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A novel weight-drop closed head focal traumatic brain injury: A candidate to translational studies?

Pedro Henrique Cassaro Lirio[a,1], Jessica Vaz Gonçalves[a,1], Walter Nunes Pontara Filho[a,1], Thamiris Alves Amancio[a,1], Juliana Tancredo Carlini[a,1], Polyana Lima Meireles Dalpiaz[a,1], Carmem Luíza Sartório[b,2], Livia Carla de Melo Rodrigues[a,1,*], Fernando Zanela da Silva Areas[a,1]

[a] *Department of Physiological Sciences, Federal University of Espírito Santo, Vitória, Brazil*
[b] *Pharmaceutical Sciences Graduate Program, Vila Velha University (UVV), Vila Velha, ES, Brazil*

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ABSTRACT

Traumatic brain injury (TBI) is a neurotrauma with a complex pathophysiology caused by an external mechanical force. This global public health problem is a leading cause of death and disability in young adults. In this scenario, many models were developed to try to simulate human TBI. The weight drop model allows the investigation of the pathophysiological cascades of TBI without surgical interference. In this protocol, a new closed-head weight-drop rat model consisting of a 48.5g weight projectile that free falls from 1.10m high onto the skull of the animals was built. We classify the present TBI model performed as moderately severe due to its mortality rate. Animals from TBI and Control (Sham) groups underwent weight for 7 days and temperature assessments within 1 hour after TBI and for 7 days. Results demonstrated that the TBI group showed less body weight gain in the days after the injury. Temperature oscillations within the first-hour post-injury and on the 3rd day after injury were observed. As the results of this study demonstrated similarity to human TBI vital parameters, this new adaptation of the Weight-drop model injury can be a suitable candidate for translational studies.

- We developed a novel closed head focal traumatic brain injury using a projectile.
- This TBI model does not require surgical intervention.
- The validation of this method demonstrates that the vital parameters of the injured rats exhibit similarities with those of TBI patients.

Specifications table
This table provides general information on your method.

Subject area:	[Neuroscience]
More specific subject area:	Brain injury
Name of your method:	Weight-drop closed-head traumatic brain injury

(*continued on next page*)

* Corresponding author.
E-mail address: livia.rodrigues@ufes.br (L.C.d.M. Rodrigues).
[1] Direção do CCS - Prédio do Centro de Ciências da Saúde, Av. Mal. Campos, 1468 - Maruípe, Vitória – Espirito Santo, Brazil
[2] Avenida Comissário José Dantas de Melo, 21 - Boa Vista II, Vila Velha – Espirito Santo, Brazil

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Resource availability:	Material table will be available in the supplementary material section

Background

Traumatic brain injury (TBI) is a neurotrauma with a complex pathophysiology caused by an external mechanical force. The damage from this injury can be divided into primary and secondary [1]. Primary damage is related to the mechanical collision of the brain and the secondary damage is characterized by neurochemical and immunoexcitotoxic processes caused by the temporal progression of the injury [2,3]. Known as the “silent epidemic”, it is the biggest cause of disability for young adults around the world. Given the importance of this public health challenge, numerous pre-clinical models were developed to simulate the TBI outcomes. These models aim to enhance our understanding of the sequelae and to explore novel treatments [4].

The Weight-drop model, first demonstrated by Feeney [5], allows the avoidance of surgical interventions, thereby providing a better understanding of the pathophysiological cascades associated with closed-head TBI. In this model, a weight is allowed to free-fall onto the animal’s head, inducing either focal or diffuse injury, depending upon the specific adaptations employed [5,6]. Also, it allows the monitoring of basic vital parameters serving as auxiliary indicators for the classification and prognosis related to TBI, closely mirroring human TBI outcomes [7].

