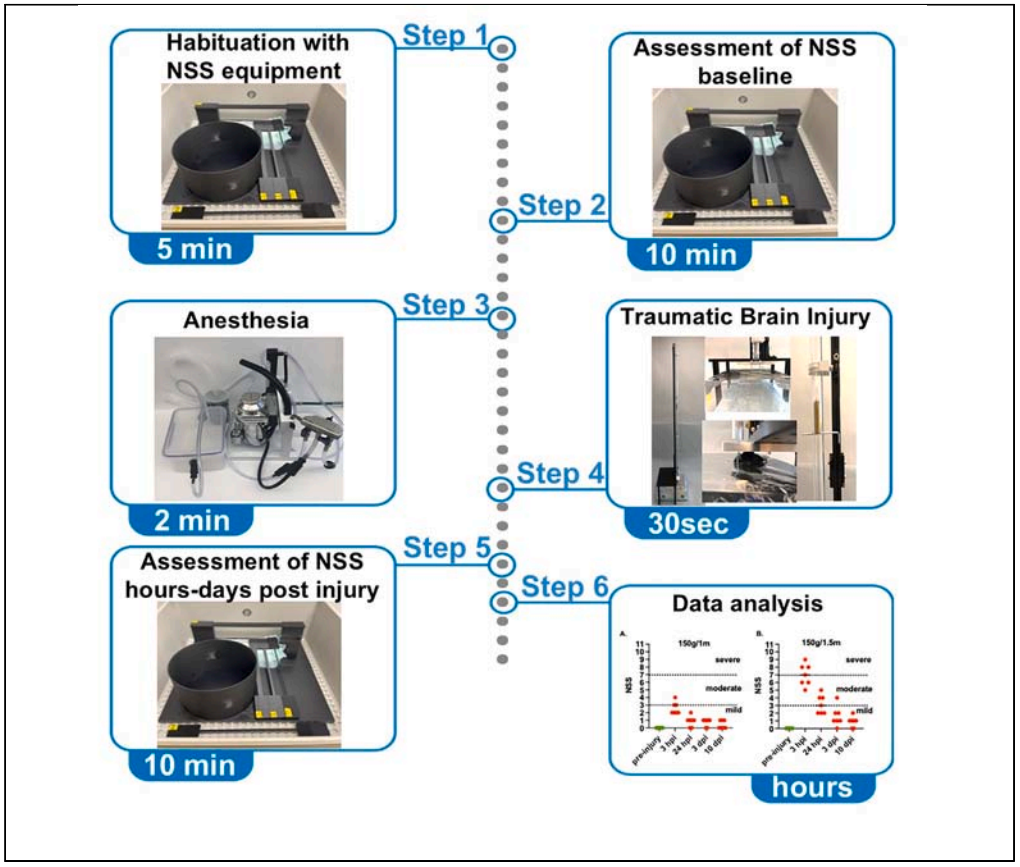
STAR Protocols

CellPress
OPEN ACCESS

Protocol

Protocol for inducing varying TBI severity in a mouse model using a closed-head, weight-drop, impact-induced acceleration mechanism



Animal models of traumatic brain injury (TBI) are critical for understanding its complex neuropathology. Here, we present a protocol to induce varying TBI severities in mice using a closed-head, weight-drop model that includes an impact-induced acceleration mechanism. We describe steps for habituation with neurological severity score (NSS) equipment, assessing NSS baseline, performing anesthesia and TBI, assessing NSS post-injury, and analyzing data. This protocol requires no prior surgical intervention and is adaptable for rat studies.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

Javier Allende Labastida, Massoud Motamedi, Ping Wu, Bartosz Szczesny

baszczes@utmb.edu

Highlights

Steps for implementing closed-head impact-acceleration TBI model

Instructions for assessing TBI severity using the neurological severity score

Guidance on setting up TBI and NSS apparatuses

Allende Labastida et al., STAR Protocols 5, 103370 December 20, 2024
https://doi.org/10.1016/j.xpro.2024.103370

STAR Protocols

CellPress
OPEN ACCESS

Protocol

Protocol for inducing varying TBI severity in a mouse model using a closed-head, weight-drop, impact-induced acceleration mechanism

Javier Allende Labastida,[1,5] Massoud Motamedi,[2] Ping Wu,[3] and Bartosz Szczesny[2,4,6,7,*]

[1]Departments of Neuroscience, Cell Biology and Anatomy, University of Texas Medical Branch, 301 University Boulevard, Galveston, TX, USA

[2]Departments of Ophthalmology and Visual Sciences, University of Texas Medical Branch, 301 University Boulevard, Galveston, TX, USA

[3]Department of Neurobiology, University of Texas Medical Branch, 301 University Boulevard, Galveston, TX, USA

[4]Department of Anesthesiology, University of Texas Medical Branch, 301 University Boulevard, Galveston, TX, USA

[5]Present address: Department of Anesthesiology and Critical Care Medicine, Johns Hopkins School of Medicine, 720 Rutland Ave., Traylor 809, Baltimore, MD, USA

