

A Projectile Concussive Impact Model Produces Neuroinflammation in Both Mild and Moderate-Severe Traumatic Brain Injury

Detailed Data Collection Methods

Animals and Traumatic Brain Injury Procedure

Four month old adult male Sprague-Dawley rats (N=30) weighing approximately 250-300g were purchased from Hilltop Lab Animals, Inc. (Scottsdale, PA, USA). Upon arrival, rats were group housed (2 rats/cage) in a temperature ($21 \pm 1^{\circ}\text{C}$) and humidity ($50 \pm 15\%$) controlled room maintained under filtered positive-pressure ventilation on a 12-h light/dark cycle (lights on 0600 ET) in the CDC-Morgantown Animal Facility and allowed to acclimate for one week prior to the experimental traumatic brain injury (TBI) procedure. Rats were given ad libitum access to food (Teklad 2918 Global 18% rat chow; Envigo, Madison, WI, USA) and water and received daily health checks from animal husbandry personnel. Rats were arbitrarily divided into 3 groups (N=10): Sham, mild TBI, severe TBI.

Prior to TBI using the projectile concussive impact (PCI) model, rats were anesthetized using an E-Z Anesthesia isoflurane vaporizer with a Sure-Seal shoebox induction chamber (E-Z Systems, Palmer, PA, USA) rats were induced with 4-5% isoflurane until unconscious and then the concentration was reduced to 2% for a total of approximately 4 minutes. Following anesthesia, the rat was removed from the induction chamber and immediately placed supine on the PCI device platform with the top of the head placed above the circular opening in the platform and gently secured in place with a rubber band to prevent head rotation/movement prior to the TBI impact. The barrel of the PCI device was aimed approximately equidistant between the eyes and ears (front to back) and centered (left to right). A ball bearing was propelled at the top of the head using 20 PSI of air pressure; mild and severe TBIs were instigated with either an aluminum (mass approximately 0.34 g) or stainless steel (mass approximately 3.47g) ball bearing, respectively. Sham animals underwent all of the same procedures as mild and severe TBI except that no ball bearing was loaded into the PCI device. Following impact, the rats were immediately removed from the PCI device enclosure and returned to their home cage for recovery; the “time to recovery” was recorded for each animal as the elapsed time from the removal from the

anesthesia induction chamber to regaining consciousness in their home cage. Pressure film (Medium) was affixed to the PCI platform by taping one edge over the opening to estimate impact forces. Pressure films were analyzed using the Topaq Pressure Analysis System (Sensor Products Inc., Madison, NJ, USA). The aluminum and stainless steel ball bearings were estimated to produce impact forces of 18.84 ± 3.69 and 181.12 ± 18.51 N, respectively.

Neurobehavioral Severity Scoring

All rats were subjected to the Revised Neurobehavioral Severity Scale (NSS-R) at baseline the day prior to TBI and 1, 6, 24, 48, and 72 hours following TBI, depending on the tissue collection time point. Briefly, performance was scored using a Likert scale of 0 to 2 (where 0 is no impairment and 2 is significant impairment) on a battery of 10 tests including: general balance, landing, tail raise, drag, righting reflex, ear reflex, eye reflex, sound, reflex, tail reflex, and paw flexion reflex. Upon completion of initial baseline testing, it was determined that the rats included in this study did not produce a “normal” response to the ear reflex, tail reflex, or paw flexion reflex tests; these tests were omitted from cumulative scoring. The individual scores on each remaining test were summed to produce a cumulative neurobehavioral score at each time point tested. All rats behaved normally (0 score) at baseline.

Tissue collection

Rats (N=5/group) were euthanized at 6 hours post-TBI by decapitation for the evaluation of cytokine mRNA expression by qPCR. Immediately following euthanasia, whole brains were removed from the skull with the aid of blunt curved forceps. Cortices, hippocampi, striatum, and cerebellum were dissected free hand on a thermoelectric cold plate (Model TCP-2, Aldrich Chemical Co., Milwaukee, WI, USA) using a pair of fine curved forceps (Roboz, Washington, DC, USA). For this study, the cortex was subdivided into 3 pieces by hemisphere: front, middle, and rear. Isolated brain tissue samples were immediately placed on dry ice and stored at -80°C . The remaining rats (N=15 or 5/group) were euthanized at 72 hours post-TBI for histology.

qRT-PCR

The total RNA from the 6 cortical areas, hippocampus, striatum, and cerebellum were isolated using Trizol® reagent (Thermo Fisher Scientific, Waltham, MA, USA) and Phase-lock heavy gel (Eppendorf, AG Hamburg, Germany), and purified using RNeasy mini-spin columns (Qiagen, Valencia, CA, USA). Total RNA (1 µg) was reverse transcribed to cDNA using Superscript III and oligo (dT)12-18 primers (Thermo Fisher Scientific, Waltham, MA, USA) in a 20 µL reaction. Real-time PCR analysis of the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and of the proinflammatory mediators, TNF α , IL-6, CCL2, IL-1 β , leukemia inhibitor factor (LIF), and oncostatin M (OSM), as well as the astrocyte marker glial fibrillary acidic protein (GFAP) was performed in an ABI7500 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) in combination with TaqMan® chemistry as previously described. All PCR amplifications (40 cycles) were performed in a total volume of 50 µL, containing 1 µL cDNA, 2.5 µL of the specific Assay on Demand primer/probe mix (Thermo Fisher Scientific, Waltham, MA, USA), and 25 µL of Taqman® Universal master mix (Thermo Fisher Scientific, Waltham, MA, USA). Sequence detection software (version 1.7; Applied Biosystems/Thermo Fisher Scientific, Waltham, MA, USA) was used to determine threshold cycle (CT) values.