Development of Video Tracking Techniques to Study the Mechanics of Head Injuries and Their Motor Effects

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Abstract

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TBI is the leading cause of disability, chronic disease, and death among the under-40 age group in the developed world. From 2002-2006, the reported incidence of TBI in the United States was almost 600 per 100,000 persons according to the CDC. Up to 80% of these injuries involve closed-head, blunt-force impacts (CDC), however many of our animal models involve open-head injury techniques. Recent models have moved to inducing an injury with a weight-drop device allowing unrestricted motion of the head following impact. This mimics head injuries seen in sports and motor vehicle accidents more accurately, but also leads to variation in the injury induction itself.

The added motion of these models requires quantification of mechanical variation in the animal's head motion post-impact to determine how much mechanical forces play a role in injury outcomes compared to physiological injury mechanisms. Using a high-speed video capture system and tracking software, a technique was developed to analyze the kinematics of animal head motion immediately post impact. Those kinematic variables can be compared to behavioral test data to determine the effects of mechanical forces on this type of injury. The development of this technique also led to the construction and development of a gait analysis system that can give increased resolution to motor deficits experienced by animals post-injury. These types of methods work towards bridging our understanding of the brain's mechanical response to loading and our knowledge of the physiological mechanisms that underlie damage to the tissue itself into mechanical modeling of clinically relevant functional measures. This research is a step towards merging the fields of engineering and neuroscience to take on one of the most complex issues in medicine: traumatic brain injury

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List of Abbreviations

ACUC	Animal Care and Use Committee
BBB	blood-brain barrier
BW	beam walk
CCI	controlled cortical impact
CDC	Centers for Disease Control
CHI	closed-head injury
CHTBI	closed-head traumatic brain injury
FPI	fluid percussion injury
FPS	frames per second
GAITOR	Gait Analysis Instrumentation and Technology Optimized for Rodents
IATBI	impact acceleration traumatic brain injury
IWM	inverted wire mesh

LED	light emitting diode
NIH	National Institutes of Health
NINDS	Nation Institutes of Neurological Disorders and Stroke
NPD	(one-step disinfectant)
PTE	posttraumatic epilepsy
RBM	rigid body motion
TBI	traumatic brain injury

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Chapter 1

Introduction

The study of head injury and its consequences is nothing new, but the physical, emotional, and fiscal burden these injuries place on those who suffer them justify the intense, continued search for answers. For as prevalent an injury as traumatic brain injuries (TBI) are, we are still only scratching the surface of this problem's complexity.
TBI is the leading cause of disability, chronic disease, and death among the under-40 age group in the developed world. From 2002-2006, the reported incidence of TBI in the United States was almost 600 per 100,000 persons (CDC, 2014), (Faul, 2015), (Thurman, 1999). This translated to roughly 1.7 million people per year, and that was only accounting for those who showed up at emergency departments, according to the CDC (CDC, 2014).

From the National Institutes of Health, TBIs include any blunt force, penetrating or acceleration/deceleration event that disrupts the normal functioning of the brain (NINDS, 2019). These injuries have been associated with cognitive dysfunction, neurological degeneration, epileptogenisis and more (Zaloshnja, 2008), (Harrison-Felix, 2006), (Bruns, 2003), (Zhang Q. &., 2003). Even mild TBIs, often classified by little or no loss of consciousness and >24 hours of post injury amnesia, have been shown to have effects on functional skills, such as new learning and memory (Gerberding JL, 2003). To study the clinical outcomes of TBI, researchers have developed a multitude of animal models that aim to mimic the damage caused to the brain during these injuries.

The earliest models consisted of open-head injuries because they allowed for precision location of the injury (Zhang Y. P., 2014), however according to the CDC, approximately 75% of reported TBIs are closed-head injuries (CHI) (CDC, 2014), (NINDS, 2019). These are injuries where the damage to the brain tissue is caused by contact with the inside of the skull as well as acceleration/deceleration of the head. There has been a push to create models that mimic these closed-head, acceleration/deceleration type injuries more closely due to their clinical prevalence (Namjoshi, 2014), (McNamara, 2020). These models typically involve impacting an animal's head and allowing unrestricted motion of the animal post-impact. This scenario inherently introduces variability into the injury induction. Where the early models were precise and repeatable, these models more accurately mimic many human injuries while including a large amount of variation (Xiong, 2013), (Namjoshi, 2014).

These newer models are valuable for their ability to duplicate injuries like those seen in sports and automobile accidents, however researchers must be able to account for the variability in the injury induction to be able to accurately draw conclusions from any results. The development of techniques to measure the mechanical variability of the impacts is essential to ensure that the appropriate measure of variability is attributed to physiological factors. These models involve unrestricted motion of a small animal, and do not allow for penetration of the skull, which limits the techniques that can be used to measure mechanical variation in these types of systems. Due to the force transfer nature of the impacts in these models, the measure of mechanical variation should be calculated from the animal itself, and not from any point on the test rig, for the most accurate results. High speed video tracking methods offer a promising solution to these problems: videography is noninvasive, does not interrupt the animal's motion and allows for tracking to be performed on the animal itself. Video tracking techniques have been employed in other types of biomechanics modeling with success in measuring variations in kinematic parameters (Namjoshi, 2014), (Tagge, 2018). My paper discusses the use of high-speed video analysis to study the kinematics of rodent head motion post-impact. This type of kinematic analysis allows for the study of the physical mechanics of these injuries, which have been found to be very important in the initial stages of the injury. The kinematics data obtained from this type of analysis can also be compared to behavior data to determine links between mechanical aspects of the injuries and clinically relevant outcomes. This type of video kinematic analysis can also be applied to the behavioral testing being performed post-injury, which is discussed here using the example of gait analysis.

Chapter 2

Review of Animal Models of TBI

As discussed above, the adoption of weight-drop models that allow unrestricted motion of an animal's head post-impact introduces mechanical variation into the injury induction. Many of the older, well-established models involve restraining the animal so there is negligible variation in the injuries due to mechanical variables (Xiong, 2013), (Namjoshi, 2014). It is important to note that in a situation as complex as traumatic brain injury, no one model can capture everything of possible interest to an investigator. Even though the models continue to evolve, tried and true methods persist because of their value for testing specific aspects of brain injury. The evolving closed-head models are especially good for modeling injuries seen in sports, motor vehicle accidents, and violent attacks (Xiong, 2013), (Marklund, 2011), (Namjoshi, 2014). These models are also the most interesting from a mechanical engineering perspective.

2.1 Methods

To best understand the body of work that already exists in animal TBI modeling I performed a comprehensive literature search using the advanced search tools of Web of Science. The following keywords were included for the initial search: animal models and brain injury. A Boolean search was used to capture articles containing any, or a combination of, the listed keywords. After removing duplicate articles, that initial search yielded 13,243 articles between the years 2000 and 2021. From that set of articles, I filtered the papers into groups by the type of model used in the experiment, review papers were excluded. The four groups represented in Figure 1 include the following animal models: controlled cortical impact (CCI), fluid percussion injury (FPI), weight drop (with restrained head motion), and impact acceleration models. Impact acceleration models are the most recent models to gain popularity, seen as an adaptation of Marmarou's model (Marmarou A. F., 1994) by Kane et al. from Wayne State (Kane, 2012). These impact-acceleration injury models have gained popularity due to their ability to more accurately replicate the most commonly reported injuries associated with TBI: motor vehicle accidents, falls and assaults (CDC, 2014), (Thurman, 1999), (Xiong, 2013). Figure 1 shows the increasing trend in published articles covering brain injury in each of the models, which coincides with the increase in reported injuries in the CDC statistics as well as the increasing publicity and interest in the science of head injuries. However, the figure also shows that these closed-head impact acceleration models are far from becoming the standard in the field. This may be partly due to the nature of the model, there is high variability associated with the injuries in these studies. It is common to assume identical induction procedures will produce equivalent injuries. This is a safe assumption with many of the other models, where the animal is secured during the injury. The motion that is

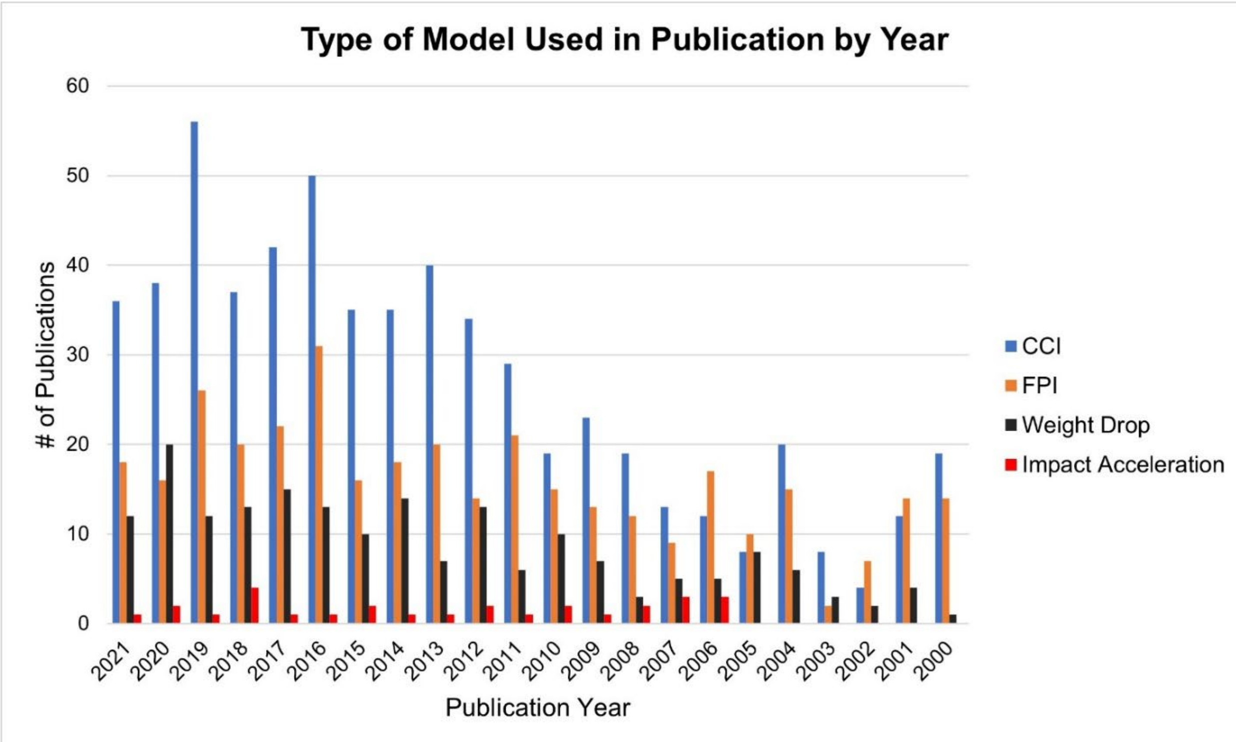

Figure 1: Results from Web of Science literature search based on the keywords: animal model and brain injury. The number of papers each year is further divided into four groups by what type of model was used in the experiment: controlled cortical impact (CCI), fluid percussion injury (FPI), weight drop (with restrained head motion), and impact acceleration.

allowed in the unrestrained models introduces the mechanical variation factor. A brief review of the most common rodent head injury models will help to elucidate the motivation behind my body of work. We will start with the oldest, most well-established models and work towards the unrestrained models. This order reflects the progression in development of rodent models, as labs work to create models that reflect clinically relevant injuries as closely as possible.

