CHIMERA ™ (Closed Head Injury Model of Engineered Rotational Acceleration) Is a Novel and Clinically Relevant Traumatic Brain Injury Model in Rodents

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ABSTRACT

Background: Traumatic brain injury (TBI) is a leading cause of death and disability in developed countries. A major challenge in TBI research is that many common experimental models do not faithfully replicate the biomechanical aspects of TBI in real-life. To address this issue, we have recently developed a rodent TBI model with high precision, reliability and translatability, called CHIMERA ™ (Closed-Head Impact Model of Engineered Rotational Acceleration). It is distinct from existing TBI models in that it delivers precise impact to the intact head in a non-surgical procedure, and allows unrestrained head movement, which facilitates integration of functional and histological outcomes to biomechanical analysis. Objectives: (1) To use CHIMERA ™ to perform mild repetitive TBI in wildtype mice at a single energy level, and characterize the biomechanical, histological and function outcomes. (2) To subject wildtype mice to single TBI at increasing energy levels, and correlate the head kinematic parameters to the behavioral and histological outcomes. Methods: In part (1), adult C57Bl/6 mice received two TBI each of 0.5J impact energy, 24 hr apart. In part (2), a single TBI of 0.1J to 0.7J impact energy was induced. Head kinematics were assessed using high-speed videography (5000 fps). Post-injury neurological outcomes were assessed by loss of righting reflex duration (immediately post-injury) and neurological severity score (1h). Axonal injury was assessed by FD Neurosilver staining (2d). Results and Discussions: In part (1), head kinematic analysis of repetitive 0.5J impacts showed that CHIMERA ™ induced a peak linear head displacement of 49.6 ± 3.5 mm, and a peak angular deflection of 2.6 ± 0.28 rad. Peak linear and angular velocities were 6.6 ± 0.8 m/s and 305.8 ± 73.7 rad/s, respectively. The head experienced peak linear and angular accelerations of 385.1 ± 52 g and 253.6 ± 69.0 krad/s^2, respectively. Injured mice showed significantly prolonged loss of righting reflex, and displayed neurological deficits. Histological analysis revealed diffuse axonal injury at various white matter areas including the optic tract. In part (2), head kinematics of 0.1J to 0.7J impacts were analyzed, and a significant correlation is observed between linear head kinematic parameters with behavior and with histology). Conclusion: CHIMERA ™ is a simple, reliable model of TBI that offers integration of biomechanics with histological and functional assessments.

INTRODUCTION

Traumatic brain injury (TBI) is defined as "an alteration in brain function, or other evidence of brain pathology, caused by an external force"(Menon et al., 2010). TBI is one of the major causes of death and disability worldwide. In North America alone, the annual incidence of TBI is over 3.5 million (Centers for Disease Control and Prevention, 2014, Canada, 2015), resulting in direct and indirect costs of over $60 billion(Finkelstein et al., 2006). Clinically, TBI is classified as mild, moderate, or severe. Apart from devastating acute consequences such as death or disability, TBI is a known risk factor for Alzheimer's Disease (Guo et al., 2000). In addition, mild TBI (mTBI), which until recently was considered to have few long-term consequences, can lead to neurodegenerative diseases such as chronic traumatic encephalopathy (CTE) with repetitive exposure. The chronic effects of repetitive mTBI has been reported by (Martland, 1928) and (Millspaugh, 1937), and recently highlighted in reports involving diagnosis of retired NFL players(McKee et al., 2009). Despite the huge socioeconomical impacts, there is still no cure for TBI.

A major reasons limiting TBI research is a lack of clinically relevant experimental TBI models that mimic human injury pathology (Namjoshi et al., 2013). Clinically, over 75% of all TBI cases are considered mTBI, which does not usually involve the penetration of the skull. Sudden head movement during impact is thought to be the major mechanism in causing tissue damage in mTBI. The rapid twisting and shearing forces that occur to the neurons and axons may result in mechanical tissue stress, leading to primary and secondary injuries. However, most common existing TBI models, such as controlled cortical impact, fluid percussion, or weight drop, do not truly represent the usual mTBI scenarios, as these experimental TBI models involve opening or compressive deformation of the skull. In addition, these models often restrain head motion during impact. For this reason, our laboratory has recently developed a more relevant TBI model called CHIMERA ^{TM} (Closed-head injury model of engineered rotational acceleration)(Namjoshi et al., 2014), which is both pathologically and biomechanically relevant to clinical TBI cases. This manuscript will first discuss the biomechanical and pathological characterization of CHIMERA TBI. It will then further illustrate how integrated analysis of biomechanics and pathologies is made possible through the CHIMERA ^{TM} model.