[6]Technical contact

[7]Lead contact

*Correspondence: baszczes@utmb.edu
https://doi.org/10.1016/j.xpro.2024.103370

SUMMARY

Animal models of traumatic brain injury (TBI) are critical for understanding its complex neuropathology. Here, we present a protocol to induce varying TBI severities in mice using a closed-head, weight-drop model that includes an impact-induced acceleration mechanism. We describe steps for habituation with neurological severity score (NSS) equipment, assessing NSS baseline, performing anesthesia and TBI, assessing NSS post-injury, and analyzing data. This protocol requires no prior surgical intervention and is adaptable for rat studies. For complete details on the use and execution of this protocol, please refer to PhD Dissertation of Javier Allende Labastida[1] and Tang et al.[2]

BEFORE YOU BEGIN

This protocol outlines a method for inducing clinically relevant, closed-head impact-acceleration Traumatic Brain Injury (TBI) in mice, adaptable for rats, without the need for pre-injury surgery. The protocol requires basic experience in animal handling and using an isoflurane vaporizer for rodent anesthesia.

TBI is a major cause of death and disability, often leading to chronic conditions and cognitive impairments.[3–5] Various animal models, like weight drop (WD), controlled cortical impact (CCI), and fluid percussion injury (FPI), aim to mimic aspects of human TBI.[6–9] While CCI and FPI offer precise control over injury severity and site, they require craniectomy, potentially increasing infection risk, and do not fully replicating clinical head injuries. Initially, the WD model involved a free-falling weight impacting the exposed dura.[10] Modifications like the Marmarou model[11] introduced closed skull injuries in rats using a steel helmet to protect the skull from fracture while allowing more severe diffuse injury due to increased force of impact, that was later adapted for mice without the helmet.[12] However, these models do not fully replicate the acceleration mechanism common in human TBI incidents during car accidents, falls and contact sports. To address this limitation, a novel model was developed allowing for rapid acceleration of the head and torso post-impact.[13] Mice are suspended on slitted aluminum foil, with the impact delivered directly to the head, allowing head

angular-acceleration followed by body acceleration while falling and resting on the collecting sponge.[13] This model had drawbacks such as potential multiple injuries due to fishing line breakage, travel distance and angle of impact, and a lack of quick TBI severity adjustment inducing variability in the severity of the injury.

Our protocol is a modification of Kane's WD model,[13] introducing an impactor that translates the weight's impact directly to the animal's head, eliminating the risk of different angles of impact, and multiple injuries. This protocol also allows for quick adjustment to different TBI severities. Details of the TBI unit and equipment for measuring TBI severity using the neurological severity score[12,14,15] are provided for self-construction or upon request.

Institutional permissions

All animal experiments described in this study were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Texas Medical Branch in Galveston and conducted following the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. Animals were housed under standard conditions, maintained on a 12-h light/dark cycle (lights on at 6:00 a.m.), and provided ad libitum access to food and water.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Isoflurane	Piramal Critical Care, Inc.	66794-017-10
Experimental models: Organisms/strains		
C57BL/6J mice, 10–14 weeks old, males	Jackson Laboratories	RRID:IMSR_JAX:000664
Other		
Aluminum foil	Fisher Scientific	01-213-100
TBI apparatus	Own	
NSS apparatus	Own	

MATERIALS AND EQUIPMENT

Apparatus for inducing TBI

The TBI apparatus used in this study is depicted in Figure 1, with detailed dimensions shown in Figure 1C. The guide tube's opening sits 10 mm over a metal plate (200 mm length, 200 mm width) containing an 11 mm diameter hole that is directly aligned with the center of the tube. The metal plate features two metal screws that serve as head positioning indicators (Figure 1A). At the guide tube's end is an impactor loosely fitted within the guide tube using a lubricant (Figures 1A and 1B). A brass plate (10 mm diameter) is glued to the bottom end of impactor to ensure uniform force distribution (Figure 1B). The custom-milled brass weight (150 g in this study) has a 20 mm diameter to prevent air compression during its free fall (Figure 1A). An open acrylic box (500 mm length, 300 mm width, 270 mm height) is positioned 30 mm below the metal plate and contains medium density foam (170 mm height). Anesthetized animals are placed chest down on a slitted aluminum foil, held by the acrylic box 100 mm above foam, with the head directly under the impactor (Figures 2A and 2B). The falling weight is released, hitting the impactor which transfers the force to the animal's head (Figures 2C and 3). The impactor's travel is limited to 20 mm beyond the original position of the mouse head's dorsal surface, preventing multiple impacts by the falling weight. To maintain the guide tube's vertical position, a freely hanging fishing line with a weighted reference is used. The materials used in constructing this unit facilitate easy cleaning (e.g., with 70% ethanol) between each animal.

