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MANUSCRIPT CITATION: Users of the protocol should also review and cite relevant papers from the attached list of papers that use this protocol.

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

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Open-field blast (OFB) model in mice

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PRECISE-TBI

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DISCLAIMER

All methods described here have been approved by the Care and Use of Laboratory Animals of the University of Missouri-Columbia, Missouri University of Science and Technology in Rolla, and by the Truman VA committees.

Use and storage of explosives is governed by the Bureau of Alcohol, Tobacco, Firearms and Explosives (BAFTE) and all training and approvals should be in place before the use.

Use of this protocol is available through the VA's Open Field Blast Core. Email VHACMOOFBC@va.gov for questions.

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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-field blast (OFB) model in mice.

Service members in theater or military training frequently are exposed to primary blast by explosive weaponry. Thus, blast-induced mTBI is the most common form of TBI, regarded as a '*Signature Wound* or *Invisible Injury'* in recent military conflicts. This highly reproducible, open-field low-intensity blast (LIB) injury in mice, the "Missouri Blast" model, uses detonating 350 g of high-energy explosive Composition C-4 (C4). Open-field LIB with C4, set at 1-m above the ground, generates the initial Friedlander waveform with blast rise time on microsecond scale, and includes interactions from the primary blast shockwave reflection off the ground. Comprehensive physical characterization includes the measurements of peak overpressure, blast rise time, positive phase duration, impulse, and velocity of blast waves. High-speed videography is used to capture the dynamic blast events and to ensure the reproducibility of the experimental blast exposures, confirming the absence of visible impact / acceleration on the blast-exposed mice in prone position. This model is scalable and allows study of varying magnitudes of primary blast injuries by placing animals at different distances away from the center of the blast. Overall, this animal model will provide a platform to enhance the understanding of the pathogenesis of blast-induced brain injury and is critical for developing new prevention and treatment strategies against the risk for later neurodegeneration and cognitive impairments. A list of publications using this protocol can be found in the attached document.

The posting of this protocol is part of the mission of PREClinical Interagency reSearch resourcE-TBI (PRECISE-TBI, 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.

ATTACHMENTS

References as of 01062023.pdf

MATERIALS

Equipment:
BAFTE approved blast site and explosives storage
Wire mesh stands for holding animals
High Frequency ICP blast pressure pencil probe for static pressure recording (e.g. PCB Model 137B25)
High Frequency ICP pressure sensor for total reflected pressure (e.g. Model 102B18)
Data Acquisition System (e.g. Synergy Hi-Techniques)
High Speed camera (E.g. Phantom V2012)
Composition C-4 (or other high explosive)
Blasting cap
Capacative Discharge Unit (e.g. Scorpion Blasting Machine)

Materials for anesthetizing:
Ketamine
Xylazine
Syringes (1ml)
Needles (25G)

BEFORE START INSTRUCTIONS

Use and storage of explosives is governed by the Bureau of Alcohol, Tobacco, Firearms and Explosives (BAFTE) and all training and approvals should be in place before use.

Animal Preparation

1 House all animals with *ad libitum* access to food and water, in a temperature-controlled housing room, that is maintained on a 12:12 hour light: dark cycle. (NOTE: The animal unit in accordance with the animal welfare act has been installed away from the blast point ensuring the animals in the sham group were not affected by explosions.)

The open field environment exposes Animals to outside conditions. Hot and cold months should be avoided when planning experiments.