2.2 Open-Head Models

Animal models can be largely separated into two groups, open-head and closed-head. These two terms are largely self-explanatory: the former involves injuries where the skull is open and the brain tissue itself is being damaged, while the latter involves injuries where the animal's skull is left intact. There are pros and cons to each type of modeling approach, and as I mentioned earlier, no single model can capture the complexity of traumatic brain injury. Open-head injury models, in general, allow for more accurate targeting of specific brain regions during injury induction (Xiong, 2013), (Marklund, 2011), (Ma, 2019). These models tend to create severe, focal injuries. They help to determine what areas of the brain are associated with functional and behavioral changes when they are damaged (SCHEFF, 1997), (Lee, 2019). They also help to look at specific physiologic mechanisms that happen downstream of the initial injury that lead to long-term deficits and neurodegeneration (Bramlett, 2015), (Xiong, 2013), (Ma, 2019). The Controlled Cortical Impact (CCI) and Fluid Percussion Injury (FPI) models are two of the most used open-head models.

2.2.1 Controlled Cortical Impact Model

The Controlled Cortical Impact (CCI) model is one of the most widely used models in the field of brain injury research. The CCI method involves performing a craniotomy to expose the dura matter over a region of interest. A device (usually pneumatic) is used to drive a rigid tip into the intact dura matter (Dixon C. E., 1991), (Osier, 2016). CCI models have been used to study cortical tissue damage, acute subdural hematoma and even coma (Xiong, 2013). This model owes its popularity to the amount of control investigators have over injury parameters when using a CCI setup. The method requires the animal to be immobilized to perform the craniotomy, then the velocity, depth and dwell time can all be set on the injury induction device (Dixon C. E., 1991), (Osier, 2016), . The CCI model easily has the most mechanical control of any of the other methods, which ensures very little variation in injury induction. However, this model mimics penetrating head injuries which are far less common than closed-head, blunt force injuries (Xiong, 2013). These models also require surgery, which stresses the animals, and anesthesia, which has been shown to have some neurological effects in rodents. These combined factors can reduce the clinical relevance of the results of these studies.

2.2.2 Fluid Percussion Injury Model

Fluid Percussion Injury (FPI) models also involve direct contact to the intact dura. Like CCI models, in FPI models a craniotomy is performed over a region of interest: classically in the midline between the lambda and bregma sutures but has also been adapted to a lateral impact at the parietal bone between the same sutures (DIXON, 1988) (Dixon C. E., 1987), (McIntosh, 1989). FPI models use a type of force transfer method: a pendulum is dropped to inject a volume of saline into the cranial cavity. This creates a pressure pulse that deforms the tissue immediately adjacent to the craniotomy (Dixon C. E., 1991) (DIXON, 1988). FPI systems are less complicated to prepare and less expensive, which makes them a popular choice (Xiong, 2013). These models still allow for some mechanical control, but less than CCI models. The only mechanical control in FPI models is the drop height of the pendulum. This is an easily reproducible measure though, which helps to keep the variation in these injuries low. FPI models face the same issues as CCI models when it comes to relevance. The method requires surgery and anesthesia and though the pressure pulse is meant to act like a blunt force impact, it acts directly on the brain tissue, which makes it less accurate in comparison to actual blunt force injuries.

2.3 Closed-Head Models

The open-head models that were discussed above involve injuring the brain tissue directly. However, CHIs are defined by leaving the animal's skull intact during the injury induction process. According to the CDC, 75% of TBIs every year are classified as CHIs. In those cases, there is no penetration of the skull. Instead, the damage to the brain is caused by blunt forces and subsequent accelerations and decelerations. With the majority of TBIs presenting to emergency departments every year being CHIs, it's clear why these models have become so popular and have been adapted into so many variations. Two of the most common forms of CHI models are piston driven impact devices and weight drop devices.

2.3.1 Piston Driven Impact Devices

Both CCI and FPI models create focal injuries to the brain tissue: areas of localized damage that we would normally associate more with a penetrating injury (Xiong, 2013), (Bodnar, 2019), (Ma, 2019). Piston Driven Impact Devices introduced a way to create a diffuse injury in rodents (Namjoshi, 2014). The method involves opening the animal's scalp and affixing a metal puck to the skull using dental cement (Bodnar, 2019). This puck works like a helmet: it distributes forces applied to it across the animal's skull to prevent a concentrated impact area that would produce a focal injury. Then, a tip of chosen material properties can be driven into the puck at a pre-determined velocity and depth (Bodnar, 2019). These types of diffuse injuries are common in blunt force incidents, where force is commonly applied over a large area of the skull. These injuries don't normally produce skull fractures, but instead are usually characterized by widespread axonal injury (Bodnar, 2019), (Namjoshi, 2014). Spreading the impact force across the puck allows investigators to still use a CCI device, while producing a different type of injury. This method does still involve surgery to place the puck, so the stress of that procedure should be kept in mind.

2.3.2 Weight Drop Impact Models

2.3.2.1 Weight Drop with Restrained Head Motion

One of the most common forms of CHI models in rodents are weight drop models. These experimental setups all involve placing an animal's head under a falling weight to produce a blunt force impact that can be adjusted in severity using the height of the drop. Some of the early weight drop models took aspects of CCI and FPI injury models and adapted them to create a method that excluded the craniotomy and simplified the induction procedure. Originally, these models still involved restraining the animal's head during the injury induction. In the Shohami model, animals' heads are fixed on a hard surface with their skull exposed through a scalp incision before a falling weight delivers a focal blow to one side of the animal's skull (Shapira, 1988). Though this model does leave the skull intact, it has a high rate of skull fracture due to the concentrated force of the blow leaving a focal injury (Xiong, 2013), (Ma, 2019). It was Marmarou et al. that introduced the soft foam surface for the animal's head to rest on during impact. That adaptation created a model for diffuse closed-head blunt force injury. The Marmarou model uses both a helmet-like puck and a soft surface to prevent skull fracture that was seen in the previous models (Marmarou A. F., 1994), (Marmarou C. R., 2009). This model can produce diffuse axonal injury (DAI) in these animals, which is a hallmark of motor vehicle accident and fall patients (Xiong, 2013), (Bodnar, 2019), (Ma, 2019).

2.3.2.2 Impact Acceleration Closed Head Injury

Marmarou's introduction of a conforming surface under the animal's head during impact started a new trend in closed-head modeling. The conforming foam allowed the animal's head to move with the blow briefly upon impact, which is a common attribute of human TBIs: in blunt force injuries the force is often applied when the head is unrestrained (Marmarou A. F., 1994). The mounting evidence that acceleration and deceleration of the brain during these injuries is a factor in the damage to the brain suggests that motion of the head during impact is an important modeling consideration (Meaney, 2014), (Zhang J. Y., 2006). Kane et al. modified the Marmarou model by exchanging the foam for an aluminum foil support (Kane, 2012). They used the same weight drop design, but now when the animal is struck it tears through the foil and falls to a foam pad in a catch box below (Kane, 2012). This new method allows unrestricted motion of the head post-impact, which mimics the type of head motion seen in motor vehicle accidents and sports collisions (Kane, 2012), (Xiong, 2013), (Ma, 2019). This new method also introduces the second impact phenomenon, where the animal is impacted and then falls and strikes the foam pad below in a second impact (Kane, 2012). The adaptations that increase this methods ability to mimic common blunt force injuries also introduce a lot of variability into the model. There can be variation in the tension of the foil, the way the foil tears and the way the animal falls and lands. All these sources of variation can affect the functional and behavioral outcomes of the animals.

2.4 Discussion

No two brain injuries are the same. That incredible variability and complexity makes modeling the problem a significant challenge. The enormous variation in brain injuries led to the development of a vast pool of models that all try to mimic specific portions of the larger whole. Researchers must choose the model that best suits their work based on the goal of their study. Table 1 gives a summary of the models discussed in this work and information that goes into selecting one for a specific study. Open-head models, such as CCI and FPI methods, are better for elucidating outcomes due to damage in very specific parts of the brain (Xiong, 2013). The open-head models tend to be used in studies looking at mechanisms downstream of the initial injury. On the other hand, CHIs are more popular for studying mechanical effects on animal outcomes (Bodnar, 2019). The evolution from the Shohami (Shapira, 1988) to the Marmarou (Marmarou A. F., 1994) to the Kane (Kane, 2012) models demonstrates an effort to create a model that comes closer and closer to mimicking what is seen in a motor vehicle accident or a sports collision. While the impact acceleration models that allow for unrestrained motion of the animal's head after impact look strikingly like something someone might see on the football field, this new method does bring with it some new challenges. The very nature of this new method introduces a great deal of mechanical variation that was not a concern in previous models (Namjoshi, 2014). We have a wealth of evidence now that links accelerations to damage in the brain (Meaney, 2014), (Zhang J. Y., 2006). Accelerations are linked to the extent of tissue damage in brain injuries, and so those mechanical variables must be accounted for in our animal models. There is a need for a method to quantify the kinematic variation of animals' heads in impact acceleration models, so that those parameters can be accounted for when looking at the functional and behavioral outcomes of the animals post-injury. The following chapter will detail the development of a technique that can be used to quantify the kinematics of a rat's head post-impact in an impact acceleration model. The kinematic data can then be used to compare to behavior data from the animals to determine if there is a link between kinematic parameters and clinically relevant measures.

Table 1 - Summary of Animal Models

Model	Injury Type	Pros	Cons	Rats & Mice?	Reported Effects	Kinematics Information Reported?
CCI	Focal	Highly reproducible, tunable, well characterized, accurate targeting	Requires craniotomy, produces a less clinically relevant injury	Yes	contusion, haemorage, sensorimotor and memory deficits, increased anxiety measures, posttraumatic epilepsy	No
FPI						
Midline	Focal	Reproducible, easily tunable, well characterized, inexpensive	Requires craniotomy, high mortality rate, produces a less clinically relevant injury, post-injury seizures	No, only rats	contusion, haemorage, sensorimotor and memory deficits, increased anxiety measures, posttraumatic epilepsy	No
Lateral	Focal, Diffuse	(see above)	This modification has a lower mortality rate	Yes	contusion, haemorage, diffuse axonal injury, sensorimotor and memory deficits, posttraumatic epilepsy	No
Weight Drop						
Feeney	Focal	Closer mechanics to human TBI	Requires a craniotomy, high mortality rate	No, only rats	contusion, haemorage, posttraumatic epilepsy	No
Shohami	Focal	Easy, inexpensive, quick, well characterized, well developed neurological testing	Lower reproducibility, chance of rebound impacts, injuries are severe	Yes	concussion, skull fracture, haemorage	No
Marmarou	Diffuse	Well characterized, similar mechanics to human TBI, lower chance of skull fracture	Lower reproducibility, chance of rebound impacts	Yes	concussion, diffuse axonal injury, sensorimotor and memory deficits, posttraumatic epilepsy (repetitive hits)	Yes
Impact Acceleratio n	Diffuse	Similar mechanics to human TBI, lower mortality, second impact modeling, no rebound injury	Needs more characterization, increased variation	Yes	Concussion, diffuse axonal injury, sensorimotor deficits	Yes

This table was constructed using information from the following sources: (Xiong, 2013) (Ma, 2019) (Dixon, Hayes, & Stefania, 2016) (Marmarou A. F., 1994) (Shapira, 1988) (SCHEFF, 1997) (DIXON, 1988) (Dixon C. E., 1991) (Hallam, 2004) (Lee, 2019) (Tagge, 2018) (Zhang Y. P., 2014) & (SCHEFF, 1997)

Chapter 3

Kinematic Analysis in Free Rotation Closed Head TBI Injury Models Using High Speed Video Tracking

In the previous chapter the field of animal modeling for the study of traumatic brain injury was introduced with a focus on modeling with rodents. As I discussed above, in some closed-head model studies the animal's head rests on a foam pad which relies on consistent mechanical properties of the foam pad to produce reliable injury outcomes (Marmarou A. F., 1994), (Marmarou C. R., 2009). These models also exhibit increased instances of skull fracture and can lead to secondary impact due to rebound of the impactor. Though these models successfully replicate the diffuse damage seen in many real world blunt TBIs, few instances of human TBI involve crushing of the skull and those tend to present a unique set of symptoms. Impact acceleration closed-head models, such as the modified Marmarou model developed at Wayne State, involve unrestricted acceleration/deceleration and rotation that better reproduce the mechanical properties across a spectrum of mild to severe human TBIs (e.g. mild sport impacts to motor vehicle accidents).

