one for Stereotaxix CCI	Leica Biosystems Nussloch GmbH
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CORRESPONDING AUTHOR

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Department:	Surgery
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Title:	Assistant Professor of Surgery
Signature:	Steven J. Schwulst
Date:	03-19-19

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Alisha D'Souza, Ph.D.
Senior Review Editor
JoVE

Dear Dr. D'Souza and Distinguished Reviewers,

Thank you for providing us with your insightful comments on our manuscript entitled "Murine Model of Controlled Cortical Impact for the Induction of Traumatic Brain Injury". We have edited the manuscript according to the editorial and reviewers' comments and recommendations, and we have listed our point-by-point responses below. Furthermore, each figure as has been submitted as a vector image file to ensure high resolution throughout production: (.svg, .eps, .ai). If submitting as a .tif or .psd, please ensure that the image is 1920 x 1080 pixels or 300 dpi. Additionally, please upload tables as .xlsx files.

Editorial comments:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

The manuscript has been thoroughly proofread.

2. Please rephrase the Short Abstract/Summary to clearly describe the protocol and its applications in complete sentences between 10-50 words: "Here, we present a protocol to …"

The Summary has been rephrased to "Here we describe a protocol for the induction of murine traumatic brain injury via an open-head controlled cortical impact".

3. Unfortunately, there are a few sections of the manuscript that show significant overlap with previously published work. Though there may be a limited number of ways to describe a technique, please use original language throughout the manuscript. Please see lines: 42-47, 48-50, 52-55.

The introductory paragraph has been rephrased to limit overlap with our previously published work.

4. Please include a single line space between each step, substep and note in the protocol section.

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There is now a single line space between each step and substep in the protocol section.

5. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets or dashes.

The numbering of the protocol has been changed as requested.

6. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” However, notes should be concise and used sparingly.

The text in the protocol section has been rewritten in the imperative tense as requested.

7. The Protocol should contain only action items that direct the reader to do something. Please move the discussion about the protocol to the Discussion.

All discussion within the protocol section has been moved to the discussion section.

8. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please ensure that individual steps of the protocol should only contain 2-3 actions per step.

The protocol section has been rephrased and rewritten to ensure it is made up almost entirely of discrete steps.

9. Please revise the protocol text to avoid the use of any personal pronouns in the protocol (e.g., "we", "you", "our" etc.).

The protocol text now avoids use of any personal pronouns.

10. Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed?

We have added more detail to the protocol steps as requested.

11. 1: Please move the details to the discussion and make this an action step.

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Moved as requested. This is now an action step.

12. 2: Please write in complete sentences. what should be the min max range in each case. How do you monitor vital signs?

This step is now written in a complete sentence with min/max range as requested.

13. There is a 10-page limit for the Protocol, but there is a 2.75-page limit for filmable content. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.

The essential steps of the protocol for the video have been highlighted in yellow.

14. Any marker studies (immunostaining, western blot, etc) to the difference between sham and experimental group?

We have included a reference to our published work demonstrating the marked difference in inflammatory infiltrate between brain-injury and sham-injury as assessed by flow cytometry—our laboratory's primary output measure (lines 445-447).

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16. Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage – LastPage, (YEAR).] For more than 6 authors, list only the first author then et al. Please do not abbreviate the journal names.

All references have been generated by the software package EndNote with the reference style set on "JoVE".

17. Figure 3, 4: Please include a scale bar.

Figures 3 and 4 now include a scale bar as requested.

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18. Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file.

Revised as requested.

Reviewers' comments:

Reviewer #1:
Manuscript Summary:
The well written manuscript provides a useful and detailed description of the controlled cortical impact model of traumatic brain injury. The manuscript is complete and provides the adequate level of detail needed to reproduce this procedure. This model is valuable to the research community, and the article describes the model as employed by the authors for several high-impact publications.

Major Concerns:
None

Minor Concerns:
It would be important to describe the considerations for control animals. A brief discussion of the authors opinion about the utility of naive controls, controls subject to anesthesia alone and/or controls subjects to craniectomy without impact would be a useful addition to the article.

We have added an entire paragraph to the discussion of the manuscript describing appropriate controls for this model including sham-injury and naïve age-matched controls. We thank the reviewer for this insightful comment and feel that the addition of this paragraph strengthens the manuscript (lines 533-540).

Reviewer #2:
Manuscript Summary:
This is a well written and organized description of an open traumatic brain injury model that will be useful to other investigators that are working in the area. There are several ways to perform this type of an injury and this protocol seems to be an excellent, reproducible injury method. The manuscript provides sufficient detail to make this transportable to other laboratories. It is appropriate for JoVE.

Minor Concerns:

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The author might want to add a sentence or two to describe why this protocol is better or more advanced than other protocols. Is it cheaper? More consistent? Overall, the protocol is clearly presented. For those interested in doing a controlled cortical impact (CCI) TBI model, this seems to be a very well thought out protocol. The video will make this even better.

The CCI model offers the investigator precise control over several biomechanical parameters when applying the injury. This allows for the greatest precision and reproducibility of any TBI model. This discussed in the last paragraph of the introduction as well as reiterated in the discussion (lines 517-518) per reviewer 2 recommendation.

There are a few suggested edits to help with clarity and understand of the procedures. Though it is assumed that the video will help with this, the surgical procedure for craniotomy needs some more text for non-surgeons. For instance, it is not clear how the drill is used to create a bone flap? Again, the video will help with this, but a description would help as well.

We have added additional text in the protocol section to help the reader gain a better understanding of how to perform the craniectomy (lines 324-328).

Please indicate the surgical adhesive that is used to close the bone flap.

The bone flap is not replaced in this protocol. The scalp is simply closed and the bone flap is discarded. This is now specifically stated in the protocol (lines 356-358).

Are sutures used to close the skin over the scalp?

This protocol describes using a commercially available veterinary adhesive to close the scalp. Sutures may be used instead if desired by the investigative group (lines 356-358).

Post-surgery analgesia, buprenorphine, is usually given for 48 hours after surgery, please add this to the protocol if that is what is done in your lab.

This protocol describes administering an extended release formulation of buprenorphine (buprenorphine-SR) that provides 72 hours of post-operative analgesia (Lines 414-415).

Sincerely,

Steven J. Schwulst, MD, FACS, FCCM
Assistant Professor of Surgery
Northwestern University