Equipment used for TBI severity assessment using neurological severity score

The neurological severity score (NSS) consists of 10 tasks, scored 0 or 1 for success or failure, respectively.[12,14,15] Each component of the NSS apparatus was 3-D printed (Figures 4A–4C), with detailed

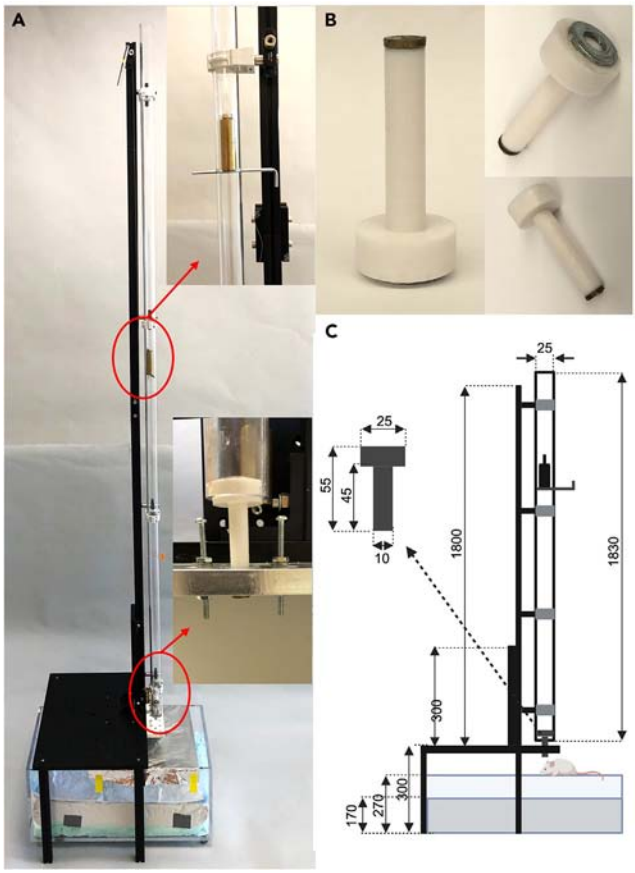


Figure 1. Apparatus for weight drop TBI model

(A) The full setup includes a vertical tube that guides a free-falling weight with an impactor attached at the end. The impactor transfers the force of the falling weight to the animal's head and limits the distance the weight travels to avoid multiple injuries. The weight is released by removing an Allen wrench tool.

(B) Close-up view of the impactor.

(C) Diagrams of the TBI apparatus, created using BioRender.com.

measurements provided in Figures 4D and 4E. The materials used ensure easy cleaning (e.g., with 70% ethanol) between animals.

The tasks are briefly described here:

Exit Circle: Tests exploration by assessing the ability to exit a 300 mm circle within 2 min.

Monoparesis/Hemiparesis: Evaluates strength by assessing gait and grip.

Straight Walk: Assesses motor function and gait.

Startle Reflex: Measures innate and unconditioned reflex in response to a startling stimulus in response to a loud hand clap.

Seeking behavior: Measures environmental interest and exploration.

Beam (7 mm × 7 mm) and Round Stick (5 mm diameter) Balancing: Tests the capability of balancing on a beam and stick, respectively.

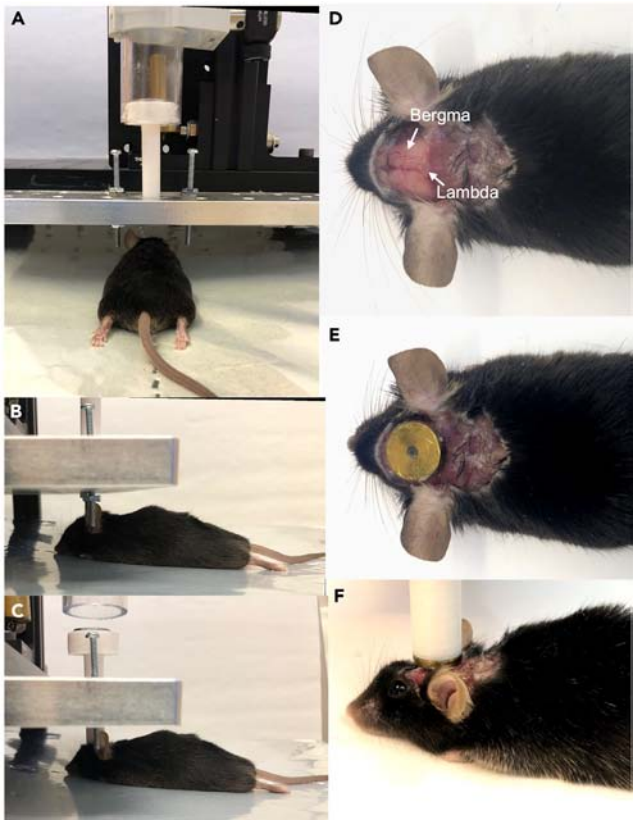


Figure 2. Setup for TBI using a mouse model

(A and B) Close-up of a mouse positioned chest down on slitted aluminum foil, with its head directly below the impactor. Two metal screws are used as markers for head positioning.

(C) Close-up of the impactor positioned between the eyes and ears.

(D) Close-up showing the bregma and lambda sutures on the exposed skull.

(E) Position of the brass disc on the exposed mouse skull.

(F) Position of the impactor on the exposed mouse skull. Note: Pre-injury surgery is not required for this model; these photographs (D–F) are for illustrative purposes only.

Beam Walk: Measures gait and balance through the ability to cross beams (520 mm length) of various widths (30 mm, 20 mm, and 10 mm).