The aspect that makes the impact acceleration models appealing, their replication of human motion during blunt force impacts, automatically introduces a new challenge: mechanical variation in the motion of the animal during injury induction. As discussed above, many of the other models restrain the animal's head during the injury or allow very little motion. That eliminates the need to account for any variation in motion of the animal during injury induction. However, in impact acceleration models there is a great possibility for variation in the animals' motion between impacts. That variation must be quantified so that the outcome of the animals can be correctly attributed to physiological and mechanical mechanisms. Methods for quantification of the mechanical variation of these models are not well developed. The task presents a challenge because this type of quantification is more commonly done on larger subjects using sensors like IMUs to measure the kinematics of the subject's motion. Rodent subjects are small for trying to use any sensor technology, and the sensors would likely need to be attached to the animals surgically to ensure they survive the impact intact. Instead, this work introduces a non-invasive video tracking method for quantifying the mechanical variation of animal head motion in rodent impact acceleration models. Analyzing the peri-impact kinematic properties allows us to better understand if and how these mechanical variables are linked to alterations in the gross brain anatomy, cellular and molecular levels, and secondary injury cascades. This TBI device-video analysis combination suite of tools allows researchers to correlate their impact outcomes to human relevant kinematic metrics (linear and angular acceleration) in impact acceleration closed-head animal models using only a high-speed camera (1000 FPS+).

3.1 Methods

3.1.1 Animal Care

All animal procedures were approved by the Animal Care and Use Committee (ACUC) at the University of California Berkeley (AUP-2017-02-9545-1). Male Sprague Dawley rats were purchased from Charles River USA (Wilmington, MA) at 49 days of age and single housed. Cages were maintained at a constant temperature and humidity with a 12-hour light-dark cycle (light 7:00 am to 7:00 pm). All animals were given ad lib access to chow and subsets had their food consumption and body weight taken daily.

3.1.2 TBI Apparatus

A rail-guided force transfer weight drop apparatus (Figure 2A) was used to induce a free rotation CHTBI like the Wayne State modified Marmarou method. A hex bolt rests on the animal's head and transfers energy from the falling weight to the targeted location (Figure 2B). The foil break-away platform was modified by fitting a U-shaped acrylic platform to rest inside of a box with a 2 cm protruding ledge allowing rubber tipped spring clamps to hold consistent tension without obstructing the camera's field of view throughout the post impact events. Perforations were made along the midline of the support foil using a circular saw blade and guide to limit variation in foil scoring and break-away resistance.

Figure 2 - Weight drop injury apparatus A. full side view of weight drop rail B. close view of weight sitting atop stopper with hex bolt pushed through

3.1.3 Injury Induction

Prior to impact, an anesthetic state was induced using 3.5% isoflurane atomized in oxygen at a flow rate of 1 L/min. At 5 minutes animals were removed to mark the fur with a non-toxic water-soluble marker superficial to the proximal edge of the mandible, scapula, and ilium, then returned to the chamber until the continually running timer reached 10 minutes. If breathing rate remained elevated or toe-pinch reflex was present animals were returned for an additional minute. Animals were then quickly moved to the perforated foil platform above a foam pad in a prone position. The bolt was positioned on the animal's head along the midline and aligned with the rat's ears to target between the lambda and bregma sutures. After confirming the toe-pinch reflex had not been regained, the weight was released from the appropriate height. Sham animals underwent the same course of anesthesia and placement on the apparatus with no weight drop. Immediately post impact, animals were returned to a clean cage in the supine position and observed for respiratory distress or convulsions and time to right was recorded.

3.1.4 High Speed Videography and Motion Tracking

Impacts were filmed along the sagittal plane at 1,000fps (X-PRI, AOS Technologies Switzerland) and saved to contain several frames prior to impact and after the animal comes to rest. Frame by frame positional data for the three marked points were analyzed using open-source point tracking software (DLTdv Digitizing Tool, Hedrick Lab-University of North Carolina).

3.1.5 Varying Input Parameters

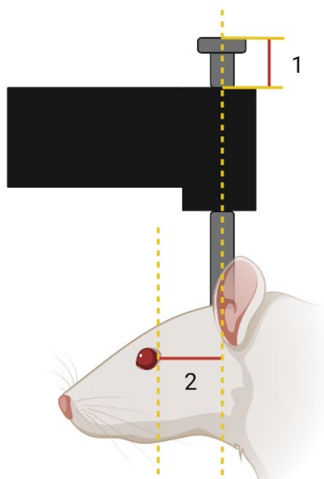

Figure 3 - Diagram showing the measurements for throw and targeting. Bolt throw is labeled 1, from the top of the hex bolt to the top of the stop. The target is labeled 2, from the back corner of the rat's eye to the midline of the bolt.

To demonstrate application of the method to TBI optimization, we systematically changed input variables relevant to the apparatus and compared the resulting impact kinematics. Impacts were administered with combinations of impactor weight (610g, 450g, 305g), bolt throw (the distance available for the impactor to transfer energy to the bolt: 1cm, 3cm, 4.5cm), and drop height (67.5cm, 135cm) to understand the effect of each.

3.1.6 Variation in Targeting and Bolt Throw

Bolt throw was calibrated for each day of impacts. Throw settings were adjusted using a sham animal. A pre-impact frame from each video was chosen and a reference object of known length in the same plane as the animal was used to calibrate pixels to real units of distance using ImageJ. To quantify variation in throw due to differences in animal anatomy, animal placement, and variations in foil platform tension, the distance between the top surface of the bolt and the top of the stopper platform was reported. To measure deviation in targeting on the animal's skull within and between groups of animals receiving the same impact parameters, the distance from the distal corner of the rat's eye to the midline of the bolt was reported (Figure 3).

3.1.7 Kinematics Calculations

The position data from tracking the high-speed videos was used to calculate peak values for linear and angular velocities and accelerations. An in-house Matlab script was used to transform the raw position data in and calibrate it into real units. That data was filtered using a low pass, 3rd order Butterworth low-pass filter. This accounts for noise in video collection and hand tracking of the point positions. Two points on the head (the eye and the mandible marking) were tracked in each video to employ rigid body motion (RBM) principles in the calculations. Any deformation of the skull of the animal was negligible compared to the gross movement of the head during the impact events, making the RBM assumptions valid. The RBM equations were necessary to calculate the angular components of velocity and acceleration. The angle between the two points that were tracked on the animal's head was ultimately used to calculate angular velocity and acceleration. The gradient function in Matlab was used to calculate the 1st and 2nd derivatives of the filtered position data. The x and y components of the position data were combined in a matrix and the gradient function was used to calculate velocity as follows:

[Vx, Vy] = gradient(Position)

The velocity data was filtered again using the same Butterworth settings and then the same gradient function was used to calculate acceleration. The same technique was used for the angular measures, but the derivatives were taken from the angle measurements between the two tracked points.

From the position data, a combination of a linear approximation and numerical derivatives were used to obtain both the linear and angular components of velocity and acceleration. For this method of calculation to produce an accurate approximation, the position must be sampled frequently enough to avoid aliasing. This dictates the required capture rate of the camera used for this method. To highlight this, a single impact was selected to calculate linear and angular acceleration for the duration of post impact events. Values were reported using positional data captured using every frame (1,000fps), every other frame (500fps), every fourth frame (250fps), and every eighth frame (125fps).

3.2 Results

The visual kinematic tracking method described above was used to analyze impact video footage for 39 male Sprague Dawley rats ranging in age from 57-77 days, and ranging in weight from 219g - 594g. The span of input parameters for these impacts was as follows: drop height 67.5cm, 135 cm; drop weight 305g, 450g, 610g; bolt throw 1.2cm - 4.8cm; and target position 0.95cm - 2.0cm. After completing the position tracking of the impact footage, the kinematics analysis produced peak linear and angular accelerations that can be found in the appendix in Table 2 and 3, respectively, grouped by parameters. Our resulting accelerations were in the range reported by Viano et al in their animal model used to study concussions in NFL players. A representative video's post impact motion tracking path is highlighted as a composite montage (Figure 4) with segments from pre-impact to the animal coming to rest. Positional data from each frame, used to calculate kinematic properties, is represented as a tracked point. Instantaneous linear and angular

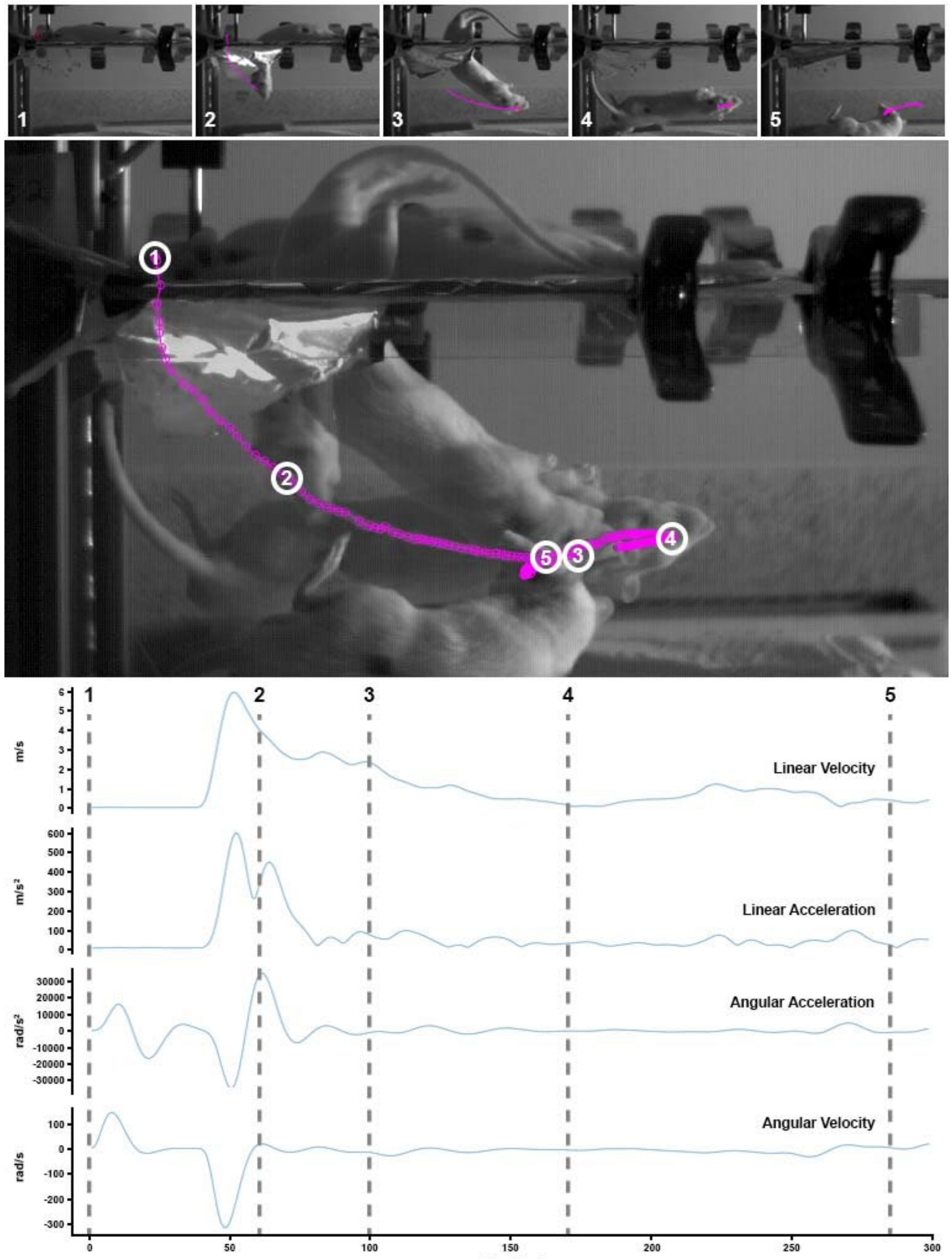

Figure 4 - The stills from the high-speed camera footage above show the path of a single animal during an impact. The colored trace in the five stills is the software tracking of the animal's eye. Below the path trace, the plots of the animal's kinematic data are shown matched to the time sequence of the impact video.

