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Short communication

Technique and preliminary findings for *in vivo* quantification of brain motion during injurious head impacts

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ABSTRACT

Computational models of the human brain are widely used in the evaluation and development of helmets and other protective equipment. These models are often attempted to be validated using cadaver tissue displacements despite studies showing neural tissue degrades quickly after death. Addressing this limitation, this study aimed to develop a technique for quantifying living brain motion *in vivo* using a closed head impact animal model of traumatic brain injury (TBI) called CHIMERA. We implanted radiopaque markers within the brain of three adult ferrets and resealed the skull while the animals were anesthetized. We affixed additional markers to the skull to track skull kinematics. The CHIMERA device delivered controlled, repeatable head impacts to the head of the animals while the impacts were fluoroscopically stereo-visualized. We observed that 1.5 mm stainless steel fiducials (~8 times the density of the brain) migrated from their implanted positions while neutral density targets remained in their implanted position post-impact. Brain motion relative to the skull was quantified in neutral density target tests and showed increasing relative motion at higher head impact severities. We observed the motion of the brain lagged behind that of the skull, similar to previous studies. This technique can be used to obtain a comprehensive dataset of *in vivo* brain motion to validate computational models reflecting the mechanical properties of the living brain. The technique would also allow the mechanical response of *in vivo* brain tissue to be compared to cadaveric preparations for investigating the fidelity of current human computational brain models.

1. Introduction

Computational brain models are now ubiquitous with biomechanical investigations of traumatic brain injury (TBI) mechanisms, tolerance, and prevention. The fidelity of computational models to the mechanics of living brain tissue is therefore critical. However, current models are almost exclusively compared to human cadaver brain displacement during head impacts (Hardy et al., 2007, 2001; Zhou et al., 2018) despite studies that have shown the mechanical properties of neural tissue change rapidly after death (Hung and Chang, 1981; Oakland et al., 2006) necessitating adjustments to measured brain mechanical properties applied to models, such as attempted in Kleiven (2007). Preparation of cadaver specimens may also invalidate brain motion data since the effect of re-pressurizing the skull is not known and can potentially introduce gases into the intracranial space (Trosseille et al., 1992).

Studies quantifying motion of living human brain tissue can do so only at very mild, non-injurious levels of head acceleration (Bayly et al., 2005). An experimental TBI animal model is essential for understanding the *in vivo* brain tissue response during injury, and the model must reflect the biomechanics of most human TBI, i.e. a closed head impact (Namjoshi et al., 2013). The Closed Head Impact Model of Engineered Rotational Acceleration (CHIMERA) is an animal model of TBI, operational for mice, rats and ferrets, that reliably induces repeatable unrestricted head kinematics following impact and produces the main pathologies of human TBI (Namjoshi et al., 2017, 2014).

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The objective of this study was to develop a technique to quantify *in vivo* brain motion during injurious head impacts using CHIMERA. The potential for fiducial targets implanted within brain tissue to migrate during a head impact event was also examined.

2. Methods

Three adult male ferrets (5–6 months old, 1.56–1.6 kg) were used in this study, which was approved by the University of British Columbia Animal Care Ethics Committee. An adult male ferret brain size is typically 35 mm anteroposteriorly, 17 mm dorsoventrally and 23 mm laterally with a mass of 8 g (Bakker et al., 2015; Barnette et al., 2009; Hutchinson et al., 2017). For each of these experiments, the following were injected subcutaneously: Buprenorphine (0.04 mg/kg) and Meloxicam (0.2 mg/kg) for analgesia, Glycopyrrolate (0.01 mg/kg) for anticholinergic properties, anti-emetic medication Maropitant (1 mg/kg), antibiotic Ampicillin (20 mg/kg), and Ketamine (6 mg/kg) and Midazolam (1 mg/kg) for sedation. The ferret was anesthetized with isoflurane via face mask and intubated. We monitored the body temperature, blood oxygen and heart activity of the animal. Stereotaxic surgery begun with exposing the cranium and burring three or four holes in the skull through which fiducials were inserted into the brain tissue. Fiducials were either 1.5 mm stainless steel spheres (1 ferret) or neutral density targets (NDTs) which consisted of 1.5 mm stainless steel spheres glued within hollowed 3.2 mm polystyrene foam beads (2 ferrets). To implant the fiducials, a solid 16-gauge needle was inserted into the brain tissue to a predetermined depth at a 45° angle from the dorsoventral axis in the coronal plane and then retracted to form a tract. The fiducial was placed at the top of the tract and pushed through to the desired depth using a flattened-tip 16-gauge needle. One fiducial was implanted through each hole and tract. A form-fitting piece of Gelfoam (Pfizer, New York, NY) was placed onto the brain surface and a quick-setting acrylic resin Cortoss (Stryker, Kalamazoo, MI) was applied to seal the skull. Stainless steel spheres (2 mm diameter) were affixed to the skull with cyanoacrylate and to a 3D-printed polylactic acid (PLA) frame affixed to the animal's canine teeth. These spheres acted as skull reference fiducials. The scalp was sutured and the animal extubated and maintained under isoflurane anesthesia for a minimum of one hour prior to head impacts, allowing the resin to set, cyanoacrylate to cure and the cerebrospinal fluid to replenish (Westerhout et al., 2011).