The beams and round stick are suspended 190 mm above the table.

STEP-BY-STEP METHOD DETAILS

Closed head, weight drop traumatic brain injury

Timing: approximately 3 min per mouse (anesthesia and injury)

The section describes the animal preparation for TBI using the closed-head, weight-drop model. This model of TBI is considered high throughput due to the short duration of the procedure and the lack of need for surgical preparation. Additionally, different TBI severities can be easily adjusted by changing the drop weight or height.

Note: In this study, male C57BL/6J mice (10–14 weeks old, > 28 g) from Jackson Laboratory were used. However, it is crucial to recognize that different strains of mice are not interchangeable. Furthermore, genetically manipulated mice (knockout, transgenic) on a C57BL/6

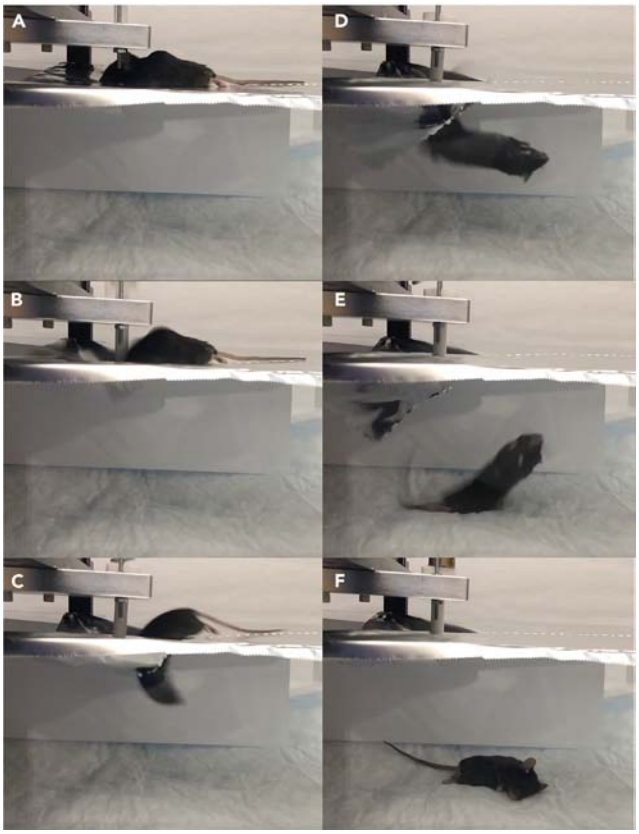


Figure 3. The closed head, weight drop TBI model with a mouse as a subject
(A–F) Sequential images from a video showing the process of TBI as described in the protocol. The impact causes a 180-degree vertical rotation of the mouse's body.

background may also exhibit varying susceptibility to injury. Therefore, adjusting the falling height and weight, followed by neurological outcome assessment by NSS, is essential to achieve the appropriate injury severity.

⚠ CRITICAL: Experiments must adhere strictly to national and institutional regulations governing the use of animals for research. It is mandatory to wear protective equipment, including gloves, face masks, and surgical gowns.

1. Carefully inspect the weight drop TBI apparatus, ensure that tube sits vertically and adjust the weight and height as needed (refer to Figure 1).
2. Score the aluminum foil sheets in the midline by making perforations using a surgical blade #15 every cm for 12 cm from the edge. Carefully and securely fasten the slit foil for animal support using scotch tape, with the slit edge facing the apparatus.

   Note: The aluminum foil is scored and should only support the mouse's body weight with minimal resistance upon impact. In this protocol, we used aluminum foil of 0.018 mm thickness.

3. Anesthetize the animals with inhalable isoflurane using a standard anesthesia machine.

   Note: Based on our experience, exposing a ~30 g wild-type C57BL/6J mouse to 4% isoflurane for 90–120 s provides adequate anesthesia. These parameters may vary depending on the mouse's age, weight, and strain.