In the present study, we introduce a novel apparatus to induce a closed-head weight-drop injury to produce moderately severe TBI in rats. We evaluated the outcomes of a 48.5g free-falling weight projectile from a height of 1.10m onto the skull of the animals causing a focal injury. Both the TBI group and the Sham group underwent weight and temperature assessments over the course of 7 days. Additionally, we conducted temperature evaluations during the initial hour post-TBI at intervals of 0’, 10’, 30’, and 60’."

Basic vital parameters such as the ones presented in this study can be key to defining the severity of TBI models. Moreover, the pursuit of a reproducible translational model regarding traumatic brain injury in rodents holds significant promise to the better understanding of the sequelae and progression of TBI.

Method details

Traumatic brain injury apparatus

The present model presents an electromagnetic system capable of sustaining a projectile with up to 48.5g and up to 12.6cm in length. In addition, aiming for complete fixation of the animal’s head and seeking to standardize the site of trauma induction, an auricular fixation from stereotaxic ear bars was adapted to the model. The equipment has the following measurements: Lower medium-density fiberboard (MDF) with 2 cm width and 15cm length; Vertical MDF with 4cm width and 15cm height; Upper MDF with 2cm width and 15cm length; 95 cm high stainless-steel tube + 15 cm free fall (totalling 110 cm from the top of the apparatus to the animal’s head); Stereotaxic ear bars measuring 20cm long and 2.5cm high with 12.5cm long fasteners. Furthermore, the apparatus has a balancing system (with adjustable height bases) to guarantee its stability in relation to the plane where it was positioned (Supplementary Fig. 1).

Projectile

The base of the projectile is 2.2cm long and 0.6cm in diameter, 2cm of which is made of stainless steel and 0.2cm of rubber (latex) with a 1mm domed tip also made of stainless steel. It is worth mentioning that the rubber structure is extremely important in the presented model to avoid severe skull fractures (Fig. 1A).

Several projectiles were tested in this study, although only the one presented was able to produce injury and mortality rates (Table 1). All projectiles used in this project had the same base, which is the structure of the projectile that touches the animal’s

Table 1
Table of tested projectiles in 8-week-old rats. In the present study, several projectiles were tested prior to selecting the one that is the most effective to induce TBI in 8-week-old Wistar rats. All the first five projectiles were not able to induce satisfactory TBI damage nor cause variation of normal behavior after injury. Diameter and length are in centimeters (cm), weight in grams (g) and energy in joules (J). Two of the 14 animals that underwent TBI induction did not survive post-injury.

PROJECTILE	MATERIAL	DIAMETER	LENGHT	WEIGHT (g)	ENERGY (J)	MORTALITY
#1	Steel	0,4cm	3,6cm	6,67g	0,072J	0%
#2	Steel	0,4cm	4,4cm	7,75g	0,083J	0%
#3	Iron	0,42cm	7,5cm	19,80g	0,213J	0%
#4	Iron	0,5cm	9,2cm	27,07g	0,292J	0%
#5	Iron	0,5cm	12cm	35,34g	0,381J	0%
#6	Brass	0,6cm	12,6cm	48,50g	0,522J	14.30%