Each ferret was subjected to 6–10 head impacts using the ferret CHIMERA device (Hutchinson et al., 2018; Namjoshi et al., 2014). The animal was placed in a supine position with the head resting on a 3D-printed PLA interface lined with 2 mm of silicon putty (Environmental Technology Inc., Fields Landing, CA), which distributed the impact load over the dorsal skull surface. A 200 g piston impacted the interface at energies of 17–56 J, causing primarily sagittal plane translation and rotation of the animal head.

Each head impact was visualized with dual image intensifiers and X-ray sources in fluoroscopy mode (85 kV and 7.5 mA), placed approximately 90° from one another (Fig. 1). Two Phantom v12.1 high-speed cameras (Vision Research Inc, Wayne, NJ) recorded the image intensifier screens at 10,000 frames per second. Pretest preparation involved capturing images of perforated metal fixed to the front of each image intensifier (for distortion correction) and images of a calibration cube with 64 stainless steel spheres (3 mm diameter) as calibration points. XMALab software (Brainerd et al., 2010) was used to correct image distortion, calibrate the 3D space defined by the intersecting fields of view, and track the fiducials. Calibration error for all tests was less than 0.3 pixels. This high-speed fluoroscopy system has previously

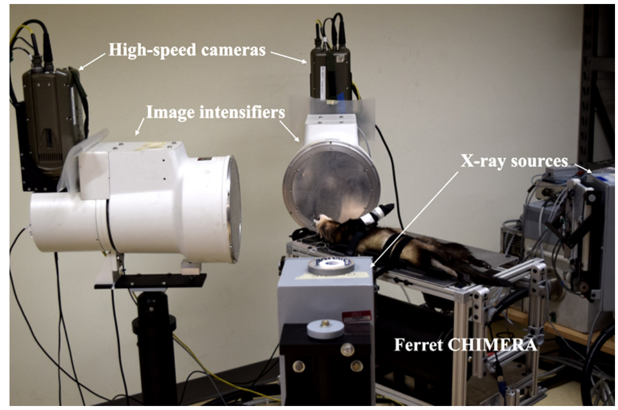

Fig. 1. High-speed fluoroscopy setup and the ferret CHIMERA device.

demonstrated fiducial tracking precision of 0.021 mm and accuracy of 0.019 mm (Lucas et al., 2018).

Following the impact experiments, ferrets were euthanized via Pentobarbital IV and perfused with phosphate buffered saline and 4% paraformaldehyde. Brains were harvested and sectioned to examine the location of fiducials, the surrounding tissue and to identify migration tracks, if present.

Tracked fiducial locations were processed in MATLAB (Mathworks, Inc. Natick, MA). Fiducial motion data were filtered with a 500 Hz cutoff, 4th-order low-pass Butterworth profile. Skull rigid body motion was determined by minimizing error between skull reference fiducial locations at each time step. Rigid body error was calculated as the mean difference between the relative distances of the markers affixed to the skull and less than 350 μm was considered acceptable based on radiostereometric analysis guidelines (Valstar et al., 2005).

Skull kinematics were calculated from skull rigid body motion and numerical differentiation. The position of the fiducial markers implanted within the brain tissue were determined with reference to the skull position to quantify skull-brain relative motion during head impacts in cases without fiducial migration.

In order to quantify brain motion with this technique, it is crucial that the implanted fiducials do not migrate within the tissue. To assess fiducial migration, it was assumed that if the markers returned to the same position relative to the skull after each head impact then they did not migrate. The average and maximum magnitude displacement difference in each pre- and post-impact fiducial position, relative to the skull reference frame, was calculated.