METHODS

CHIMERA ^{TM} TBI and experiment animals

A detailed description of CHIMERA ^{TM} is published in(Namjoshi et al., 2014). In short, during TBI, the animal is under isoflurane anesthesia and rests in a supine position on the animal bed. The body of the animal is strapped to the animal bed but its head is freely moveable. Its head is aligned to a pneumatic impactor based on crosshair marks on the head plate. Upon triggering the system, a pneumatically-driven piston (with an impactor tip of 5 mm) travels vertically upward and impacts on the mouse head at the parietal region, causing a rotational motion of the mouse head along the sagittal plane. The impact energy can be adjusted by

controlling the air pressure in the compressed air tank. Wildtype C57B/6 mice of 4-month old of age were used in the studies. In the characterization experiments, the mice received two sham injury (isoflurane exposure and restraint only) or TBI each of 0.5J impact energy at 24 hours apart. In the correlation study, the mice received a single TBI of 0.1, 0.5, or 0.7J impact energy.

Biomechanical analysis

Kinematic analysis were performed on head motion captured with a high speed video camera (Q-PRI, AOS Technologies, Switzerland) at 5000 (characterization experiment) or 9000 (correlation experiment) frames per second. Head motion was tracked using two markers – a "cheek" marker achieved by applying a paint or a sticker to the side of the animal head, and a "nose" marker achieved by tying a piece of dental floss around its snout. Video analysis was assisted by the software ProAnalyst (Xcitex Inc, MA). The X- and Y coordinates of the markers were tracked frame by frame and processed with a 400 Hz low-pass Butterworth filter. Velocity and acceleration were determined by discrete differentiation of the position data, and the resultant linear velocity and acceleration were calculated as the magnitude of the X- and Y-components of the cheek marker. The rotation of the mouse head during TBI was determined by measuring the angle between the line joining the cheek mark and the snout mark, and the horizon. The kinematic parameters of the mouse head was scaled to human equivalent using the equal stress / equal velocity approach(Viano et al., 2009) with a λ of 13.8 [λ = (mass of human brain / mass of mouse brain)$^{1/3}$].

Behavioral and histological analyses

Detailed procedures were published in (Namjoshi et al., 2014). In summary, the duration of loss of righting reflex was reported as the interval between isoflurane discontinuation and the time when the animal righted itself. Neurological deficits were assessed based on published protocols(Viano et al., 2009). The higher the score indicates more deficits. Motor performance was assessed using accelerating rotarod. The mice were trained to a baseline of 210 sec before injury. The lower the post-injury performance indicates greater motor deficits. At the designated time points, the mice were euthanized and brain samples harvested after perfusion with ice-cold PBS and heparin. The brains are fixed with 4% paraformaldehyde for 2 days and sucrose protected before cryosection at 40 um. Axonal injury is assessed using Neurosilver staining (FD NeuroTechnologies, MD) and microglia response is assessed using Iba1 immunohistochemistry (Wako, JP), as previously described.

RESULTS

CHIMERA ^{TM} induces mTBI relevant to the clinical scenario

CHIMERA ™ induces reproducible impact to the mouse head with no restriction to head movement (Figure 1). It has precise control of impact energy ($r2 > 0.9996$, Figure 2). To investigate the reproducibility of TBI-induced head motion, two mTBI at 0.5J impact energy were induced to seven mice in two consecutive days. High speed imaging and head kinematic analysis show that CHIMERA ™ can induce reproducible head motions (Figure 3). The average peak linear velocity and acceleration were 6.6 m/s and 385.3 g, respectively, with coefficients of variation (CV) of 5% and 13%, respectively. The average peak rotational velocity and acceleration were 305.8 rad/s and 253.6 k rad/s^2, respectively, with CV of 20% and 21%, respectively. These parameters were then scaled and compared to those of published human mTBI reports. These values were comparable, suggesting that the tissue stress experienced by mice undergoing mTBI by the CHIMERA is comparable to that occurring to human.