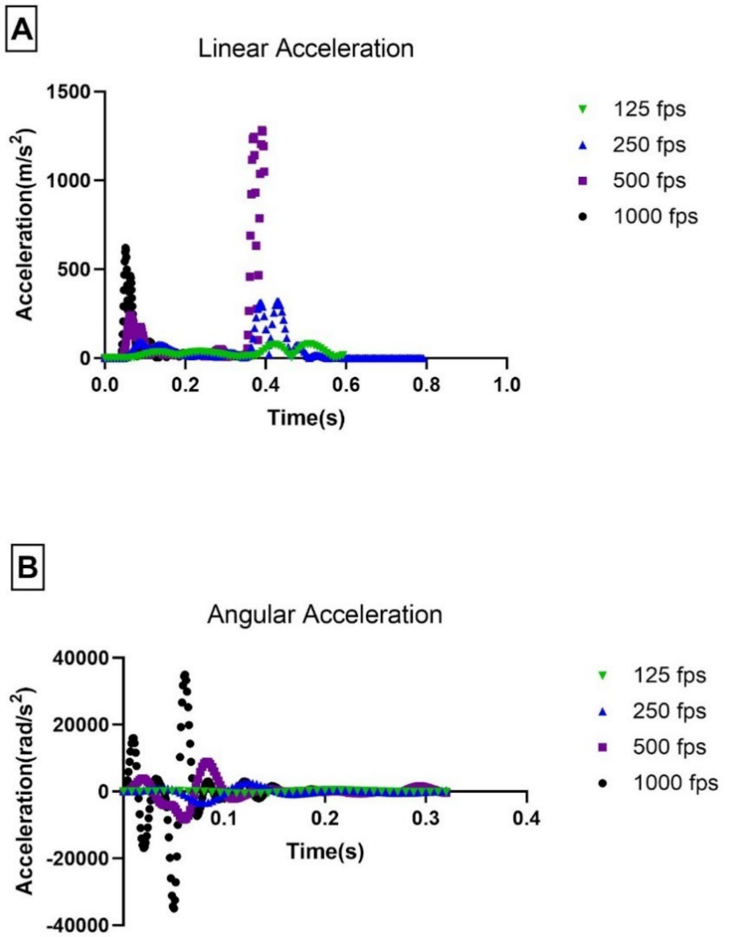

Figure 5 - A-B. Linear and angular acceleration plots for the same animal video processed at different capture speeds to show the importance of frame rate for this method. The slower the capture rate, the less position data the camera can capture, which makes the numerical derivative approximation less accurate. The plots show that low capture speeds stretch the peaks out temporally, shift them to longer times, and create artificial high and double peaks. artificial high and double peaks.

acceleration calculations for each tracked point throughout the impact are shown in Figure 5 for the same video analyzed at 1,000, 500, 250, and 125fps. Figure 5 shows the importance of capture speed to the method, insufficient capture speeds under-sample the motion of the animal and create artefacts like temporal stretching. Previous studies report that these impacts occur in the range of 12ms-15ms, which makes the 1,000fps sampling rate appropriate. The kinematic data revealed variation in the linear and angular acceleration of the animals impacted with the same input parameters (450g, 135cm, 3cm) that can best be seen in Figure 6. The previous assumption that many groups employed, that impact mechanics show no variation among animals, is proven incorrect here. The results from varying input parameters show there is no significant effect on post-impact kinematics for any variable: drop height, drop weight, or bolt throw. We had predicted that mechanical outputs, like the accelerations we're interested in, would increase with increasing drop height and weight. Increasing the bolt throw theoretically provides the system with more distance for the weight to transfer force to the animal's head, so we had predicted that an increased throw would produce higher kinematics values as well. However, in Figure 6 the kinematics results show no significant change in any of the variables when they are adjusted. This suggests that the weight drop system that was used is less predictable in terms of injury severity calibration than previous studies had suggested with their systems. There may be other variables that still need to be controlled or quantified, such as the tension of the support foil. The foil acts as the counterforce when the animal is impacted. It is currently taped in place and scored by hand. The variation in resistance the animal experiences from the foil may be obscuring the effects of changing the input parameters.

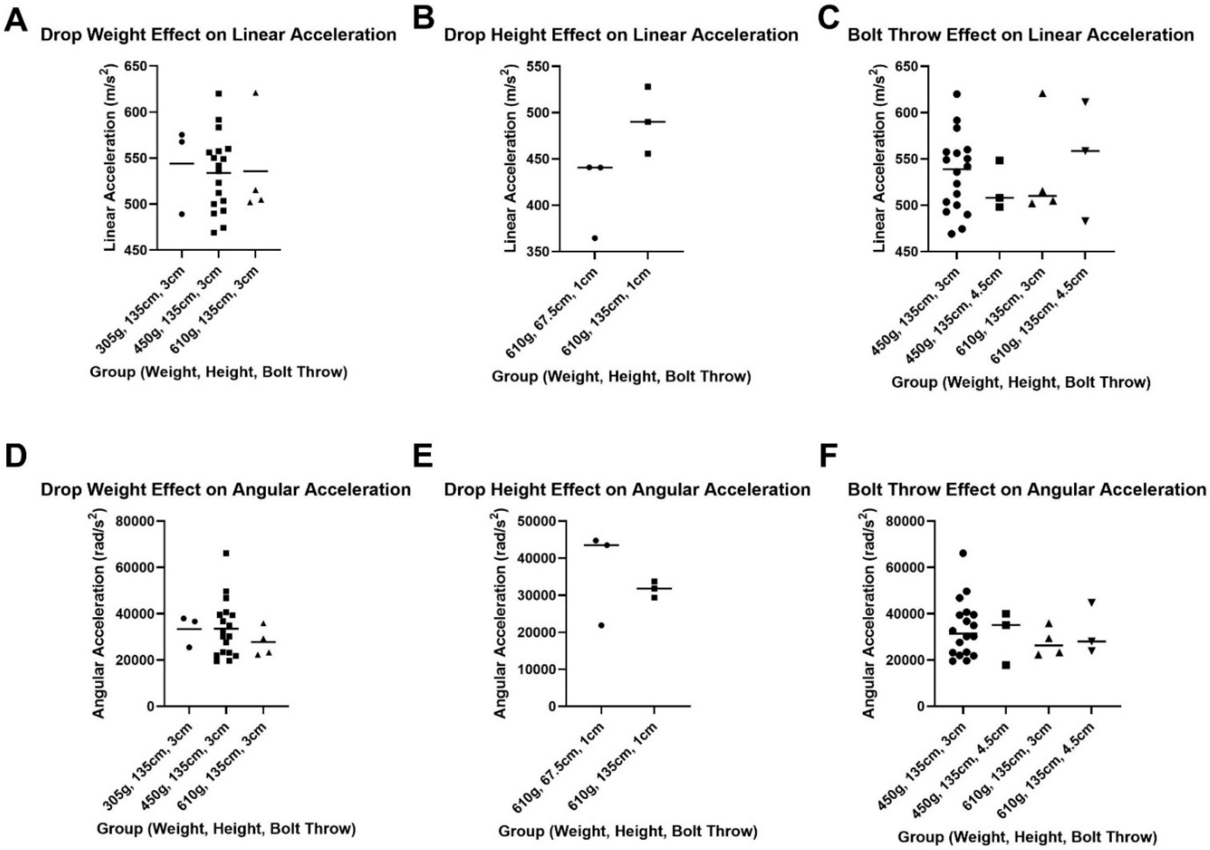

Figure 6 - A-C. Varying input parameters drop height, drop weight and bolt throw A-C. effects on linear acceleration D-F. effects on angular acceleration. There are no significant effects on head kinematics from changing system inputs.

3.3 Discussion

With the increased usage of these unrestrained weight drop models of CHTBI, inquiry into the mechanical variability associated with these models is valuable. It is common practice to assume that using a consistent set of input parameters in these rail-guided drop impact systems produces an identical injury. Further, there has been a prevailing assumption that these rail-guided weight drop devices produce results that are comparable across devices and groups when the same parameters are used. However, the results above show that that may not be the case. This leads to questions about how much the variation in the injuries, from a single device and across devices, affects the results of these TBI studies and how to best account for that variation. The nature of the model design lends itself to variability in impact mechanics - this model was developed to more accurately mimic human CHI, which display a great amount of mechanical variability. The importance of measurements such as linear and angular acceleration in relation to TBI functional outcomes were discussed above. Namely, that higher values of these parameters can be associated with increased functional impairment following a CHTBI. This indicates that measurement of mechanical variability is vital when conducting studies using this type of CHTBI model.

We were able to use a non-invasive imaging technique to measure variation in head kinematics of rats immediately post-impact, which gave us an opportunity to explore some of the common assumptions related to these weight drop systems. Typically, for studies on CHTBI there is no data reported for any mechanical variation. However, Figure 7 shows the existence of that variation and poses the question of how to determine what part it plays in the overall outcome of the injury.