3. Results

During stereotaxic surgery, the ferrets showed sudden, transient episodes of sinus bradycardia which appeared to be in response to cerebrospinal fluid pressure changes, typically when the dura was punctured. One required no further intervention and completed the CHIMERA procedure, while two underwent cardiac arrest. Atropine and epinephrine was administered IV resuscitating one animal that then completed the surgery and TBI procedure with no further complications. One could not be revived, at which time the surgery was stopped, skull sealed and the TBI procedure carried out with the *ex vivo* specimen approximately one hour post mortem.

Fiducial markers were successfully implanted within the brain tissue of ferrets to predetermined coordinates and affixed to the skull and dentition of each animal, (e.g. Fig. 2a). Fiducial locations were tracked during the post-impact trajectory of the supine head (Fig. 2b). Rigid body error for the skull reference markers was

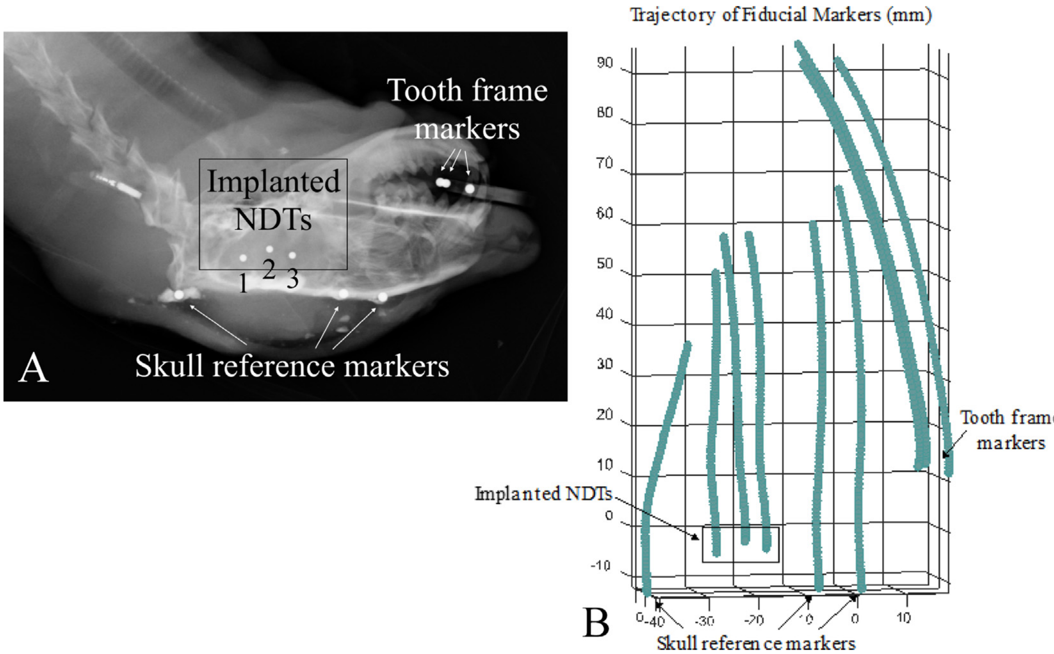

Fig. 2. (A) X-ray image of the skull and dentition reference and implanted brain fiducials (NDTs 1–3) and (B) the tracked trajectory of the fiducials in the laboratory frame of reference, from the bottom to the top of the figure, during a CHIMERA head impact (right). Note the same orientation of the fiducial markers in both figures.

within the acceptable margin for all tests. For an impact energy of 17 J, peak linear skull acceleration was 1103 g and peak rotational velocity 121.6 rad/s, while at 56 J, peak kinematics were 2017 g and 187.8 rad/s.

Average and maximum difference between any pre- and post-impact position of the 1.5 mm stainless steel beads, relative to the skull reference plane, over six impacts was 1.07 mm and 4.47 mm, respectively, suggesting migration of the fiducials from their implanted positions. For NDTs, the corresponding average and maximum was 0.21 mm and 0.62 mm. Inspection of the tissue after sectioning showed further evidence of migration of the 1.5 mm steel spheres within the brain tissue, whereas no fiducial migration was evident for NDTs (Fig. 3).

Given the observed migration of the implanted 1.5 mm stainless steel spheres, their relative motion was not tracked. Since the primary motivation for this study was protocol development, no comparison between NDT motions implanted within the *in vivo* and deceased brain tissue can be made since fiducials were not in identical locations. Preliminary relative skull-brain motion results are described below for one *in vivo* specimen, which exhibited greater displacements at increasing levels of impact energy and peak head kinematics. Trajectories of the NDTs relative to the skull in the sagittal plane for a 17 J and 56 J impact are pictured in Fig. 4. NDT motion lagged that of the skull, first travelling dorsally within the skull as the skull accelerated ventrally before returning towards their initial position after peak skull kinematics.