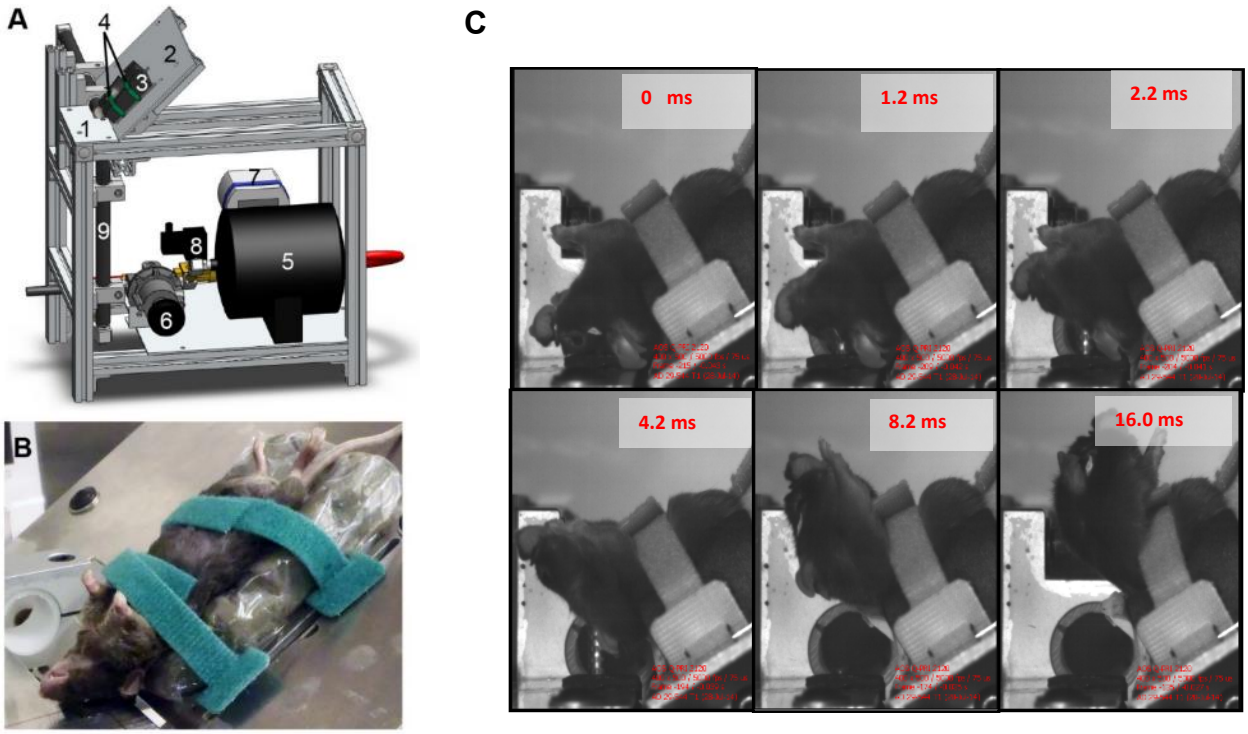


Figure 1 The CHIMERA ™ device induces unrestricted head motion during TBI. (A)The picture depicts the CHIMERA ™ device. Various parts are labeled with numbers as follows: 1. head plate, 2. body plate, 3. animal bed, 4. Velcro straps, 5. air tank, 6. air pressure regulator, 7. digital pressure gauge, 8. two-way solenoid valve, 9 vertical piston barrel. (B) Close-up view of animal strapped on the holding platform. Restraint is applied only to body but not the head. (C) TBI-induced head motion was captured at 5000 fps. Velcro straps were applied to mouse body only, allowing free head movement after impact. The head deflects along the sagittal plane, which subsequently returned to its original position. (Adapted from (Namjoshi et al., 2014))