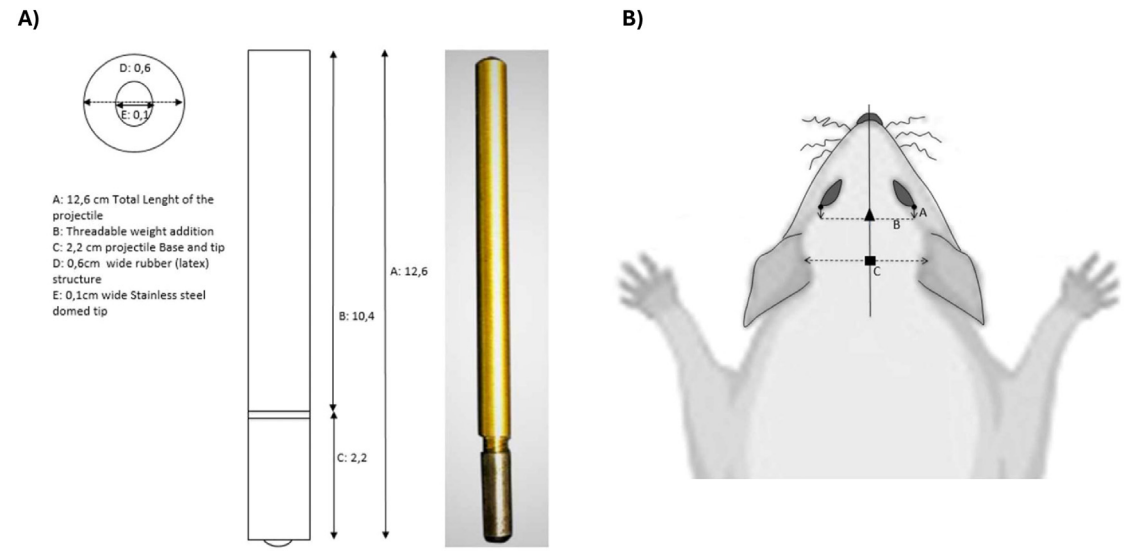

Fig. 1. A) Projectile used in the model. On the right, an image from the projectile used to induce TBI on 8-week-old Wistar rats was made of brass, weighs 48,5g and is 12,6cm long. On the left side a schematic representation of the projectile parts and measurements. Moreover, all the detailed information about the projectile is given in Item 2 of the protocol description. 1B) Schematic representation of bregma location. A: Horizontal line 0,2cm posterior to the corners of the right and left eyeballs. B: Horizontal line between the most anterior parts of the right and left helix. C: Vertical line at the midpoint of A and B. Triangle: Bregma location and impact site. Circle: Lambda location. The distance from point A to B is 0.8cm.

head. The difference between the projectiles used in the project was their removable part. In addition, the materials in which they were produced, diameter and length, also varied. Moreover, all weight additions (or removable parts) have a threadable tip, allowing correct attachment to the base.

NOTE: After carrying out the tests, the only projectile able to reproduce a moderately severe traumatic brain injury that presented subdural bleeding, formation of an inflammatory process, pain behavior in the animals and degree of mortality was the presented projectile (Fig. 1A), which corresponds to an average of 20%±3% of the total body weight of the 8 weeks old rats. This projectile was the one chosen to carry out the following procedures.

Bregma location without surgical intervention

Bregma, the meeting point of the coronal and sagittal fissures, was found in a superior view, where 2 horizontal lines and a sagittal line (from the midpoint of the 2 previous lines) were marked (Fig. 1B). Horizontal line 1: between the middle parts of the right and left helix (lambda location). Horizontal line 2: corresponding to the coronal suture: 0.2 cm posterior to the posterior corners of the right and left eyeballs. Sagittal line, which corresponds to the sagittal fissure: midpoint of the previous 2. Bregma, the projectile impact site, corresponds to the intersection of line 2 with the sagittal line. For confirmation, the distance from lambda to bregma was measured, in which 0.8cm was the parameter used for 8-week-old Wistar rats.

Protocol

Animals

Male Wistar rats (n=26; 8 weeks old) weighting 245-285g and obtained from the institutional animal care facility of Federal University of Espirito Santo were used in this study. The animals were housed with ad libitum access to food and water in groups of four to five per cage (50 cm × 60 cm × 22 cm) until the procedure and returned after the induction of TBI. Room temperature was maintained at 23 ± 1 ˚C with 12:12h light/dark cycle and controlled humidity. The Guide for the Care and Use of Laboratory Animals and Directive 2010/63/EU and the internal guide were followed in all experiments. This study was approved by the local Ethics Committee on Animal Use for Research under the protocol number 12/2021.

Procedures prior to the induction of TBI

1. Take the home cage of animals to the procedure room.
2. Perform deep anesthesia with Ketamine (75mg/kg) and Xylazine (5mg/kg) intraperitoneally (ip).
3. Wait patiently until animals don't respond to pain stimulus in paw and/or tail pinch using a Tissue Forceps or "Mouse Tooth" Forceps.