4. Discussion

To our knowledge, this study is the first to report tracking of fiducials implanted into a living brain in response to impact,

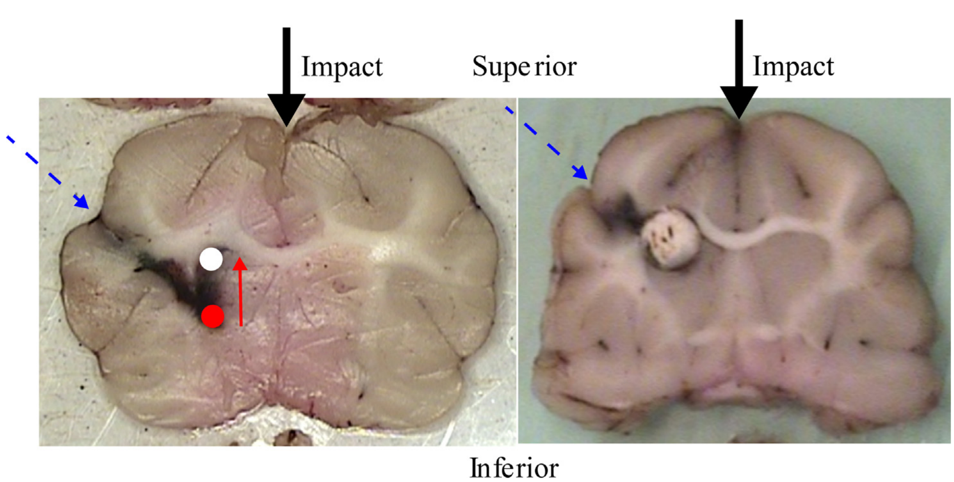

Fig. 3. Coronal sections of the brain showing the 45° angle needle path for fiducial implantation (dashed blue arrows) and the primary direction of impact (solid black arrows). Fiducial migration was noted for the 1.5 mm steel spheres in the left image which shows the initial implanted fiducial location (red circle), final position upon tissue dissection (white circle) and direction of migration opposite the direction of impact (red arrow) while no migration was noted for the NDTs in the right image. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