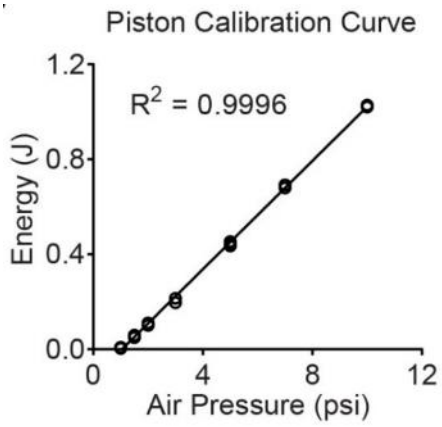
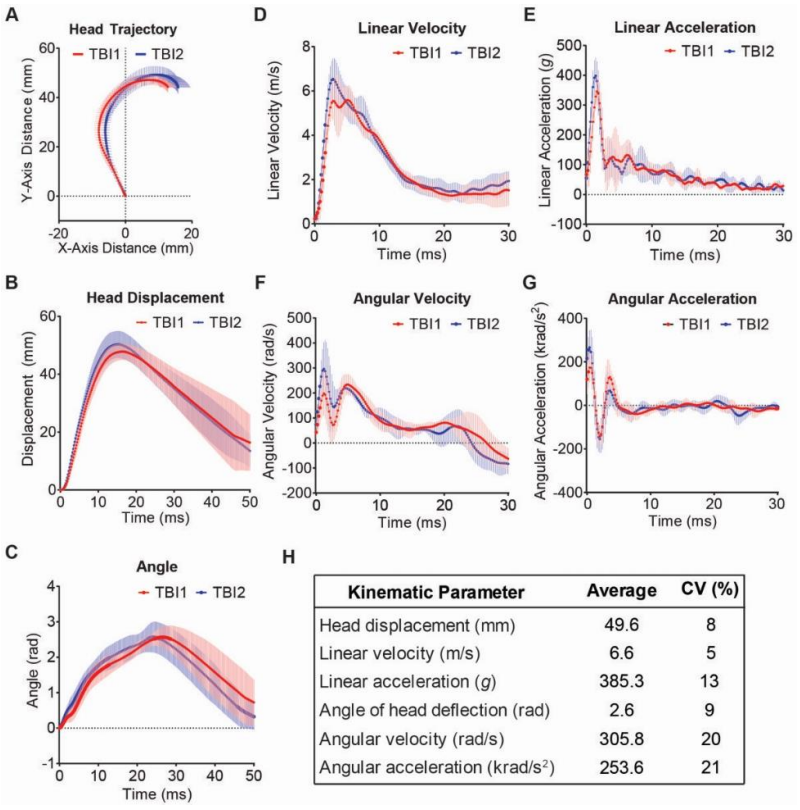


Figure 2 CHIMERA ^{TM} impact energy can be precisely controlled by adjusting air pressure. Air pressure-energy calibration curve was obtained by driving a 50 g piston at increasing air pressure values and calculating the resultant impact energy. The graph depicts three measurements for each air pressure value. (Adapted from (Namjoshi et al., 2014))



Kinematic Parameter	Average	CV (%)
Head displacement (mm)	49.6	8
Linear velocity (m/s)	6.6	5
Linear acceleration (*g*)	385.3	13
Angle of head deflection (rad)	2.6	9
Angular velocity (rad/s)	305.8	20
Angular acceleration (krad/s^2)	253.6	21

Figure 3 Head kinematics during repetitive TBI.
Head kinematic parameters during impacts were assessed in 7 mice subjected to repetitive TBI. Data are represented as the means for each impact. (A) Head trajectory during the maximum acceleration phase in the sagittal plane following impact. (B) Head displacement-time graph following impact. (C) Head deflection is measured as the angle between the snout, side marker

and the horizontal plane. Linear head velocity and linear head acceleration are depicted in (D) and (E), respectively. (F) and (G) show angular head velocity and angular acceleration, respectively. Data in (A) are represented as mean ± 95% CI in both X- and Y- direction, respectively. Data in B-G are represented as mean ± 95% CI. (H) Summary of peak values of kinematic parameters averaged across all 8 rTBI mice. The coefficient of variation (CV) was calculated as the average of day 1 and day 2 peak values from all available recordings. (Previously published in (Namjoshi et al., 2014))