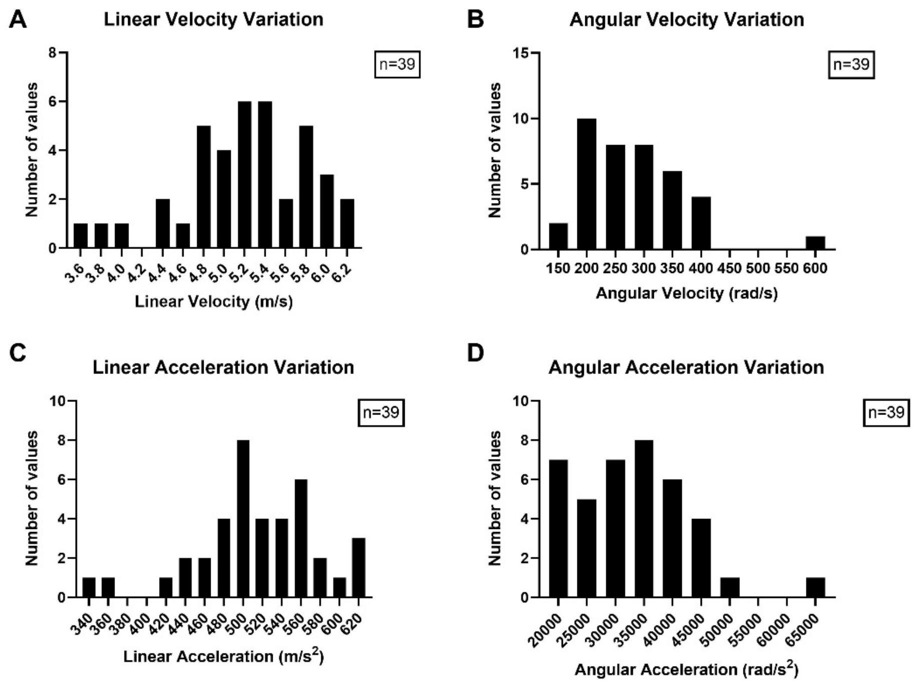

Figure 7 - The variation in linear and angular components of velocity and acceleration for all 39 animals in the study. These plots show the method's ability to capture the variability in the kinematics of the animals' heads during these injuries.

Chapter 4

Linking Kinematic Parameters to Clinical Outcome Measures in Traumatic Brain Injury Models

In the previous chapter a novel method for quantifying mechanical variation in impact acceleration weight drop models of CHTBI in rodents was described. The validation of this method is significant, however being able to link the mechanical data this method can produce to clinically relevant functional outcomes is a powerful application. There has been a separation in the study of brain injury from the perspective of mechanics and that of physiology. Mechanical models have tended to focus on the instant of injury and gross deformation of the brain structures in terms of stresses and strains (Gabler, 2018) (Laksari, 2020) (El Sayed, 2008). These models are often very situationally complex, to mimic real world scenarios, and provide a wealth of data through an abundance of sensors and computation models. The physiological side of the equation has approached the problem using animal models. These models are limited in their complexity and data collection methods due to the live animal component (Xiong, 2013) (Gabler, 2018). However, they have one large advantage over the mechanical models, longitudinal data.

As I mentioned above, mechanical modeling has revealed that certain mechanical parameters, like linear and angular acceleration, are linked to increased damage to the brain in blunt force injuries (Zhang J. Y., 2006) (Meaney, 2014) (Rowson, 2012). This data is not able to directly link those mechanical parameters to functional measures used clinically to determine patient outcomes. The kinematic tracking method presented above provides an opportunity to bridge that gap by obtaining mechanical parameter data from a longitudinal model where animals can be evaluated at several time points to compare functional and mechanical data.

For this study, behavioral testing was used as functional measures to determine if the animals showed a deficit following the injury. In human TBI, behavior changes are also a measure used clinically to determine functional outcome (Bramlett, 2015) (Wilson, 2017). In the case of human TBI the behavioral assessments can be complex and detailed because the testing can be performed orally. However, in animal testing the subjects can not verbally communicate how they feel so we must design tests that take their natural behaviors into account. Then deficits in that behavior are captured clearly by the test method. In this study a modified version of a behavioral battery called the Neurological Severity Score (NSS) (Tagge, 2018) was used to evaluate the animals' behavior before and several times after the impact to determine if those functional measures displayed any changes associated with the single weight drop impact. I was able to take the data from the behavioral testing and compare that with the data from the kinematics data from above to determine if there were any links between the mechanics of impact induction and functional outcome in this model.

4.1 Methods

4.1.1 Animal Care

All animal procedures were approved by the Animal Care and Use Committee (ACUC) at the University of California Berkeley (AUP-2017-02-9545-1). Male Sprague Dawley rats were purchased from Charles River USA (Wilmington, MA) at 49 days of age and single housed. Cages were maintained at a constant temperature and humidity with a 12-hour light-dark cycle (light 7:00 am to 7:00 pm). All animals were given ad lib access to chow and subsets had their food consumption and body weight taken daily.

4.1.2 Behavioral Testing

Beam Walk (BW), and Inverted Wire Mesh (IWM) tasks (Tagge, 2018) were used to evaluate a subset of animals receiving impacts of 450g, from 135cm, and 3cm throw for short term deficits by measuring performance pre, 30min and 24hr post-impact. All tasks were reported as the average and standard deviation of 3 scorers blinded to the animal's condition.

4.1.2.1 Beam Walk (BW)

The BW apparatus is a 3.8 x 128cm textured acrylic beam with an open 20cm x 16.5cm x 14cm (depth x width x height) dark box at one end mounted 90cm above the floor with a tarp draped below the beam to prevent potential fall injuries. For each trial, rats were placed at the end of the beam facing the dark box and given up to 45s to traverse the beam until entering the box or falling. For two days prior to impact rats were trained on the task until they could complete the task in less than 45s without assistance (up to four trials per day). Animals were allowed to remain in the box for 30s as reinforcement after completing the task. Animals were guided to the box for reinforcement if they did not complete the task in the allotted time. Trials were recorded and scorers were blinded to animal status. Time for the animal's nose to enter the box, number of foot faults, partial falls (two limbs off, but recovered), and falls were scored.

4.1.2.2 Inverted Wire Mesh (IWM)

Two acrylic frames with external dimensions of 45cm x 45cm and internal dimension of 35cm x 35cm were fastened by bolts to secure 1.25cm wire mesh for the testing arena. Rats were placed in the center of the wire mesh and once all 4 paws were secure, the arena was inverted by flipping animal head-over-tail over a draped tarp. Time from inversion to release was recorded.

4.1.3 Statistical Analysis

The animals used in the statistical analysis were from the following groups (drop weight, drop height, bolt throw): 450g, 135cm, 3cm; 450, 135cm, 4cm; 610g, 135cm, 1cm; 610g, 135cm, 3cm; 610g, 135cm, 4cm. The total number of animals used in the analysis was 31. The animals that were included were selected because of the completeness of their data sets.

Based on the results in Chapter 3 indicating that the bolt throw had no significant effect on the kinematics data, the animals were separated into two groups: Group 1[450g, 135cm,3_4cm] n=21; Group 2[610g, 135cm, 1_3_4cm] n=10. This separation was done on the suspicion that there would be a significant difference in animal weight between the two groups. This was confirmed with a simple t-test: Group 1 is significantly heavier in terms of animal weight (p=0.006). Based on this, the rest of the analysis was adjusted for animal weight.

Regression analysis was used to determine if relationships exist between any of the head kinematic parameters and behavioral outcomes. The following head kinematic values were used as potential predictive values: peak linear velocity, peak angular velocity, peak linear acceleration, peak angular acceleration, and targeting position.

4.2 Results

T-tests were used to evaluate the differences in all kinematic peak parameters (linear velocity, angular velocity, linear acceleration, angular acceleration), all behavioral scores (pre, 30min, 24hr) and death rate between both groups. There were no significant differences in any measures between the two groups, which confirms that the predetermined inputs to the weight drop system for this study had no effect on kinematic outcomes and likely no effect on functional outcomes either.

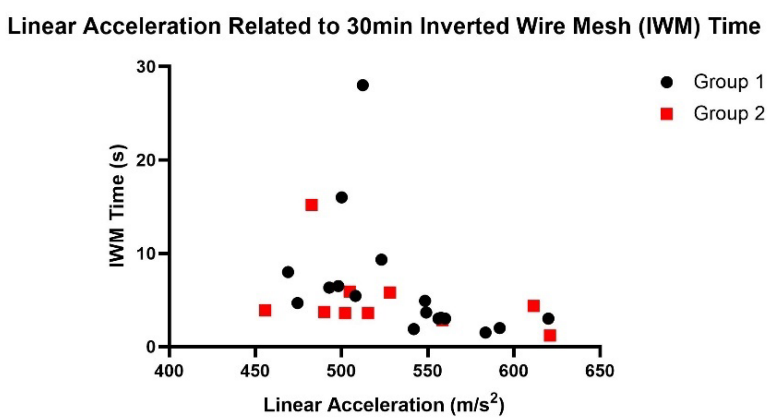

Figure 8 - Single and multiple linear regression show the ability of increased peak linear acceleration to predict poor outcome on the IWM test, as indicated by less time being able to hang on.

Regression analysis revealed several significant predictive relationships between kinematic parameters and behavior outcomes. Univariate linear regression demonstrated that higher peak linear acceleration at impact is predictive of shorter time, poorer performance, on the IWM at the 30min time point (Figure 8). Multiple linear regression was able to detect relationships at both the 30min and 24hr time points. It showed that the 30min BW score is significantly negatively affected by both peak angular acceleration and targeting position. Those same parameters are also significant predictors of slower BW time at the 30min time point (Figure 9). The IWM test at 30min was shown, again, to be affected negatively by peak linear acceleration. The multiple linear regression also revealed predictive relationships at the 24hr mark. Interestingly, both BW outcomes can be predicted to be worse by higher values of angular velocity and acceleration and larger values of targeting position. At the 24hr mark the regression no longer shows any predictive relationship for the IWM test.

4.3 Discussion

These results present a promising step towards bridging the gap between mechanical and physiological modeling of brain injuries. The visual tracking method presented above takes classic methods used in other mechanical testing applications and applies them to an increasingly popular modeling approach in rodent models of brain injury. This mechanical testing approach is a great match for paring with animal modeling because it is non-invasive and is not disruptive of the area where the actual injury is occurring. The confirmation that the kinematics data obtained from the method can be used to predict short term behavioral outcomes in the injured animals is further confirmation of the importance of methods such as this.

