

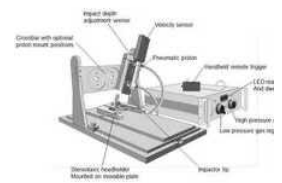
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Open-Skull Controlled Cortical Impact (CCI) model in rats

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Controlled Cortical Impact Model of TBI



C Edward Dixon Ph. D.¹, Nico Osier Ph. D.²

¹University of Pittsburgh; ²The University of Texas at Austin

PRECISE-TBI



Monique Surles-Zeigler

University of California, San Diego

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We use this protocol and it's working

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Abstract

This is a protocol to describe the materials and methods utilized by the submitter to perform preclinical traumatic brain injury (TBI) using the open-skull controlled cortical impact (CCI) model.

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



- 1 Examine the CCI device and its maintenance logs to ensure that it is in proper working order and the maintenance schedule has been adhered to (see Note 1).
- 2 Place the rat into the chamber and induce anesthesia using isoflurane (4.0%) in a 2:1 mixture of N₂O:O₂.
- 3 Ensure adequate anesthesia is achieved by use of the toe pinch test or a similar method.
- 4 Intubate the test animal (see Note 2).
- 5 Place the intubated rat into the stereotaxic frame and stabilize the head using an incisor bar and bilateral ear bars.
- 6 Place all physiologic monitoring equipment needed for data collection and maintaining body temperature, such as a temperature probe, pulse oximeter, etc.
- 7 Reduce the isoflurane to the maintenance dose (2.0%); if signs of regaining consciousness are noted (e.g. whisker movement), gradually increase the dose.
- 8 Shave the rat's head over the surgical site using hair trimmers.
- 9 Apply a sterile drape over the rat to maintain aseptic technique, exposing only the surgical site.
- 10 Apply antiseptic solution (e.g. betadine) to the scalp using sterile gauze.
- 11 Use a sterile scalpel to make a 20 mm long midline incision on the rat's scalp.
- 12 Use sterile microdissecting forceps, periosteal elevator, and a cotton-tipped applicator to expose the skull by carefully reflecting the skin, fascia, and muscle.
- 13 Use the pneumatic drill to create a circular bone window (i.e. craniectomy), 7 cm in diameter (approximately 1 mm larger than the tip diameter), and centered between the

- lambda/bregma (anterior-to-posterior) and coronal ridge/sagittal suture (medial-to-lateral) (see Note 3).
- 14 Use microdissecting forceps to gently lift and remove the bone flap without disrupting the dura mater; discard the bone flap.
 - 15 If the craniectomy is not large enough for unobstructed tip clearance, enlarge it with sterile rongeurs.
 - 16 Extend the shaft of the CCI device manually to verify correct positioning of the test animal within the stereotaxic frame such that the impactor tip is centered within the bone window (see Note 4).
 - 17 Gently zero the impact tip to the cortical surface, ensuring the piston is statically pressured and in the full-stroke position.
 - 18 Carefully withdraw the impactor tip and set the piston assembly to 2.8 mm, or the desired depth of injury specified in your protocol.
 - 19 Use the remote to actuate the device.
 - 20 Use sutures or another method (e.g. staples; glue) to close the surgical site and apply lidocaine or a similar topical drug to minimize pain.
 - 21 Discontinue the anesthesia and extubate the rat.
 - 22 Loosen the ear bars, remove the teeth from the incisor bar, and lift the animal from the stereotaxic frame.
 - 23 Depending on the experimental goals, monitor any post-operative outcomes of interest such as the righting reflex.
 - 24 Supervise the rat while it recovers from anesthesia to the point that spontaneous locomotion returns.
 - 25 Return the rat to its cage and resume standard housing and husbandry.
 - 26 Continue to monitor the rat for pain and administer analgesic per institutional and federal guidelines for the treatment of laboratory animals and in accordance with your IACUC



protocol.

NOTES

- 27 Note 1: Test fire the device to verify the piston fires freely. Compare the velocity sensor to the device's speed setting to verify consistency.
- Note 2: Use a laryngoscope if necessary, to facilitate cannula insertion.
- Note 3: Exercise care to standardize the size and shape of the craniectomies across test animals.
- Note 4: When verifying the position of the impactor tip, exercise care to not disrupt the dura.