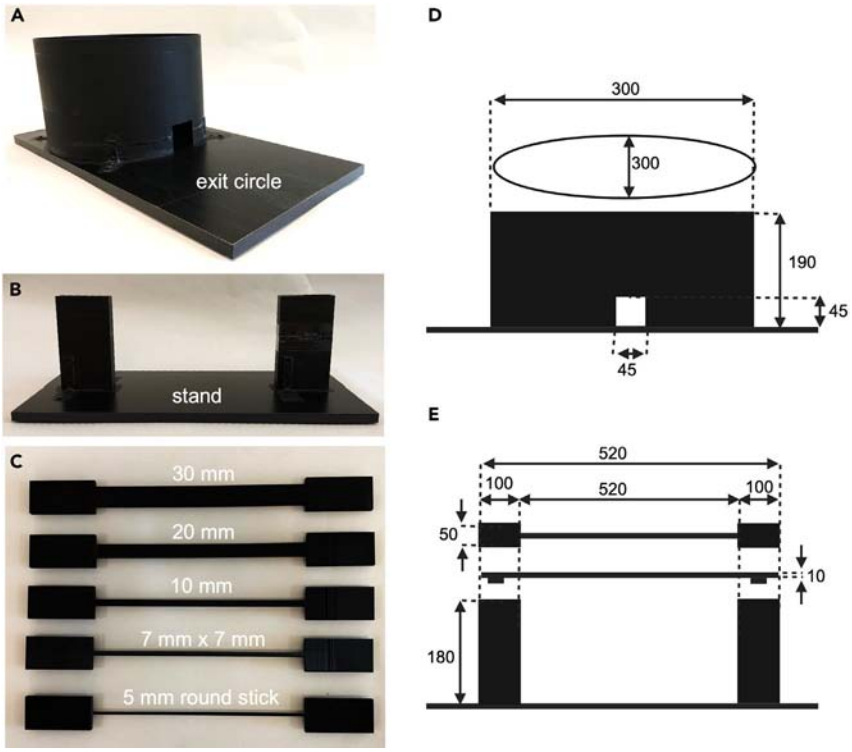


Figure 4. Setup for NSS assessment

(A) Exit circle test.

(B and C) Stand and interchangeable beams used for the beam balancing, beam walk, and round stick balance tests.

(D–E) Diagrams of the NSS apparatus created with BioRender.com.

4. Monitor the depth of anesthesia by performing a toe pinch using tweezers. Stop anesthesia as soon as pain reflex is lost. Adequate anesthesia should result in no response to extremity (no flexion or withdrawal).
5. Place the mouse chest down on a slit piece of aluminum foil, with the head positioned directly in the path of the impactor, allowing injury between the bregma and lambda (in the midline between the eyes and the ears, refer to Figure 2).

   Note: Washable, non-toxic paint can be applied to the tip of the impactor before injury to precisely mark the site of impact on the aluminum foil. The same paint can be used to mark the impact site on the head on the animal. Impact beyond lambda will significantly increase TBI severity and mortality.

6. Administer head trauma by releasing the weight with quick retraction of the trigger (Allen Wrench tool, Figure 1).

   Note: The brass weight will freely fall and hit the impactor, transferring the impact to the mouse's head.

7. Immediately after impact, the mouse will rupture the aluminum foil and fall freely onto the foam cushion positioned at the bottom of the acrylic box (refer to Figure 3).

   Note: The impact-induced acceleration results in a 180° vertical rotation of the mouse's body. The impactor should not travel more than 20 mm beyond the original position of the dorsal

Table 1. Spreadsheet for assessment of TBI severity using NSS

Project						Investigator				Date		
ID												
Exit circle	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Seeking behavior	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Monoparesis/hemiparesis	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Straight walk	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Startle reflex	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Beam balancing	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Beam walk 3 cm	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
2 cm	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
1 cm	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Round stick	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Total	–	–	–	–	–	–	–	–	–	–	–	–

surface of the mouse's head. Figure 3 displays several still pictures captured from a video recording of the injury showing 180° vertical rotation of the mouse body.

8. Transfer the injured animal onto a heated blanket to maintain temperature.

Note: Monitor the time of recovery (rightening reflex). Depending on the injury severity, recovery time varies, with the rightening reflex recovering up to 10 min for severe TBI.

9. Place mice back into the cage in the temperature-controlled room with 12-h light and dark cycles.

Note: Animals should be closely monitored within the first several hours. This step is critical, as based on our recent report, we observed up to 15% mortality within first 24 h in severe TBI.[2] Moreover, based on CT images we detected frequent skull fractures and hematomas.[2] No skull fractures and lack of mortality were observed in mild TBI.

Assessment of TBI using neurological severity score

Timing: 20 min per mouse

The section details the behavioral procedure of the neurological severity score (NSS) and its use to assess neurological deficits and determine the severity of head trauma. The NSS includes 10 tasks that primarily evaluate motor skills and balance, along with alertness and seeking behavior. Prior habituation is necessary for this assessment.

Note: Evaluating neurological impairments is crucial for determining TBI severity. The neurological severity score (NSS) mirrors the Glasgow Coma Scale used in clinical settings but primarily assesses motor function.[12,14,15] Originally consisting of 25 tasks, NSS has been condensed to 10. NSS demonstrates close correlation with MRI and histological examination, rendering it a dependable indicator of brain injury.[15]

⚠ CRITICAL: To minimize stress during NSS evaluation, mice should be acclimated to the equipment for 5 min before injury (day −2). Additionally, animals should undergo pre-injury testing to establish an NSS baseline (day −1). Any pre-injury neurological deficits should be documented, and NSS should be adjusted accordingly (ΔNSS).

Note: Neurologically healthy animals should complete each task during NSS evaluation. It is recommended to use a spreadsheet for recording NSS assessments (Table 1).

10. Task 1: Exit Circle. Place the mouse in a circle and monitor the time it takes to exit.

    Note: Healthy mice exit within 2 min (0 point); otherwise, 1 point is given.

11. Task 2: Seeking Behavior. Observe the mouse exploring the circle for 2 min.

    Note: Healthy mice display exploratory and sniffing behavior, receiving 0 points; otherwise, 1 point is added.

12. Task 3: Monoparesis/Hemiparesis.
    a. Evaluate paw's dragging while walking.
    b. Hold mouse by tail and test the ability to grip anatomic forceps.