4. NOTE: Do not induce TBI while the animal still responds to those stimuli, otherwise it will cause pain spams.
5. Plug in the TBI apparatus and after verifying anesthesia success proceed to the induction of TBI

Induction of TBI

1. With the animal deeply anesthetized, place it in the middle of the apparatus, where there should be a marker or aim, marking where the projectile will land.
   NOTE: The right measurements and marks of the apparatus should be done prior to inducing the TBI, since it will define the success on the induction of TBI in the chosen area.
2. Insert the stereotaxic ear bars into the animal; this procedure confirms the constraint of the animal's head, avoiding any involuntary movement of the head.
3. To find the bregma without using surgical procedures, follow the steps described above.
4. Make sure that the animal's head is still perpendicular to the solid base of the apparatus, so the projectile can properly hit the aim.
5. After constraining the head of the rat, carefully place the projectile on top of the apparatus.
   NOTE: You should not press the projectile down, otherwise it will fall instantly from the top. That can inapt the procedure of TBI induction and if that happens, the animal should not be considered for assessments afterwards.
6. Press the activation pedal to release the projectile.
7. Promptly after falling onto the skull of the rat, remove the stereotaxic bars and place the animal into individual cages for assessments.
8. To perform Sham TBI, all the procedures should be done the same as previously described but the drop of the projectile into the rat's skull. Also, topic anesthetics could be used to simulate the injury.
9. Return the rat to its home cage after the effect of anesthesia cease and it has recovered the ability to feed and has normal behavior.
10. Clean the apparatus and projectile with Alcohol 70% after each TBI induction.

Basic vital parameters assessments

1. Right after the induction of the TBI, return the animal to an individual clean cage to assess the temperature.
2. Carefully introduce the digital thermometer rectally.
3. Wait till the temperature stabilizes and carefully remove the thermometer from the animal.
4. Repeat this procedure at 10 minutes, 30 minutes and 60 minutes post-injury.
5. Daily, during the 7 days of assessment, place the animal on a digital scale and wait until stabilization for daily body weight assessment.
   NOTE: Preferably at the same time of the day (e.g. Morning).
6. In the same way as previously described, to assess daily rectal temperature, steps 1-3 were followed.
   NOTE: Since the animal is fully awake, the correct management and containment will be needed to avoid any unnecessary distress and pain to the animal.

Method validation

For 7 days, the animals in the control (SHAM) and TBI group were monitored, and daily weighing was performed, respecting the same period of the day for all measurements (Fig. 2A).

The animals submitted to traumatic brain injury in the presented model showed, through Two-way ANOVA, a lower weight gain ($P < 0.05$) in comparison to the control group (CONTROL: 293.6±4.99; TBI: 284.6± 2.19, $F(1, 22) = 5.237$). It is possible to infer that the difference between the body weight of the groups is related to the acute consequences caused by traumatic brain injury, such as possible mild skull fractures and hemorrhages. Weight loss in animal models of TBI was already reported in previous studies of different models of TBI, with the appearance of behavioral deficits related to the damage caused by the event in the acute period [8].

Right after traumatic brain injury induction, during the first hour, the rectal temperature was measured at four different times: 0 minutes (immediately after TBI), 10 minutes, 30 minutes and 60 minutes (Fig. 2C). As a control, the temperature of the same animals was measured before TBI induction (CONTROL: 37,56±0.17, 0′: 38.09±0.18, 10′: 37.73±0.17, 30′: 37.54±0.14, 60′: 37.34±0.17, $F(2{,}418, 14{,}51) = 17{,}09$, $p < 0.0001$). Immediately after TBI, a statistically significant difference ($p < 0.01$) was demonstrated in comparison to the control. Differences between the times 0 minutes and 10 minutes ($p < 0.01$) were also identified. Temperature oscillations are also demonstrated in studies conducted in humans, which also have a similar response pattern [9].

