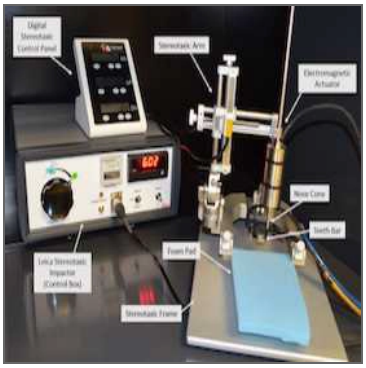

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Closed-head controlled impact model in adult rats- VandeVord Lab

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PRECISE-TBI

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DISCLAIMER

None

ABSTRACT

This is a protocol to describe the materials and methods utilized to perform preclinical traumatic brain injury (TBI) using a closed-head controlled impact model in adult rats. The model utilizes an electromagnetic actuator to deliver an impact to the intact scalp and has been optimized for use in adult rats. A skull template is used to mark the impact site and the injury parameters can be adjusted according to the desired severity of impact using the control box.

The posting of this protocol is part of the mission of the PREClinical Interagency reSearch resourcE-TBI (PRECISE-TBI, precise-tbi.org) to improve clinical translation of therapeutics by providing an online catalog and standardized protocols to reduce the variability of model usage between laboratories.

IMAGE ATTRIBUTION

White MR, VandeVord PJ. Regional variances depict a unique glial-specific inflammatory response following closed-head injury. Front Cell Neurosci. 2023 Feb 15;17:1076851. doi: 10.3389/fncel.2023.1076851. PMID: 36909284; PMCID: PMC9996631.

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Protocol status: Working
We use this protocol and it's working

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MATERIALS

- Leica Impact One control box, [RRID:SCR_025114]
  - **(Disclaimer: This product has been discontinued. Similar equipment may be purchased from Neuroscience Tools [Neuropactor TBI Instrument for CCI, CHI, SCI, Neurochimera])**
- Neuroscience Tools Neuropactor stereotaxic actuator (with compatible injury tips), [RRID:SCR_024868]
- Harvard Apparatus digital stereotaxic coordinate panel,
- stereotaxic frame,
  Similar to Harvard Apparatus Digital U-Frame Stereotaxic Instruments (cat # 17-1806),
- 1.25cm thick foam pad,
- isoflurane delivery system with nose cone,
- skull template (Please see Note 4),
- heating pad,
- eye lubricant,
- electric razor

1 Choose impactor tip based on diameter of choice (See Note 1) and tightly screw the tip into the bottom of the actuator before mounting the actuator on the stereotaxic crossbar.

2 Connect the actuator to the impactor control box and connect the control box to a power source (outlet).

3 Connect the stereotaxic coordinate display to the stereotaxic frame.

4 Examine the device and check maintenance logs to ensure it is working properly prior to use (see Note 2).

5 Use adjustment knob on the control box to set the desired velocity for impact.

6 Use dial on the control box to set the desired dwell time for impact (See Note 3).

7 On the base of the stereotaxic frame, place a 1.25 cm thick foam pad large enough for the animal's body to lay flatly on.

8 Secure an anesthesia nose cone to the bite bar of the stereotaxic frame.

9 Place the rat into the anesthesia chamber and induce anesthesia using isoflurane (4.0%) in 100% O2.

10 After 5 - 15 minutes of anesthesia exposure, remove rat from the chamber and place on the foam pad within stereotaxic frame in prone position. Immediately secure teeth to bite bar and affix nose cone on the animal to maintain isoflurane anesthesia.

11 Apply eye lubricant to the rat's eyes.

12 Shave the animal’s head from between the eyes to between the ears.

13 Ensure the animal’s head is resting on the foam pad and is level in all directions by adjusting the height of the bite bar.

14 Align a skull template along the scalp and mark the site of injury using a marker (see Note 4).

15 With the tip in the “Extend” position (as set using switch on control box), move the tip to Bregma on the skull template and zero out all stereotaxic coordinates on display.

16 Remove the skull template from the rat’s scalp.

17 Move the tip to the marked site of impact and firmly zero the tip against the animal’s scalp. Record M/L and A/P coordinates and zero out D/V coordinates at the plane of the scalp.

18 Use switch on control box to set tip in the “Retract” position.

19 Lower the tip to the desired depth of impact according to the D/V stereotaxic coordinates.

20 Ensure the animal is adequately anesthetized by use of toe pinch or a similar method.

21 Flip down “Impact” switch on control box to trigger the impact (see Note 5).

22 Start a stopwatch at the time of impact to begin measurement of righting response time.

23 Immediately remove the animal from the stereotaxic frame and nose cone. Place the animal on its back on a heating pad until it is awake and able to right itself. Examine for any health concerns.

24 Supervise the rat while it recovers from anesthesia to the point that spontaneous locomotion occurs.

25 Stop stopwatch when the animal rights itself on all four paws and record the righting response time.

26 Return the rat to its home cage and continue to monitor per institutional and federal guidelines for the treatment of laboratory animals and in accordance with your IACUC protocol.

27 Resume standard housing and husbandry.

Notes

28 Note 1: Tip set includes tips ranging in diameter from 1mm to 5mm. We use a 5mm tip for our experiments.
Note 2: Test fire multiple impacts to be sure the device fires and retracts the tip properly.
Note 3: The lowest place digit on the dwell time dial sets tenths of a second and the next highest place digit sets number of seconds, so a setting of “03” = 300 ms.
Note 4: The skull template is used to ensure consistency of the site of impact across injuries by allowing for the approximation of the location of Bregma without opening the scalp. The skull template should be the top of a skull from a rat of the same age and breed. At the desired location of impact, a small hole should be drilled through the skull, wide enough to fit an Ultra Fine Point Sharpie or similar marker through to mark the site of impact.
Note 5: Sham animals should undergo all the same procedures with the exception of triggering the impact.