    Note: Dragging paw's and/or failure to grip anatomic forceps results in 1 point. Otherwise, 0 points is added.

13. Task 4: Straight Walk. Assess walking ability and alertness on a flat surface.

    Note: A healthy mouse intrinsically explores surrounding and displays a normal, straight walking pattern (0 point added). Any deviation from normal walking patterns (wobbly gait, dragging of one or more paws) adds 1 point.

14. Task 5: Startle Reflex. Place mouse on empty, flat surface. Clap hands loudly and monitor the mouse's reaction.

    Note: Normal reflex will produce a bounce or wince (0 points added) movement. Failure to react adds 1 point.

15. Task 6: Beam Balancing. Place the mouse on a beam (7 mm × 7 mm). Leave the mouse on a beam for at least 10 s.

    Note: Failure to balance for 10 s adds 1 point, otherwise 0 points added.

16. Tasks 7–9: Beam walk.
    a. Place the mouse on one end of a 30 mm wide and 320 mm long beam. Observe the mouse's ability of crossing the beam.

    Note: Due to intrinsic seeking behavior, healthy mouse cross the beam within 3 min (0 points). Failure to cross adds 1 point and stop the test. Add 2 additional points for failure of crossing 20 mm and 10 mm beam tasks.

    b. If the 30 mm beam is crossed, proceed to 20 mm beam.

    Note: Healthy mouse will cross 20 mm beam within 3 min (0 points) and if failed to cross add 1 point and stop the task. Add additional 1 point for failing 10 mm beam test. It is important to stop further testing if the mouse did not cross 30 mm or 20 mm beam walk and 3 or 2 points needs to be added, respectively.

    c. If 20 mm beam has been crossed, proceed to 10 mm beam task.

    Note: Healthy mice will cross 10 mm beam within 3 min (0 points). If failed add 1 point.

17. Task 10: Round Stick Balance. Leave the mouse on a round stick (5 mm in diameter) for at least 10 s.

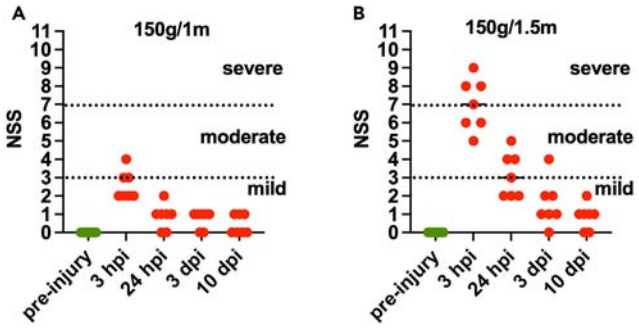


Figure 5. Weight drop mouse model for mild and moderate/severe TBI

Neurological deficits were assessed using the NSS in mice injured by a 150 g weight dropped from (A) 1 m and (B) 1.5 m. NSS was measured before injury and at 3 h post-injury (hpi), 24 hpi, 3 days post-injury (dpi), and 10 dpi. *N* = 8 per group.

Note: Mouse should "perch" by grasping the beam with hind and front limbs with tail on one side and head on the other. Failure to balance adds 1 point.

⚠ CRITICAL: Summing up points from all tasks yields the total NSS score. Scores of 1–3 indicate mild TBI, 4–6 moderate TBI, and 7–10 severe TBI. Healthy, uninjured mice usually score 0 in NSS testing. Task 10 may be the most challenging, with around 5% of wild type C57BL6/J mice aged 10–14 weeks failing to perform it. Therefore, assessment of the baseline NSS is critical, and any pre-injury NSS >0 should be considered during post-injury recovery evaluation.

EXPECTED OUTCOMES

We induced a closed-head impact-acceleration TBI in male C57BL/6J mice (10–14 weeks old, weighing over 28 g) by dropping a 150 g weight from either 1.0 m or 1.5 m onto the center of their heads (between bregma and lambda). The mice were placed in the prone position on a scored slit of aluminum foil (Figures 2A and 2B). Neurological deficits to determine injury severity were assessed using the NSS at 3 h, 24 h, 3 days, and 10 days post-injury (*n* = 8 per experimental group).

For mice subjected to the 150 g weight dropped from 1 m, we determine mild TBI in 7 out of 8 mice and moderate TBI in 1 out of 8 animals at 3 hpi, with NSS scores: 2, 2, 2, 2, 2, 3, 3, 4 (Figure 5A). NSS scores improved by 24 hpi, but deficits persisted in 3 out of 8 animals, particularly in the round stick balance, even at 10 dpi. No mortality was reported.