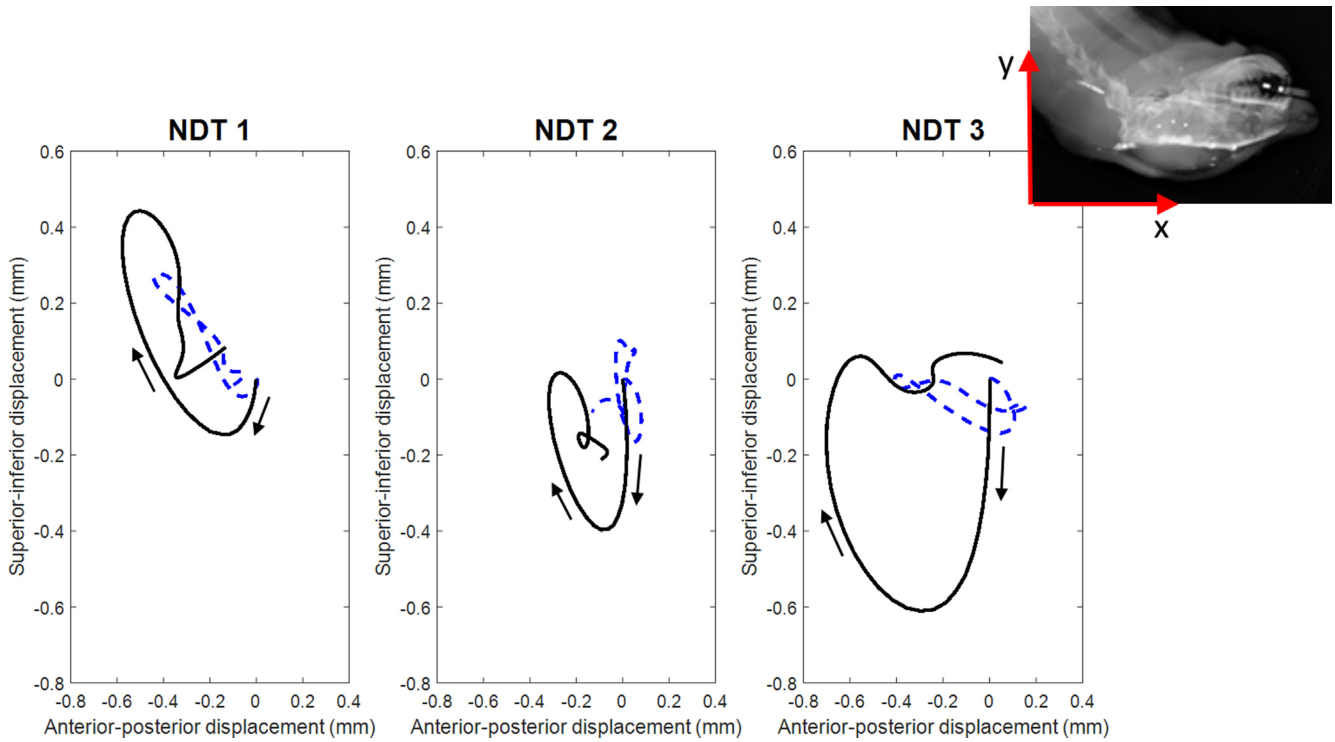

Fig. 4. Sagittal plane trajectories of the implanted NDT 1–3 (shown in Fig. 2) relative to the skull, beginning at the origin, for a 17 J impact (dashed blue line) and 56 J impact (solid black line). The positive × axis is the anterior direction of the head and the positive y axis is the inferior direction of the head, as shown top right. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

demonstrating an experimental technique and anesthetic regime for studying displacement of ferret brain tissue *in vivo*. Importantly, head impacts delivered in this study reflect the closed head injury biomechanics of most human TBI and are injurious, with matched severity impacts in non-surgical animals, performed at another site, exhibiting MRI abnormalities at three hours post-impact (Hutchinson et al., 2018).

As mentioned, current computational models of the human brain have been exclusively validated against cadaveric neural tissue experiments (Giordano and Kleiven, 2016; Kleiven, 2006; Mao et al., 2013). The extent to which the cadaveric brain maintains biomechanical fidelity to living tissue using preparation techniques such as brain and cerebral vasculature perfusion is presently unknown. This question could be answered in the future using the techniques described in this study.

A number of recent studies have examined the effect of implanted target mass on the motion of neural tissue during impact. Added mass of up to 10 times NDT mass to specific nodes of the SIMon brain model and to a spinal cord computational model, increasing the target densities, showed a negligible effect on their displacement during simulated impact experiments (Drake et al., 2017; Lucas et al., 2018). The results of the present study suggest that use of NDTs (without added mass) is necessary for visualizing brain motion for the applied head impact conditions since 1.5 mm steel spheres (~8 times brain density) damaged the tissue and migrated from their position. Simulating a heavier fiducial by increasing point node mass may not account for greater contact pressure and potential for related damage that we observed to the surrounding tissue after impact. Careful examination of the pre- and post-impact positions of the implanted fiducials, as well as inspection of the brain tissue, should be undertaken in studies of this type to ensure no migration is occurring. Tissue inspection is recommended given the assumption that fiducials return to exactly the same position may not be valid, noting pre- to post-impact differences of several millimeters were considered acceptable in human cadaver experiments (Hardy et al., 2001).

Due to its investigational nature, this study is limited by a small sample size but demonstrates a technique ready for implementation in a larger study. Despite small specimen numbers, brain motion was seen to lag behind that of the skull (Fig. 4) in agreement with previous studies that have quantified brain motion (Alshareef et al., 2017; Hardy et al., 2007). The technique detailed here has the advantage of high sampling frequency but is limited by requiring line of sight to each fiducial. Large head accelerations required for injurious impacts to the ferret meant a minimum fiducial size was required for accurate tracking, hence the NDTs are relatively large compared to the ferret brain size (Fig. 3). Since this marker size may affect localized brain motion and limits the number of NDTs implantable in a single specimen, a larger animal model may mitigate these issues. Confirming the quantified *in vivo* brain motion using sonomicrometry (Alshareef et al., 2017), where line of sight is not required, would also enhance the reliability of the data. Tissue damage at implantation tracts is a necessary limitation to techniques quantifying internal brain deformation during injurious head impacts. Future studies might consider whether recovery surgery is possible, allowing for tract healing prior to head impact experiments.

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Declaration of Competing Interest

This work was not commercial in nature and was funded as described in the Acknowledgements above. Author PAC works part time in a consulting company that may benefit from being associated with this study. None of the remaining parties have a conflict of interest with the submitted work.

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