The TBI group showed a statistically significant decrease in body temperature on day 3 (Fig. 2B) when compared to the control group (CONTROL: 37,71±0,08; TBI: 36,81±0,13, $F(1, 12) = 21{,}21$, $p < 0{,}001$). The decreased temperature culminates with the inflammatory process peak and could also imply metabolic changes or hypothalamic damage through TBI secondary damage [10].

Many models of traumatic brain injury were developed for decades. Notably, none of them can mimic the complexity of human TBI to date. Although, closed-head TBI models, like the one presented, is one step closer to translating into one of the best models to understand the complex pathophysiology and deficits from TBI [11]. In addition, closed-head injury models seem to develop behavior and neuropathological outcomes similar to the ones presented by TBI patients [12]. The present Weight-drop model, as mentioned,

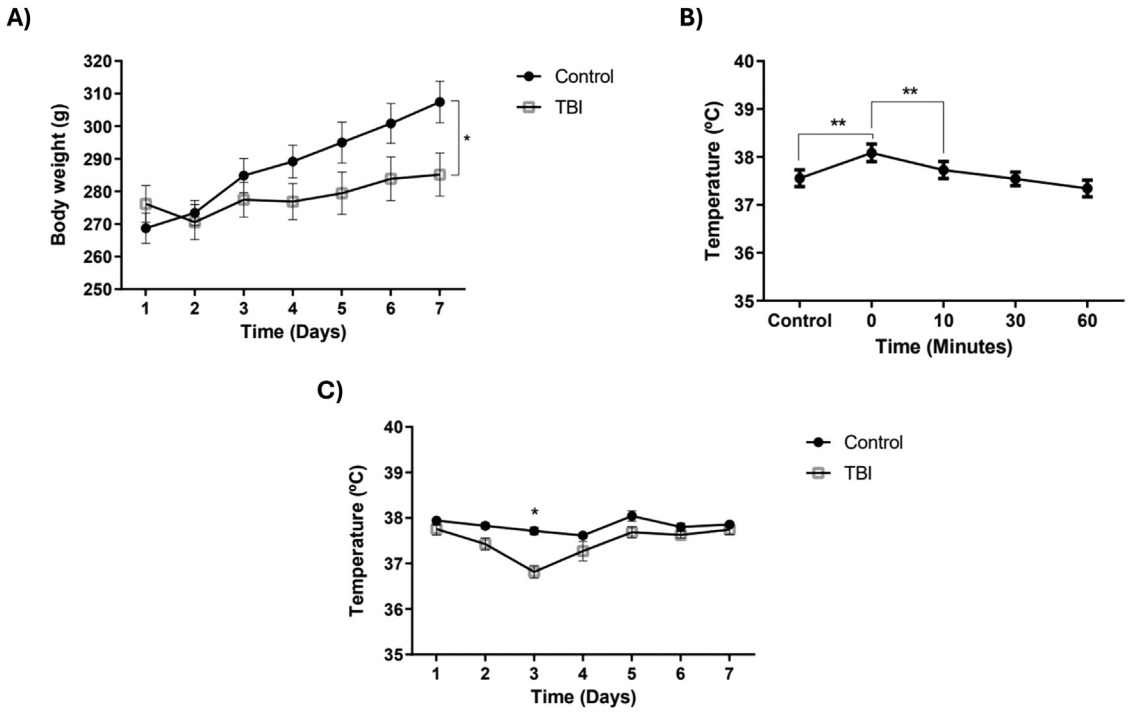

Fig. 2. A) Differences in body weight gain. All rats in the Sham and TBI group were daily weighted for 7 days during the morning. The graph represents the body mass in relation to the time of 7 days (n=12) per group. Two-Way ANOVA for repeated measures and Bonferroni post hoc test for multiple comparisons. The TBI group had less weight gain in the 7 days of assessment ($p > 0.05$). Also, on days 6 and 7, there was a significant difference between groups ($p = 0.04$ and $p = 0.01$, respectively). B) Temperature oscillation at different times within 1 hour post-injury. Graph representing rectal temperature after traumatic brain injury at different times. Immediately after TBI induction the animals had a significant increase in rectal temperature. 10 minutes after TBI decrease in the temperature was also demonstrated (n=7) per group. One-way ANOVA for repeated measures, Bonferroni test for multiple comparisons. C) Daily temperature oscillations within 1 week post-injury. Graph representing daily rectal temperature after traumatic brain injury for a week. Mainly on the 3rd day post-injury, TBI-induced animals showed a significant decrease in temperature in comparison to the control group. Data presented as means ± SEM (n=7) per group. Two-way ANOVA for repeated measures, Bonferroni test for multiple comparisons. $^{*}p<0.05$ ** $p<0.01$.

was adapted from Feeney [5]. To date, there are countless variations of this method to induce TBI, however, to the best of our knowledge, this is the first to use projectiles in closed head weight-drop model to induce moderately severe TBI in rats.

In this study, we developed a closed-head Weight-drop model TBI apparatus that is capable of causing focal injury by constraining the head of the animal. This allows the visualization and assessment of vital parameters and a broad range of outcomes without surgical intervention. As mentioned, keeping the skull or scalp of the animals intact prior to TBI, can mimic closer outcomes to human TBI [13]. The presented model and the demonstrated results of less gain of weight and oscillations of temperature right after the TBI and within a week corroborate with clinical parameters of TBI patients [7].

