

MAY 05, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.rm7vzb2r5vx1/v1

Protocol Citation: Bruce G. Lyeth Ph. D. 2023. Fluid Percussion Model in rats - Lyeth Lab. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.rm7vzb2r5vx1/v1>

MANUSCRIPT CITATION: Van, KC and Lyeth BG. Lateral (Parasagittal) Fluid Percussion Model of Traumatic Brain Injury. In F. H. Kobeissy, C.E. Dixon, R.L. Hayes & S. Mondello, eds. Injury Models of Central Nervous System: Methods and Protocols. Humana Press: New York, pp. 231-251, 2016.

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

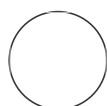
Created: Feb 26, 2023

🌐 Fluid Percussion Model in rats - Lyeth 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 the fluid percussion model in rats.

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

Figure and legend are copyrighted by "In F. H. Kobeissy, C.E. Dixon, R.L. Hayes & S. Mondello, eds. Injury Models of Central Nervous System: Methods and Protocols. Humana Press: New York, pp. 231-251, 2016."

Figure 1. Schematic diagram of fluid percussion injury device (Model 01-B Custom Design & Fabrication). Luer-lock outlet port (1), Luer-lock bubble removal port (2), transducer housing (3), fluid cylinder (4), fill port (5), Piston and O-rings (6), oscilloscope (7), transducer amplifier (8), protractor (9), and impact hammer (10).

Last Modified: May 05,
2023

PROTOCOL integer ID:
77626

Keywords: PRECISE-TBI,
Fluid percussion model, Rat,
Traumatic Brain Injury, TBI,
preclinical TBI model

- 1 Check that the fluid percussion device has been cleaned and calibrated according to the maintenance schedule (see Note 1).
- 2 Examine the fluid percussion device for proper alignment of pendulum striker with the cylinder's piston.
- 3 Perform multiple pendulum drops with outlet closed to detect the presence of any air bubbles in the system - remove air bubbles if detected.
- 4 Place the rat into the anesthesia induction chamber and induce anesthesia using isoflurane (4.0%) in a 2:1 mixture of N2O:O2 for 4 minutes.
- 5 Ensure adequate anesthesia is achieved by use of the toe pinch test or a similar method.
- 6 Intubate the test animal (see Note 2).
- 7 Reduce the isoflurane to the maintenance dose (2.0%); if signs of regaining consciousness are noted (e.g., whisker movement, paw withdrawal from toe pinch), gradually increase the dose.

- 8** Place the intubated rat into the stereotaxic frame and stabilize the head using an incisor bar and bilateral ear bars.
- 9** Place a rectal temperature probe to monitor body temperature and maintain within normal range using feedback heating pad and/or heat lamp.
- 10** Place all physiologic monitoring equipment needed for data collection (e.g., pulse oximeter, etc.).
- 11** Shave the rat's head over the surgical site using hair trimmers.
- 12** Apply antiseptic solution (e.g., betadine) to the scalp using sterile gauze.
- 13** Apply a sterile drape over the rat to maintain aseptic technique, exposing only the surgical site.
- 14** Use a sterile scalpel to make a 20 mm long midline incision on the rat's scalp.
- 15** Use sterile micro dissecting forceps, periosteal elevator, and a cotton-tipped applicator to expose the skull by carefully reflecting the skin, fascia, and muscle.

Use a trephine or drill burr to create a circular bone window (i.e., craniectomy), 4.8 mm in

- 16** diameter (see Note 3). For lateral (parasagittal) fluid percussion center the craniectomy between Lambda/Bregma (anterior-to-posterior) and lateral ridge/sagittal suture (medial-to-lateral). For central fluid percussion center the craniectomy between Lambda/Bregma (anterior-to-posterior) taking care not to rupture the sagittal sinus vein.
- 17** Use micro dissecting forceps to gently lift and remove the bone flap without disrupting the dura mater; discard the bone flap.
- 18** Carefully examine the craniectomy to ensure that the craniectomy is free of any bone fragments on along the ventral edge of the craniectomy. Use a bone curette to carefully remove any bone fragments.
- 19** Drill two burr holes (one anterior and one posterior to the craniectomy using a manual drill (pin vise with #47 drill bit) for placement of two anchoring screws.
- 20** Carefully secure a screw (2:56, 2.1 mm diameter roundhead machine screw) into each burr hole taking care not to extend the tip of the screw below the posterior surface of the skull.
- 21** Apply Super Glue (cyanoacrylate adhesive) to the lip of the injury cap and place into the craniectomy (see Note 4).
- 22** Make a mixture of dental cement (crosslinked flash acrylic) with medium consistency and place around the injury cap and anchor screws using the wood end of a cotton-tipped applicator (see Note 5).
- 23** When the dental cement has cured (hardened; ~10 minutes) insert an extension tube into the female Luer of the injury cap and fill with sterile normal saline. Disconnect the rat from the ventilator and carefully connect the female Luer end of the extension to the fluid percussion device male Luer outlet (see Note 6).
- 24** Release the pendulum to produce the TBI and start a timer (stopwatch).

- 25 Disconnect the rat from the fluid percussion device, remove the extension tube, and place the rat prone on the surgical table. Reconnect the ventilator.
- 26 Unscrew the anchor screws and remove the injury cap-dental cement structure. Use sutures or staples to close the surgical incision site and apply lidocaine or a similar topical drug to minimize pain.
- 27 Discontinue the anesthesia and extubate the rat when spontaneous breathing has reemerged.
- 28 Depending on the experimental goals, monitor any post-operative outcomes of interest such as the righting reflex.
- 29 Closely monitor the rat until spontaneous locomotion returns.
- 30 Return the rat to its home cage and resume standard housing and husbandry.
- 31 Continue to monitor the rat for pain and administer analgesics per institutional and federal guidelines for the treatment of laboratory animals and in accordance with your IACUC protocol.

Notes

- 32 Note 1: Rotate the device's piston to verify that the piston moves freely. Test drop the pendulum several times to verify the correct magnitude of pressure pulse and to verify the absence of air

bubbles in the system.

Note 2: Use a pediatric laryngoscope to facilitate insertion of endotracheal intubation tube.

Note 3: Exercise care to avoid damage to the dura and standardize the location of the craniectomies across test animals.

Note 4: Check the interior of the injury cap to ensure that excess cyanoacrylate adhesive is not obstructing any portion of the craniectomy.

Note 5: Minimize the amount of dental cement applied to the skull. An exothermic reaction occurs as the cement cures which could deleteriously heat the skull and brain if excessive cement is used.

Note 6: Take care not to introduce air into the system when connecting the rat to the fluid percussion device.