For mice injured by the 150 g weight dropped from 1.5 m, we observed moderate TBI in 3 out of 7 mice and severe TBI in 4 out of 7 animals at 3 hpi, with NSS scores: 5, 6, 6, 7, 8, 8, 9 (Figure 5B). One animal died from the injury. NSS scores improved by 24 hpi, but deficits persisted in 5 out of 7 animals, including an inability to pass round stick balance and 10 mm beam walk tasks, even at 10 dpi. In addition to ~15% mortality rate, consistent skull fracture and hematomas were observed in severe TBI.[2]

The described protocol and TBI apparatus allow for easy adjustments based on variations in dropped height and weight, facilitating the testing of different TBI severities. Enhancements to this model include preventing multiple injuries by utilizing an impactor instead of a fishing line, which may break or worn out over time. This protocol also enables relatively high throughput, requiring less than 10 s to perform TBI on anesthetized animals, which may be crucial for testing pharmacological interventions. Additionally, by suspending animals on the slit of aluminum foil, variability in the supporting surface is minimized compared to other weight drop models. Furthermore, this

model closely mimics common human TBI scenarios resulting from falls, car accidents and contact sports, accounting for acceleration/deceleration forces during free-falling animals. Lastly, the described TBI apparatus can be easily adapted for use with rats.

LIMITATIONS

Several reports have utilized the model described in this protocol (closed head, weight drop TBI using unrestrained animals). In line with our findings, Kane et al. (2012) reported that a single injury by a 95 g weight dropped from 1 m induced "very mild TBI," allowing for the study of repeated mild TBIs.[13] Although the neuropathology induced by this model is expected to resemble other weight drop models (such as Marmarou or Flierl models[11,12]), it is essential to verify sequential neuropathological events in future studies. Additionally, our study exclusively employed male mice, requiring further experimentation with female mice.

Moreover, while rodent brains share structural and functional similarities with humans, there are notable differences. Rodent brains are considerably smaller and have a lower mass relative to body mass. Additionally, rodents possess disproportionately less white matter than gray matter compared to humans.[16] Therefore, replicating in rodents the tissue-level forces to simulate rotational acceleration in larger human brains may pose challenges.[17] Although research with rodents offers economic, practical (due to the availability of transgenic animals), and ethical benefits, TBI, like other rodent models of various pathologies, presents common translational challenges that constrain its application.

TROUBLESHOOTING

Problem 1

Unexpected high mortality rate (protocol step 9).

Potential solution

We noted an increased mortality rate when the impactor struck the head of the animal beyond lambda, closer to the mouse brainstem. To mitigate this, we marked the tip of the impactor with washable, non-toxic paint, made a mark on the aluminum foil, and positioned the mouse head between bregma and lambda, closer to bregma accordingly (Figures 2D–2F).

Additionally, it is important to consider that different mouse strains and transgenic animals may exhibit varying susceptibility to injury. We suggest conducting tests using adjusted height and weight separately, rather than concurrently.

We have recently reported that injury caused by 150 g dropped from 1.5 m resulted in ~15% mortality, constant (<90%) skull fracture and hematomas.[2]

Problem 2

Inconsistent injury site (protocol step 6).

Potential solution

It is crucial to ensure proper anesthesia of the animal before injury. Insufficient anesthesia may lead to head/body movement before injury. To prevent this, prolong the duration of anesthesia, work efficiently, and monitor the depth of anesthesia by conducting a paw or rail pinch reflex assessment using tweezers. Adequate anesthesia should abolish this reflex showing no response to stimuli. In our recent publication,[2] exposure of ~30 g C57BL/6J male mice to 4% isoflurane for 90–120 s was sufficient to induce adequate anesthesia. However, these parameters may vary depending on age, mass and mouse strain.

Problem 3

Large variability in NSS (protocol step 17).

Potential solution

While minor deviations in NSS are anticipated with specific weight and height adjustments, typically within +/−2 points (mirroring clinical setting), greater variability may arise from inconsistent injury site, varying depths of anesthesia, or the inclusion of mice with diverse genetic backgrounds or transgenic animals.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Bartosz Szczesny (baszczes@utmb.edu).

Technical contact

Questions about the technical specifics of performing the protocol should be directed to the technical contact, Bartosz Szczesny (baszczes@utmb.edu).

Materials availability

For additional information and requests for resources, please contact Bartosz Szczesny (baszczes@utmb.edu).

Data and code availability

For additional information about the data generated in this protocol, please contact the lead investigator, Bartosz Szczesny (baszczes@utmb.edu). This study did not generate new code.

ACKNOWLEDGMENTS

We are thankful to Jamal Saada and Tony Z. Tang for their help in the construction of the TBI units and measuring NSS. This work was supported by National Science Foundation (NSF) project number 2136421 and grants from TIRR/Mission Connect (to B.S.) and Coalition for Brain Injury Research project number P62301 (to P.W.).

AUTHOR CONTRIBUTIONS

Writing – original draft, B.S.; writing – review and editing, J.A.L., M.M., P.W., and B.S.; figure preparation, B.S.; performing experiment, J.A.L.; conceptualization and model development, J.A.L. and B.S.; funding acquisition and supervision, P.W. and B.S.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Allende Labastida, J. (2019). Characterization and Therapeutic Evaluation of a Traumatic Brain Injury Model in Compliance with Common Data Elements. Doctoral Dissertation (The University of Texas Medical Branch at Galveston), pp. 89–150.