Highlighting the procedure of the protocol itself, one of the most critical phases are as follows: (1) Make sure that the animal is deeply anesthetized prior to inducing TBI and for so, use the “mouse tooth” forceps to assess the pinch reflex of the tail and pawn, (2) Placement of the stereotaxic ear bars have to be positioned carefully and equally on both ears. The incorrect placement of the bars can not only cause unnecessary wounds to the animal but can also miss the area of impact, (3) After TBI, watch the animal for a minimum of 1 hour or until they are able to feed and the effect of anesthesia cease, (4) For correct measurement of the temperature throughout the days and right after TBI, the accurate immobilization of the animal is necessary to avoid any kind of excessive distress and pain. Worth mentioning that any abrupt movements of the animal while assessing temperature could cause bleeding.

The weight (and/or length) of the projectile can be modified. Since this present study used only Wistar rats with a starting weight of 245-285g and 8 weeks old, any modifications of these starting points can change the outcomes observed. Also, it can differ the presented effectiveness of the projectile. Furthermore, due to the non-surgical intervention (closed head), the bregma location parameters were related to previous studies using the method presented above [14]. The variation of these anatomical parameters between animals is negligible, particularly using the demonstrated projectile, the impact of the chosen area should be done without major problems, especially because of the diameter of the tip.

With the above, Weight-drop models are still valid to date and fulfill most of the expected outcomes from human TBI. Although it is still a challenge to develop an ideal translational method to induce TBI, we conclude that the presented model can be a suitable

candidate for translational studies searching the understanding of TBI pathophysiology and outcomes, since our results corroborate with clinical findings.

Limitations

The mortality rate of this model can only express data to this very range of body weight and age of the rats. Any changes in these two starting points or the projectile, as mentioned before, will also vary the results obtained.

Ethics statements

The Guide for the Care and Use of Laboratory Animals and Directive 2010/63/EU and the internal guide were followed in all experiments. This study was approved by the local Ethics Committee on Animal Use for Research under the protocol number 12/2021.

CRediT author statement

PHCL - Acquisition of data, analysis, interpretation and drafting the article.
JVG - Acquisition of data, drafting the article.
WNPF - Acquisition of data.
TAA - Acquisition of data.
JTC - Acquisition of data.
PLMD - drafting the article and revising.
CLS - drafting the article and revising.
LCMR - drafting the article, revising and final approval for submission.
FZSA - Conception of the study and final approval for submission.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mex.2024.102806.

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