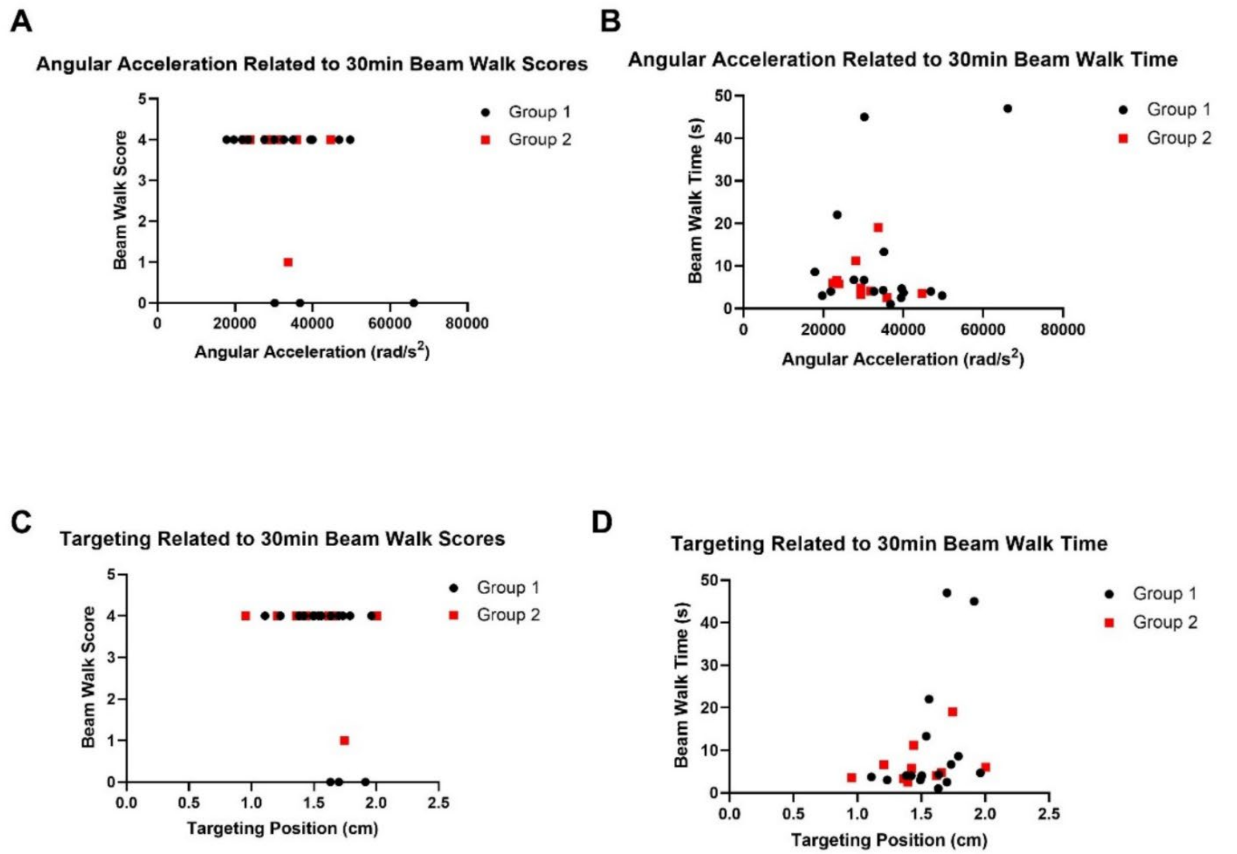

Figure 9 - Predictive relationships between kinematic values and BW test scores and times. Higher peak values of the angular components of acceleration are predictive of poor performance on the BW test in both the score and time measures at the 30min timepoint. Increased targeting location measure is also predictive or poor performance in both BW measures at the 30min timepoint.

It was interesting to see that the statistical analysis seems to confirm that none of the input parameters to our weight drop system seem to have any effect on, not only the kinematic data, but the functional outcomes either. This suggests that further investigation of our system may be needed to validate injury severities in terms of reported variables from other labs. The animals in this study were injured when they were older than intended, which could be a factor in the lack of significance of the input parameters. The predictive relationships with targeting position are not all that surprising because larger values of targeting position indicate the animal is being stuck further toward the back of the skull and brain stem. The ideal target location is midline between the lambda and bregma sutures, however this can be difficult to control with the limited time to position an animal and different investigator placing the animals. This data shows the importance of that value though and indicates the possible need for a design control to cut down variation there. Probably the most interesting take away from the regression analysis is the consistent separation in association between BW and IWM and their respective kinematic predictive values. IWM was shown to be related to the linear kinematic values at 30min in both single and multivariate analysis, where BW was related to angular values at the 30min and 24hr mark in multivariate analysis. The apparent specification of certain kinematic parameters being associated with the outcomes of different behavioral tests is very interesting. This could indicate that the mechanical parameters are associated with different physiological process, which would align with what mechanical models have shown us in terms of damages patterns to brain tissue associated with different mechanical loading. Being able to apply these relationships to design of equipment used to protect someone's head could be revolutionary. Having sensor data about a blow someone took to the head and being able to have some ability to predict what functional changes and the extent of them would be an incredible step in diagnostics. This method could greatly improve our ability to predict patient outcomes and classify the severity of an injury.

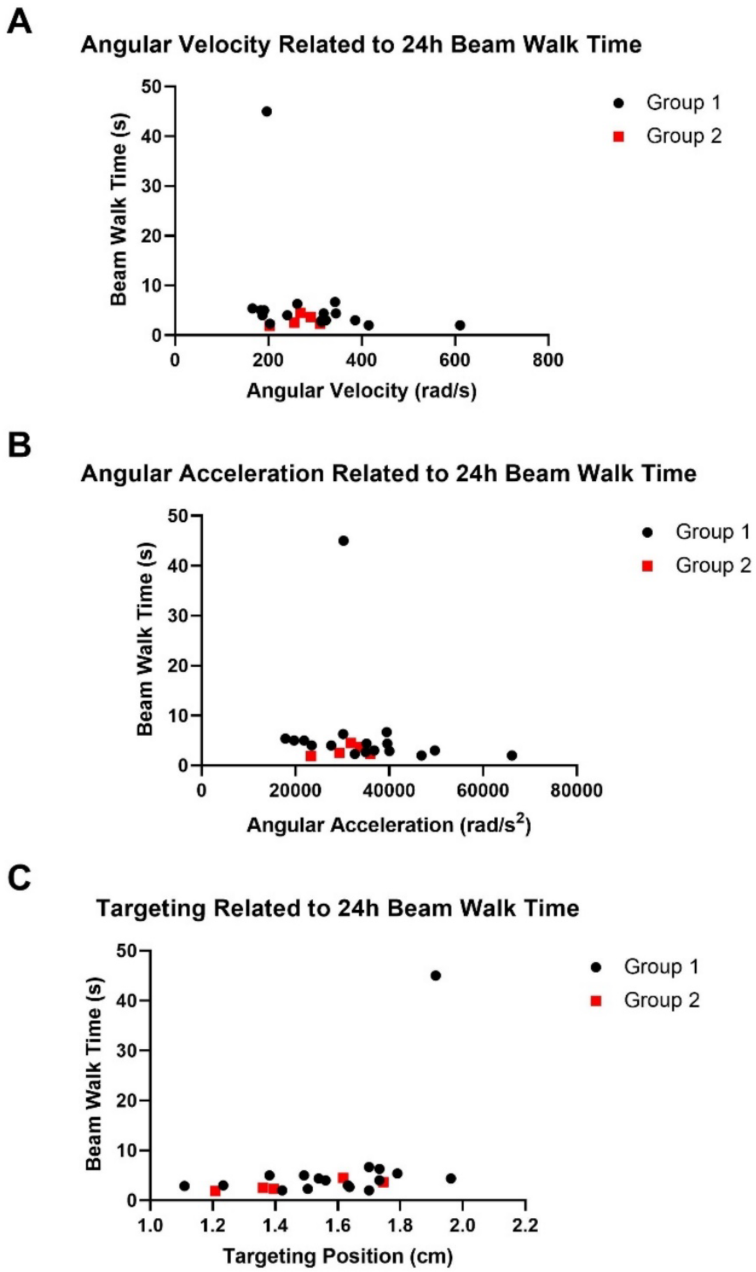

Figure 10 - Predictive relationships between kinematic values and BW test times at the 24hr time point. Higher peak values of angular velocity and acceleration are associated with poor performance on the BW time measure again at the 24hr mark. Again, increased targeting measurements are predictive of poor BW times as well.

Chapter 5

Gait Analysis for Animal Modeling of TBI

Behavioral testing is an important part of TBI science. Behavioral assays are some of the most common tests investigators use to assess changes after an insult to the brain because they are quick to administer, cost-effective and they're relatable to us (Shultz, 2020) (van der Staay, 2006). In the previous chapter I discussed two behavioral assays: the beam walk and the inverted wire mesh test. Those tests both focus more on an animal's motor function: their ability to ambulate, their coordination, as well as their balance and dexterity (Russell, 2011). Those two assays are very popular because they are quick to run and score, and they have been proven to show changes in animals before and after injury (Gibson, 2013) (Hallam, 2004). However, these tests are granular in terms of their scoring and they do not produce significant data to analyze. The beam walk only counts how far the animal makes it on the beam (the BW score) and how long the animal is on the beam (the BW time). The BW score is 0-4, zero being that the animal falls from the beam. With only two measures and the score being so discretized, it is difficult to catch more subtle changes in an animal's behavior after an injury. Though the BW test may catch changes in more severe injuries, it may miss changes in mild or moderate injuries.

Increasing the specificity of the testing method will help to capture the more subtle changes in behavior. One way to accomplish this with motor function is through motion capture gait analysis (Jacobs, 2018) (Kappos, 2017). This method takes principles from the kinematic tracking analysis from Chapter 3 and applies them to a new type of motion. By creating an arena where the animal's sagittal and ventral views can be seen as it walks along a path, we can analyze the position of the feet and limbs during motion. Using open-source machine learning software, we can use the position data from the video tracking to look at different parameters relevant to motor function: stride length and width, duty factor, maximum contact area, etc. (Jacobs, 2018) (Kappos, 2017) (Cheng, 1997) (Deumens, 2007). This is an exciting extension of the video tracking work I discussed above.

5.1 Methods

5.1.1 Animal Care

All animal procedures were approved by the Animal Care and Use Committee (ACUC) at the University of California Berkeley (AUP-2020-11-13859). Male and Female Sprague Dawley rats were purchased from Charles River USA (Wilmington, MA) at 6 weeks of age and housed two per cage. Cages were maintained at a constant temperature and humidity with a 12-hour light-dark cycle (light 7:00 am to 7:00 pm). All animals were given ad lib access to chow and given seven days to acclimate in their home room before any study activities were undertaken.

5.1.2 Gait Walkway Protocol

Rat gait is tested on a linear walkway enclosed with clear, acrylic walls and flooring (6” x 48” x 12”) (Figure 11). This is a modification of the walkway used by Jacobs et al. in their study on rat movement with spinal injuries (Jacobs, 2018). LED lights are placed along the flooring and along a panel that is 3 ft overhead. Animals are positioned at one end of the walkway and removed after either crossing a total of 4 times or 2min has been reached. To record the behavior, a panel of mirrored acrylic is stationed underneath the walkway at a 45-degree angle, and a camera is positioned to capture the whole mirror in frame. Figure 11 shows the gait analysis walkway with LED lights set to red to increase the contrast of the animal’s footprints. Between animals, the walkway and removable barrier are cleaned with NPD. Rats are trained on the walkway by completing two trials each day for two days before the day of the injury. Rats were tested on the gait walkway 30min pre-injury, 30min post-injury and then 24hr, 48hr and 72hr post-injury before being sacrificed for dissection.

Figure 11 - Gait analysis walkway with acrylic arena installed and LED lights turned on. The mirror sitting underneath the walkway is elevated to just under the walkway during testing but is positioned on the floor here for ease of view.

5.1.3 TBI Apparatus

The injury induction system used in this study was a modified version of the force transfer weight drop tower used in the study from Chapter 3. This system uses the same rail-guided weight but incorporates a new stop that keeps the head of the bolt in contact with the weight all the way through the impact. This ensures maximum force transfer. The Friedman lab from Dalhousie University was consulted about the installation of the support foil in this type of model. Their technique for scoring the foil was adopted to achieve consistency in our impacts and between studies (Parker, 2021). The same catch box with foam landing pad from the previous study was used here. The impact parameters were also in accordance with what the Friedman laboratory had produced moderate injuries with in similarly aged rats: 450g weight dropped from 120cm (Parker, 2021).

5.1.4 Injury Induction

For this study all animals were exposed to a single impact, with the parameters listed above, at least seven days after arriving at our facility and before the animals reached ten weeks old. Animals were anesthetized using 3.5% isoflurane atomized in oxygen at 3L/min for induction of unconsciousness and the 1L/min for maintenance. All animals were exposed to 5min of isoflurane, except one male and one female who were used to adjust the bolt throw and had to be exposed to the anesthetic for an extra period before impact. At 5min each animal was removed from the anesthesia box and the toe-pinch reflex was checked before the animal was placed on the support foil and the bolt placed on the animal's head. The target for the bolt was midline between the lambda and bregma sutures for all the animals. The toe pinch reflex was checked once more before the weight was released to strike the animal. Immediately after the impact the animal was placed supine in their home cage with a camera recording their recovery. Animal cages were kept on a heating pad during recovery.