2. Tang, T.Z., Zhao, Y., Agarwal, D., Tharzeen, A., Patrikeev, I., Zhang, Y., DeJesus, J., Bossmann, S.H., Natarajan, B., Motamedi, M., and Szczesny, B. (2024). Serum amyloid A and mitochondrial DNA in extracellular vesicles are novel markers for detecting traumatic brain injury in a mouse model. iScience *27*, 108932. https://doi.org/10.1016/j.isci.2024.108932.

3. Masel, B.E., and DeWitt, D.S. (2010). Traumatic brain injury: a disease process, not an event. J. Neurotrauma *27*, 1529–1540. https://doi.org/10.1089/neu.2010.1358.

4. Maas, A.I.R., Menon, D.K., Manley, G.T., Abrams, M., Åkerlund, C., Andelic, N., Aries, M., Bashford, T., Bell, M.J., Bodien, Y.G., et al. (2022). Traumatic brain injury: progress and challenges in prevention, clinical care, and research. Lancet Neurol. *21*, 1004–1060. https://doi.org/10.1016/S1474-4422(22)00309-X.

5. Najem, D., Rennie, K., Ribecco-Lutkiewicz, M., Ly, D., Haukenfrers, J., Liu, Q., Nzau, M., Fraser, D.D., and Bani-Yaghoub, M. (2018). Traumatic brain injury: classification, models, and markers. Biochem. Cell. Biol. *96*, 391–406. https://doi.org/10.1139/bcb-2016-0160.

6. Johnson, V.E., Meaney, D.F., Cullen, D.K., and Smith, D.H. (2015). Animal models of traumatic brain injury. Handb. Clin. Neurol. *127*, 115–128. https://doi.org/10.1016/B978-0-444-52892-6.00008-8.

7. Ma, X., Aravind, A., Pfister, B.J., Chandra, N., and Haorah, J. (2019). Animal Models of Traumatic Brain Injury and Assessment of Injury Severity. Mol. Neurobiol. *56*, 5332–5345. https://doi.org/10.1007/s12035-018-1454-5.

8. Marklund, N. (2016). Rodent Models of Traumatic Brain Injury: Methods and Challenges. Methods Mol. Biol. *1462*, 29–46. https://doi.org/10.1007/978-1-4939-3816-2_3.

9. Dewitt, D.S., Perez-Polo, R., Hulsebosch, C.E., Dash, P.K., and Robertson, C.S. (2013). Challenges in the development of rodent models of mild traumatic brain injury. J. Neurotrauma *30*, 688–701. https://doi.org/10.1089/neu.2012.2349.

10. Feeney, D.M., Boyeson, M.G., Linn, R.T., Murray, H.M., and Dail, W.G. (1981). Responses to cortical injury: I. Methodology and local effects of contusions in the rat. Brain Res. *211*, 67–77. https://doi.org/10.1016/0006-8993(81)90067-6.

11. Marmarou, A., Foda, M.A., van den Brink, W., Campbell, J., Kita, H., and Demetriadou, K. (1994). A new model of diffuse brain injury in rats. Part I: Pathophysiology and biomechanics. J. Neurosurg. *80*, 291–300. https://doi.org/10.3171/jns.1994.80.2.0291.

12. Flierl, M.A., Stahel, P.F., Beauchamp, K.M., Morgan, S.J., Smith, W.R., and Shohami, E. (2009). Mouse closed head injury model induced by a weight-drop device. Nat. Protoc. *4*, 1328–1337. https://doi.org/10.1038/nprot.2009.148.

13. Kane, M.J., Angoa-Pérez, M., Briggs, D.I., Viano, D.C., Kreipke, C.W., and Kuhn, D.M. (2012). A mouse model of human repetitive mild traumatic brain injury. J. Neurosci. Methods *203*, 41–49. https://doi.org/10.1016/j.jneumeth.2011.09.003.

14. Khalin, I., Jamari, N.L.A., Razak, N.B.A., Hasain, Z.B., Nor, M.A.B.M., Zainudin, M.H.B.A., Omar, A.B., and Alyautdin, R. (2016). A mouse model of weight-drop closed head injury: emphasis on cognitive and neurological deficiency. Neural Regen. Res. *11*, 630–635. https://doi.org/10.4103/1673-5374.180749.

15. Tsenter, J., Beni-Adani, L., Assaf, Y., Alexandrovich, A.G., Trembovler, V., and Shohami, E. (2008). Dynamic changes in the recovery after traumatic brain injury in mice: effect of injury severity on T2-weighted MRI abnormalities, and motor and cognitive functions. J. Neurotrauma *25*, 324–333. https://doi.org/10.1089/neu.2007.0452.

16. Zhang, K., and Sejnowski, T.J. (2000). A universal scaling law between gray matter and white matter of cerebral cortex. Proc. Natl. Acad. Sci. USA *97*, 5621–5626. https://doi.org/10.1073/pnas.090504197.

17. Cullen, D.K., Harris, J.P., Browne, K.D., Wolf, J.A., Duda, J.E., Meaney, D.F., Margulies, S.S., and Smith, D.H. (2016). A Porcine Model of Traumatic Brain Injury via Head Rotational Acceleration. Methods Mol. Biol. *1462*, 289–324. https://doi.org/10.1007/978-1-4939-3816-2_17.