CHIMERA ™ TBI induces clinically relevant behavioral and histological changes

Two mild CHIMERA ™ TBI over two consecutive days in mice induces behavioral and biological deficits that recapitulate those commonly observed in clinical scenarios. Compared to sham-operated mice, the injured mice displayed consistently prolonged loss of righting reflex ($p<0.001$, Figure 4A), which is analogous to loss of consciousness in humans. The injured mice also displayed increased neurological severity score from 1h to 7d post-injury ($p<0.001$, Figure 4B). Histological analysis revealed that TBI induced punctate and fibrous argyphilic structures in various white matter areas ($p<0.001$, Figure 5A and 5C) including the olfactory nerve layer, corpus callosum, and the brachium of superior colliculus, indicating persistent diffuse axonal injury. Iba1 immunohistochemistry revealed that microgliosis occurred at the same white matter areas ($p<0.005$, Figure 5B and 5D). The density of microglial cells increased and the microglial morphology changed to a more hypertrophic and bushy phenotype, suggesting microglial activation at the injured axons.

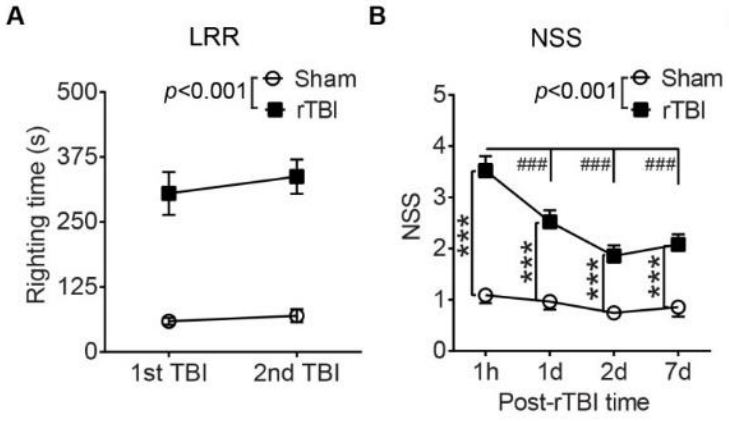


Figure 4 CHIMERA ™ rTBI induces behavioral deficits.
(A) Duration of loss of righting reflex (LRR) was assessed immediately following the sham or TBI procedure. Cohort size: Sham, N = 31; rTBI, N = 39. (B) Neurological severity score (NSS) was assessed at 1h, 1d, 2d, and 7d post-rTBI. Cohort size: Sham (1 h: N = 34, 1d: N = 31, 2d: N = 35, 7d: N = 21); rTBI (1 h, 1d and 2d: N = 42, 7d: N = 25). (Adapted from (Namjoshi et al., 2014))