5.1.5 Videography

Animals were filmed on the gait walkway using a GoPro Hero 8 (GoPro; San Mateo, CA). The videos were captured using the 120fps setting with linear field of view. The linear field of view setting is crucial or distortion on the edges of the video will need to be considered before any distances can be analyzed. The camera was placed parallel to the walkway at a distance to just capture the edges of the walkway. The height of the camera was set to capture the foot strike of the rat while also capturing all the paws in the ventral view in the mirror. These videos will need to be cropped in frame and length, as well as thresholded, before the next analysis steps.

5.1.6 Dissection

Upon death each animal was prepared for dissection by shaving the head. Photos were taken of any discoloring or bruising on the skin in the region of the impact. Using a scalpel an incision was made from between the eyes to the rear of the skull. The scalpel was used to separate the skin from the skull. After peeling the skin back photos were taken of any skull fractures or blood below the skull.

5.2 Results

Eight Sprague Dawley rats, four male and four female, were exposed to a single head impact using drop weight and drop height parameters: 450g and 120cm, respectively. Impacts occurred while animals were between 42 and 63 days old. One male died immediately post-impact and one female had to be sacrificed at the end of testing 24hr post-impact due to loss of greater than 30% of body weight.

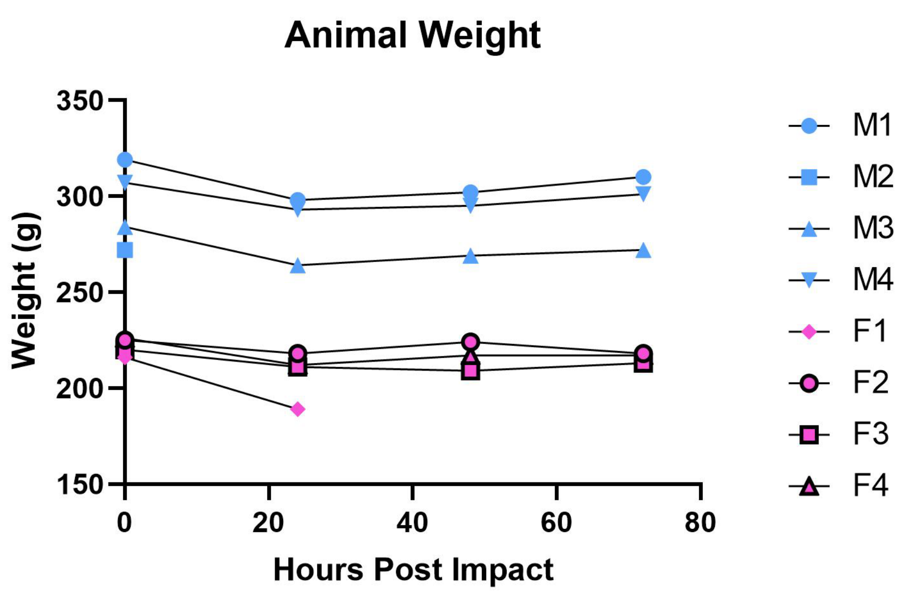

Figure 12 - Animal weight over the course of the study. Weights taken once per day starting the day of impacts and ending 72hrs after.

All animals' weight was tracked starting the day of the impact and continuing through the 72hr study. The weights can be seen in Figure 12. There is a drop in each animal's weight at the 24hr mark. This is consistent with other head injury studies and the drop in weight can be attributed to loss of appetite, dizziness and loss of coordination, and increased fatigue in the animals immediately post-impact. The animals usually recover their weight, which is seen here. The time each animal was exposed to isoflurane and the time for each animal to right itself after impact was compared between the sexes. There was no significant difference in either the exposure time to isoflurane or the time-to-right between the groups (Figure 13).

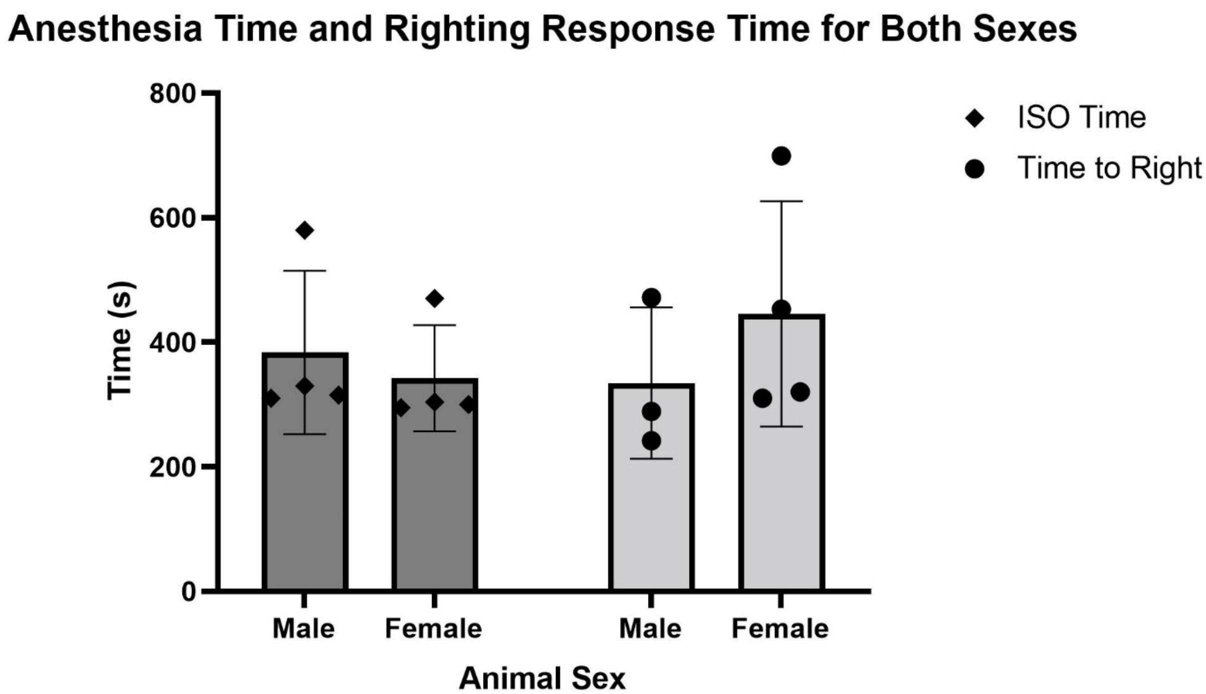

Figure 13 - The isoflurane exposure time and time it took animals to right themselves after impact recorded for each sex. Male 2 died immediately after impact and is therefore not represented on the time-to-right plot. There were no significant differences between sexes for either measurement.

Preliminary analysis has revealed promising indications that the gait walkway will be able to detect changes, such as shifting pressure patterns on the animal's feet when they walk before and after injury. Figure 15 shows still images from unprocessed gait video of the female rat that had to be sacrificed at 24hrs. That animal had a profound motor deficit and will serve as a test case for the continued gait analysis effort.

The dissection revealed that each animal had bruising to the scalp where the bolt had been driven into the animal's head. Under the scalp, all animals also displayed obvious bleeding on the brain even when sacrificed at the 72hr mark. Figure 14 shows the bruising and bleeding documented for each animal. There was no evidence of skull fracture on any of the animals. Note that Male 2 died immediately after impact and Female 2 was sacrificed at the 24hr mark.

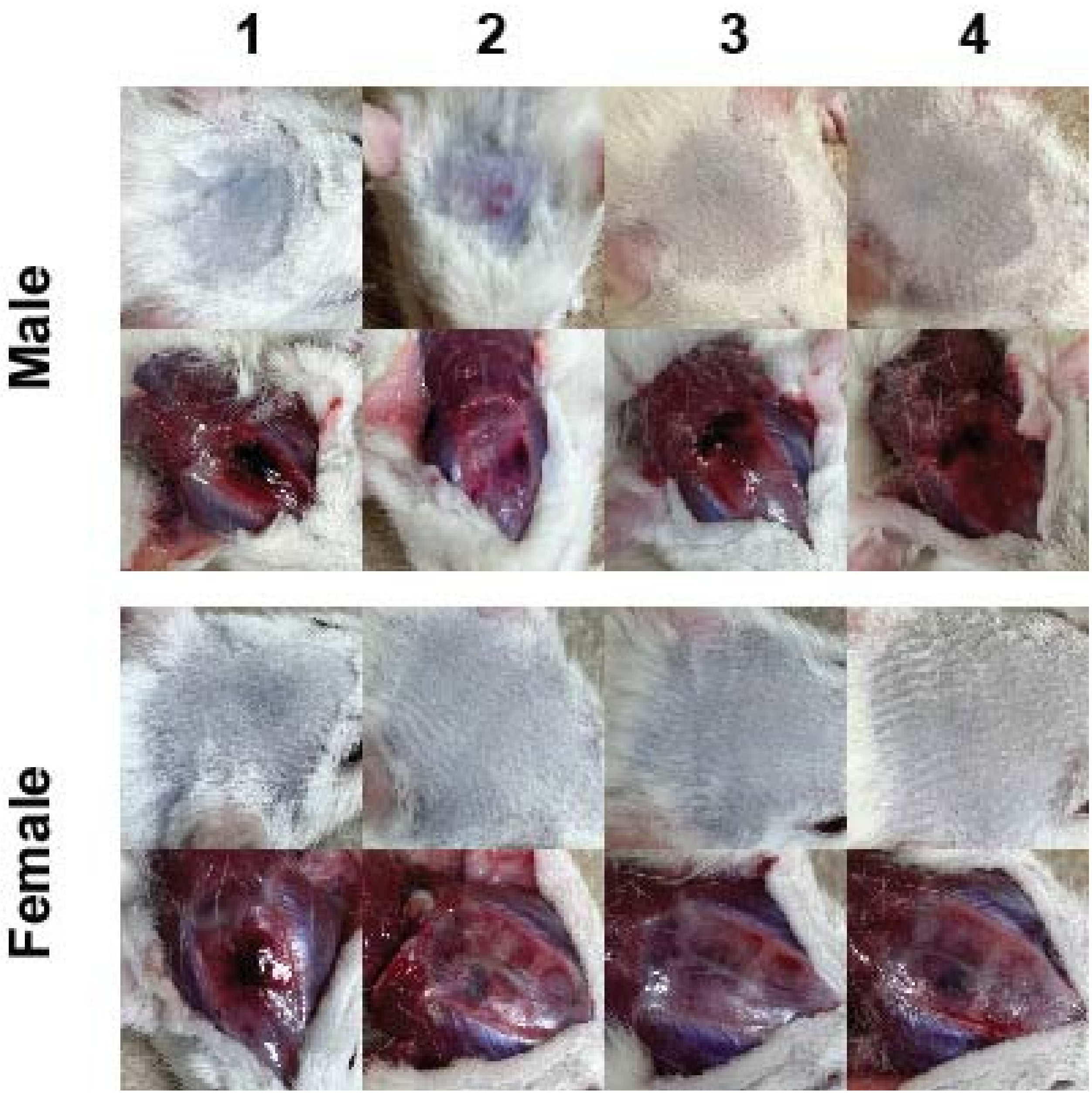

Figure 14 - Images of each animal post-sacrifice. Each animal has evidence of bruising on the scalp where the bolt contacted the head during impact. Each animal also shows evidence of bleeding on the brain. Male 2 was dissected at 30min due to death after impact. Female 2 was dissected after sacrifice at 24hr for significant weight loss. All other animals still show signs of brain bleed at 72hrs.