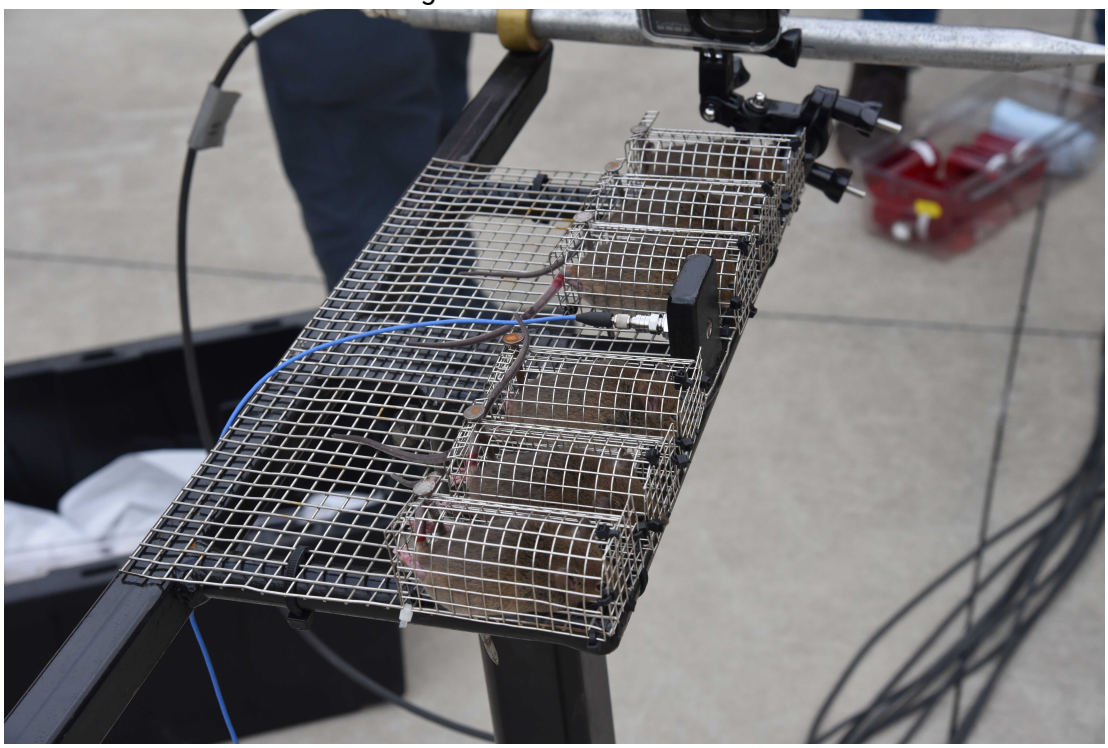
Open-Field Blast Setup

2 The open-field blast experimental site is located within the well-equipped Energetics Research Facility at Missouri University of Science & Technology, on the University Experimental Mine in Rolla.

3 Set the blast conditions for calculated peak overpressure using a blast simulator such as the

Army Blast Effects Calculator (BEC) software or Kingery Bulmash online calculator.

4 Place metal mesh platforms at calculated distances away from the charge stand location allowing the blast waves to travel unimpeded in all directions. Charge stands are made for animals to be 1 meter above the ground.



Mice in prone position placed in metal mesh platforms allowing unimpeded blast wave travel

5 Place incident (static) pressure pencil gauges from PCB piezotronics (137B series) on the side of each metal mesh platform pointed towards the charge to measure changes in pressure with a minimum sampling rate of 1 MHz. Flush-mount (PBC piezotronics 102B series) pressure sensors are placed facing the charge and screwed into a metal plate of similar size to the animal to measure total (static plus dynamic) reflected pressure.

6 Connect sensors to a data acquisition system (DAS), Synergy Hi-techniques, or similar DAS with constant current input option, with coax cables and input calibration data. Connect a break wire trigger to spare channel or trigger from pressure rise. DAS should be in a sheltered location away from the blast site.

7 Setup the high-speed cameras to record the passage of the blast waves and their effects on the animals. The MREL camera is set to 2,000 frames per second (fps) and focused on the animals. The Phantom camera is set to at least 20,000 fps and centered on the animal stand with sufficient surrounding spaces to capture the propagation of the blast waves. The cameras are

triggered by a wire placed around the cap and set to record on a decrease in voltage as the wire breaks during detonation. A short pre-trigger is required due to trigger mechanism. If manually triggering then have a long pre-trigger and triggered after sound is heard.

8 Test all the instrumentation to ensure proper connections and that changes in pressure are observed on the DAS by tapping each sensor.

9 Once all the instrumentation is in the proper position, the anesthetized mice will be ready to place on the platforms (see the section of Induction of Primary Blast Injury).

10 Place high-energy Composition C-4 (C4) explosive in a spherical configuration packed to a density close to 1.6 g/cc and taped for extra confinement 1 meter (m) above the ground for interaction of the incident and ground reflection waves. Reduce the height of the charge to expose animals to only the Mach Stem region if desired.

11 See induction of primary blast injury section

12 After the blasting site is clear of all personnel other than blaster in charge, the electric blasting cap is inserted, with the break wire attached to it with electrical tape. The blasting cap with number 8 strength or higher with the break wire is inserted into the C4 sphere. Once all personnel are accounted for and at a safe distance, the instrumentation can be set to capture mode and C4 can be initiated with a Scorpion capacitive discharge unit.

Induction of Primary Blast Injury

13 Prior to the blast experiment, anesthetize the mouse through intraperitoneal (i.p.) injection of approximately 5 µl/g body weight using a Ketamine/Xylazine mixture (25 mg/mL ketamine and 1.25 mg/mL xylazine) until it is non-responsive to a paw or tail pinch. (NOTE: Blast exposures are conducted between 10 A.M.–12 P.M. during spring and fall seasons at the Experimental Mine). Environmental conditions need to be recorded (temperature, wind speed/direction, and humidity).

14 Place the animals on the positioned metal mesh platform facing the explosive source in a prone position, with their head and body longitudinally oriented along the direction of shock wave propagation.

15 Ensure no remaining personnel are on the blast site.

16 Detonate the C4 explosive.

17 Record the blast dynamic event by pressure gauges and high-speed cameras during the blast exposure.

18 Ensure the blast event is completed. Remove the animals from the metal mesh platform and return to the original cage.

19 Once the animals are able to spontaneously move and recovery from anesthesia complete, continuously monitor them for at least 15 to 30 minutes (min).

Induction of Sham Injury

20 Sham group undergo the identical procedures as the blast group only without blast exposure.

21 Once the animals are able to spontaneously move and recovery from anesthesia, continuously monitor for at least 15 to 30 min.