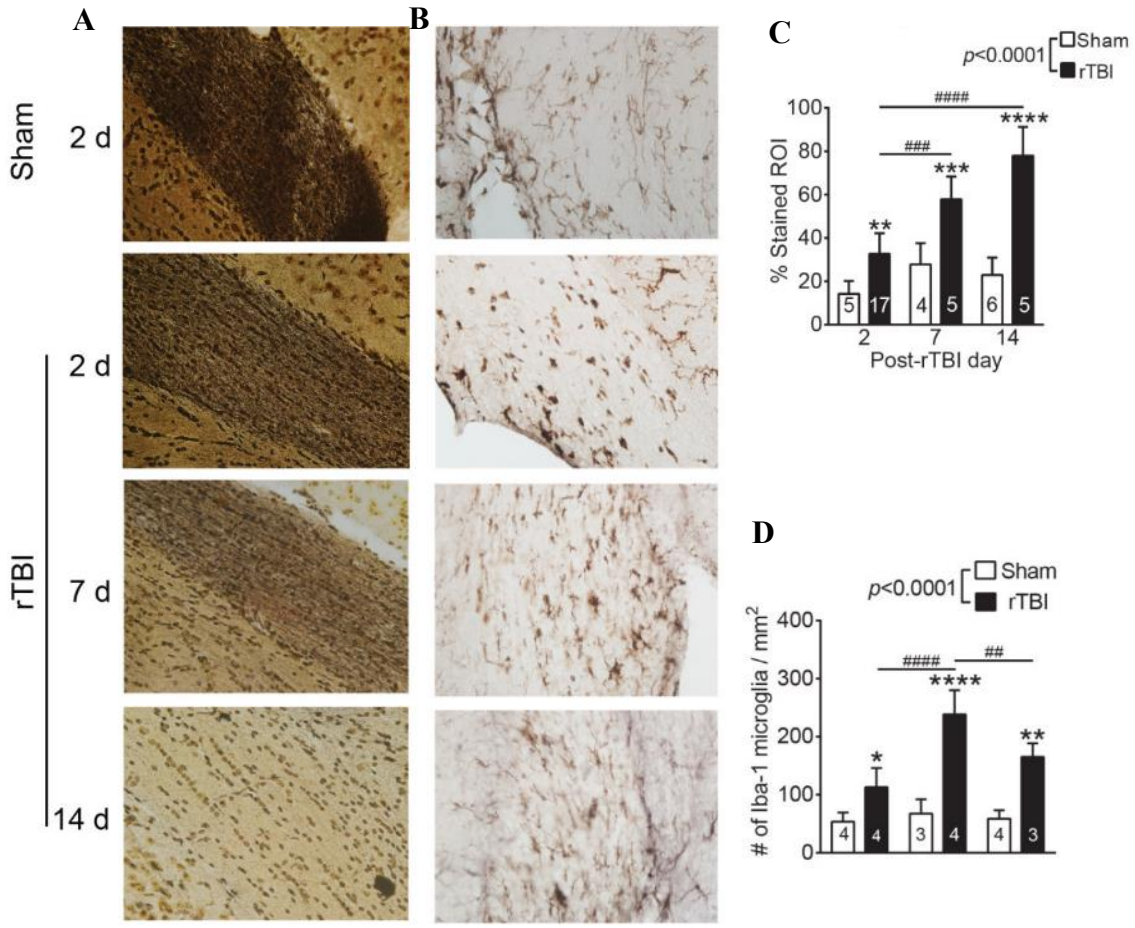


Figure 5 CHIMERA [TM] rTBI induces diffuse axonal injury and white matter inflammation. (A) Axonal degeneration was assessed by silver staining at 2, 7, and 14d post-rTBI. 40X-magnified images of coronal sections showing the optic tract was used as an example of white matter injury. Quantification of % stained area is shown in (C). (B) Microglia were stained using Iba1 immunohistochemsitry at 2, 7, 14d post-rTBI. Optic tract is shown as an example area of white matter microglial activation. Quantification of the density of microglia is shown in (D) (Adapted from (Namjoshi et al., 2014))

Head motion induced by CHIMERA [TM] TBI correlates with behavioral and pathological outcomes

After establishing that impacts by CHIMERA [TM] induces free head motion, and that CHIMERA [TM] TBI induces clinically relevant pathologies, we then proceeded to integrate the biomechanical and pathological analyses, by varying the impact energy of a single TBI and assessing the correlation between head motion and injury outcomes. When impact energy was increased from 0.1J to 0.5J and 0.7J, both the post-injury duration of loss of righting reflex and Day 2 axonal injury were increased (n=2 per group). Most importantly, we observed a significant positive correlation between kinematic parameters and behavioral outcomes: duration of loss of

righting reflex correlated with peak linear head velocity ($p<0.05$ and $r^2 > 0.69$), and trended towards correlating with linear head acceleration (Figure 6A & 6B). Further, the area of axonal argyphilic staining correlates with linear head velocity and linear head acceleration ($p<0.05$ & $r^2>0.78$) (Figure 6A & 6B). A larger study cohort size is being investigated.