5.3 Discussion

This study uses both male and female animals, which is uncommon in studying animal models of TBI. Data on female animals in brain injury studies is underrepresented, which makes studies like this one so important. It was interesting that neither sex reached unconsciousness noticeably faster when exposed to the isoflurane. The females were lighter overall, which led us to believe they would need less anesthesia, but that did not

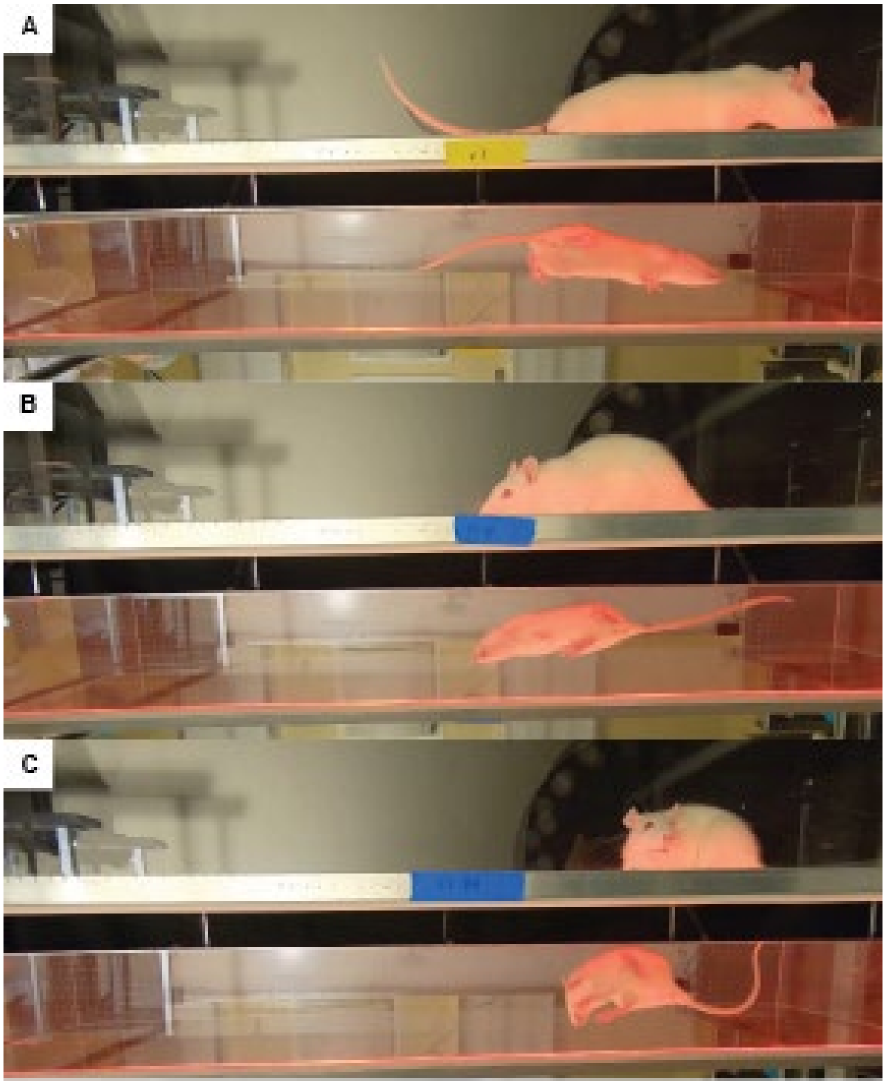

Figure 15 - Raw video footage of Female 1 A. pre-impact B. 3min post-impact and C. 24hr post-impact. This animal was visibly impaired at the 30min mark, stumbling and contacting walls of the arena. The animal was unable to ambulate at all at the 24hr mark and had to be sacrificed. This preliminary data does seem to show agreeance with observations. The pattern of pressure on the animal's feet seems to be distinctly different from video to video, which could indicate loss of balance and coordination.

end up being true. We were also surprised to see that the females did not experience a significantly increased time-to-right. With both sexes exposed to the same injury parameters, we expected the females to experience more severe signs of injury – including increased time-to-right. The animals we tested are in the juvenile stage and males and females develop on different timelines, which is part of the reason an injury like this could affect the sexes differently: different levels of skull ossification, different hormones, etc. In human TBI there has been evidence that females are more susceptible to the effects of brain injury (Gupte, 2019), however in this study that does not appear to be the case.

Another one of the interesting findings of this study was the presence of bruising and bleeding on the brain in each of the animals. In the previous study detailed in Chapter 3, we were attempting to mimic a single moderate TBI based on input and post-impact observations from other studies. In our previous work we had been inducing injury with rats that were 10-11 weeks old, instead of 8-9 weeks. In that phase of the rat's life a large amount of development happens very quickly, including ossification of the skull which can protect the animal from these types of blunt force injury. When we were using the older animals, we had no mortality and no evidence of bleeding on the brain with any of the animals with any of the input configurations that we used. The combination of inputs and support foil technique from the Friedman lab (Parker, 2021) and the younger animals allowed us to replicate the mortality and damage reported by other groups consistent with a moderate injury.

The gait video data is in the preliminary stages of analysis. The footage is in 120fps raw format. That data must be cropped in space and time: the video image must be cut down to only include the rat walking and the walkway underneath and then clips of single passes of the animal across the walkway must be extracted from each video. The clipped videos need to have correction run to remove shadows and glares, then the video can be thresholded to increase the contrast of the animal's footprints. Once the video processing has been completed the GAITOR analysis suite out of the University of Florida's Orthopedic Biomedical Engineering Laboratory (Jacobs, 2018). This analysis tool is a machine learning software that can be trained to track an animal's footprint, tail, nose (ventral) and foot strike (sagittal). From the position data the software has analysis toolboxes to analyze gait parameters such as: duty factor, foot strike times, stride length, step widths and velocity of the animal (Jacobs, 2018). As stated above, the preliminary video data looks promising. The video footage of Female 1 that had a profound motor deficit at the 24hr mark shows a visible change in the pattern of pressure from the pre-impact test to the 30min test and the 24hr test (Figure 15). In the 30min test the animal's weight looks to have shifted to the animal's toes, indicating loss of balance. This was confirmed by visual stumbling and contact with the side of the walkway during the 30min test. Hopefully the more complex analysis will provide data to confirm those observations.

Chapter 6

Conclusions and Future Directions

This work describes methods that seek to bridge the mechanics of head injuries to physiological outcomes with clinical relevancy. Mechanical modeling of TBI is not new but moving beyond the pure study of the tissue response to loading to applying data collection methods familiar in mechanical applications and using that data paired with basic dynamics to investigate links to behavior changes following a brain injury is a whole new path for combining the two fields of study.

Impact acceleration animal models of brain injury mimic many commonly occurring forms of human TBI. These models involve unrestrained motion of the animal's head upon impact. This is analogous to blunt force human head injuries where the head is struck and then moves away from the blow. These blunt force, unrestrained injuries involve mechanical variation: differing velocities and accelerations, for example. That mechanical variation is important, because previous studies have shown that those kinematic properties are linked to injury severity. This work provides a method to perform the quantification of mechanical variation in weight drop impact acceleration models. The method detailed above provides a quick, non-invasive, easily installed system for capturing video data of the impacts as they occur. The analysis method for the video data uses open-source software and basic dynamics calculations, which makes the process cost-effective and accessible.

The ability to take that kinematics data and compare it directly to behavior data from animals before and after an injury allowed us to determine if there were predictive relationships between mechanical values and clinically relevant functional measures. This is what helps take a step forward in bridging mechanics and physiology. If we can use sensors that collect kinematics data in safety equipment for the head and use readings from that equipment after a blow to the head to predict what types of functional outcomes could be a concern.

The video tracking methodology also extends to behavioral testing methods. The same methods used in the kinematic tracking study can be improved using video tracking analysis. The beam walk test is commonly used to evaluate a rodent's locomotion and balance in response to brain injury. The test is normally evaluated visually, which limits the detail of the scoring. However, the non-invasive video tracking used in the kinematics study can increase the detail of the scoring for the test. The walkway used for this method is simple and inexpensive. The video processing is detailed, but the analysis can be completed using open-source software. The study we conducted revealed that younger animals displayed signs of injury more aligned with what collaborators reported with the same input parameters. The preliminary gait video looks promising for being able to catch motor deficits post-injury. One animal in the study was profoundly impaired and can act as a test case to develop the analysis process.

The gait study used both male and female animals, which is uncommon in animal studies. In future studies with both methods female cohorts should be included. The kinematics method should continue to be built up in sample size, especially using animals injured at 8-9 weeks. That is the age that can be compared to other study results and can be used to work on optimization on the weight drop apparatus. Female cohorts should be added to both studies because the sex differences in outcomes to brain injury are still little understood. Including female animals in these studies will help to increase the pool of data for female animal model responses to brain injury, specifically closed-head impact acceleration injuries. The gait footage collected is still in raw form and needs to be processed before being analyzed. The video processing will focus on isolating the animal in the frame and removing any glare or shadows. The analysis software is a deep neural network that pairs tracking of the sagittal foot strike tracking with the footprint tracking to provide calculations of gait parameters of interest: duty factor, stride length, stride width, maximum pressure area, etc. The goal is to be able to compare those parameters between different time points to determine if this method would be viable as a behavioral assay for this application.

Ultimately, the methods discussed here bring us a step closer to combining the power of our understanding in mechanics and physiology to get closer and closer to solving one of medicine's most complex questions: how do we best combat the effects of traumatic brain injury?

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Appendix

Table 2 - Linear Acceleration Values from Kinematic Analysis

Impact Parameters **weight (g), height (cm), throw (cm)**	**Range** **(m/s^2)**	**Mean** **(m/s^2)**	**SD** **(m/s^2)**
All (n = 39)	344.0 - 620.9	514.8	61.8
305, 135, 3 (n = 3)	489.3 - 575.4	544.1	47.6
450, 135, 3 (n = 18)	469.0 - 620.0	534.0	41.7
450, 135, 4.5 (n = 3)	498.1 - 548.5	518.2	26.7
610, 67.5, 1 (n = 3)	364.5 - 441.0	415.4	44.1
610, 67.5, 3 (n = 2)	344.0 - 417.4	380.7	52.0
610, 135, 1 (n = 3)	455.7 - 528.1	491.3	36.2
610, 135, 3 (n = 4)	502.0 - 620.9	535.8	57.0
610, 135, 4.5 (n = 3)	482.7 - 611.5	550.9	64.7

Table 3 - Angular Acceleration Values from Kinematic Analysis

Impact Parameters weight (g), height (cm), throw (cm)	Range (rad/s^2)	Mean (rad/s^2)	SD (rad/s^2)
All (n = 39)	17,858 - 66,162	32,901	10,045
305, 135, 3 (n = 3)	25,523 - 37, 996	33,408	6,859
450, 135, 3 (n = 18)	19,564 - 66,162	33,596	12,271
450, 135, 4.5 (n = 3)	17,858 - 40,011	31,004	11,642
610, 67.5, 1 (n = 3)	21,909 - 44,756	36,725	12,846
610, 67.5, 3 (n = 2)	34,014 - 38,563	36,289	3,217
610, 135, 1 (n = 3)	29,337 - 33,710	31,619	2,193
610, 135, 3 (n = 4)	22,391 - 35,913	27,737	6,261
610, 135, 4.5 (n = 3)	23,876 - 44,680	32,205	11,004