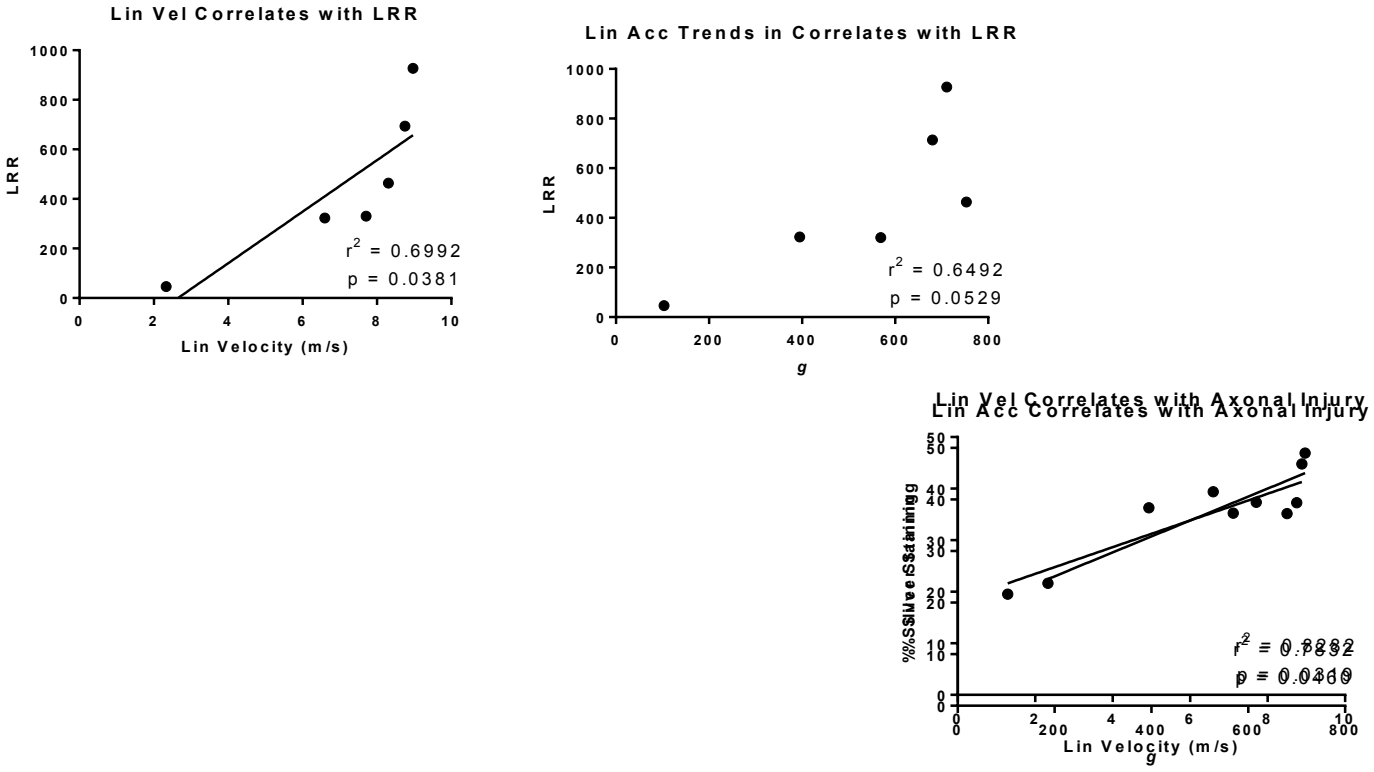


Figure 6 CHIMERA [TM] TBI induces head kinematic parameters that correlate with behavior and histological outcomes.

CHIMERA [TM] TBI impact energy of 0.1, 0.5, and 0.7 J were used. (A) Linear velocity shows a significant correlation with LRR duration. (B) Linear acceleration shows a trend towards correlation with LRR duration. (C) Linear velocity shows a significant correlation with axonal injury at optic tract. (D) Linear acceleration shows a significant correlation with axonal injury at optic tract.

DISCUSSION

Our results show that head motion induced by CHIMERA [TM] correlates with post-TBI neurological deficits and axonal injury. This finding provides further support to the idea that sudden head motion during TBI is responsible for inducing tissue damage and results in behavioral deficits. Head motion is considered a better correlate to injury outcomes than the

impact energy, because even though CHIMERA ™ offers reproducible head impacts, factors such as animal-to-animal variation (e.g. differences in head mass) may act as a confounder to the resultant head motion. Results from this study highlights the importance of biomechanical analyses in TBI research. Future directions involve increasing sample size, examining the effects of more levels of impact energy and investigating how other injury parameters, e.g. the plane of motion, may affect injury outcomes. Rotational kinematic parameters are also being studied. However, as rotational kinematic parameters require more than one track mark, the measurement error will inevitably be greater than that of linear kinematic parameters. Thus an even large sample size may be required to achieve sufficient statistical power to analyze rotational kinematic data.

CONCLUSIONS

CHIMERA ™ is a simple, reliable model of TBI that offers integration of biomechanics with histological and functional assessments.

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