Open Access Theses                    Electronic Theses and Dissertations

2008-01-01

# Evaluation of Sensorimotor Deficits and Compensatory Mechanisms Following Traumatic Brain Injury Using Three-Dimensional Kinematic Analysis in Rodent Models

Connie Elka Myerson
*University of Miami*, c.myerson@umiami.edu

Follow this and additional works at: https://scholarlyrepository.miami.edu/oa_theses

UNIVERSITY OF MIAMI


EVALUATION OF SENSORIMOTOR DEFICITS AND COMPENSATORY
MECHANISMS FOLLOWING TRAUMATIC BRAIN INJURY USING THREE-
DIMENSIONAL KINEMATIC ANALYSIS IN RODENT MODELS


By

Connie Myerson


A THESIS


Submitted to the Faculty
of the University of Miami
in partial fulfillment of the requirements for
the degree of Master of Science


Coral Gables, Florida

May 2008

UNIVERSITY OF MIAMI


A thesis submitted in partial fulfillment of
the requirements for the degree of
Master of Science


EVALUATION OF SENSORIMOTOR DEFICITS AND COMPENSATORY
MECHANISMS FOLLOWING TRAUMATIC BRAIN INJURY USING THREE-
DIMENSIONAL KINEMATIC ANALYSIS IN RODENT MODELS


Connie Elka Myerson


Approved:

_____
Dr. Edward J. Green
Associate Professor of Psychology, Neuroscience

_____
Dr. Terri A. Scandura
Dean of the Graduate School


_____
Dr. Philip M. McCabe
Associate Chairman, Dept. of Psychology
Professor of Psychology, Neuroscience

_____
Dr. Helen M. Bramlett
Assistant Professor
Neurological Surgery

MYERSON, CONNIE E.                                    (M.S., Clinical Psychology)

<u>Evaluation of Sensorimotor Deficits and</u>                      (May 2008)
<u>Compensatory Mechanisms Following Traumatic</u>
<u>Brain Injury Using Three-Dimensional</u>
<u>Kinematic Analysis in Rodent Models.</u>

Abstract of a thesis at the University of Miami.

Thesis supervised by Edward J. Green, Ph.D.
No. of pages in text. (75)

Three-dimensional kinematic analysis was used to precisely quantify alterations in gait and compensatory behaviors in rat performance on beamwalk and treadmill tasks following moderate traumatic brain injury.  Measures included limb height, joint angles, adduction, flexion, and swing/stance phase duration.  Injury-associated changes on the treadmill included postural and hip angle change, and increases in hip height and adduction. The beamwalk presented as a more sensitive measure when coupled with kinematic analysis, as differences between injury groups were evident on measures including knee, ankle, elbow, and mid hip height.  Animal response was diverse, possibly reflecting individual compensatory strategies which varied among injured animals. Kinematic analysis was ultimately shown to be a useful tool in characterizing and dissociating initial impairment, compensation, and recovery.

**TABLE OF CONTENTS**

Page

Chapter

Chapter 1: Rationale

The purpose of traumatic brain injury research is to prevent human injury following a traumatic assault, as well as monitor, characterize, and facilitate recovery. Accordingly, the methods by which those injuries are assessed and methods proven effective are of utmost importance. The use of 3-D kinematic gait analysis may allow for objective and precise report of gait characteristics, some of which were previously undetectable, and may improve upon or enhance earlier measures.

For years a variety of behavioral assessment methods have been used to investigate different locations and severities of injury, and to pinpoint certain motor or sensory systems. However, these existing behavioral measures display several limitations. Most methods fail to address mechanisms of compensation, and measure only the endpoints of certain tasks. Endpoint measures fail to characterize the full range of interim behaviors, and are therefore unable to distinguish whether the displayed behaviors are due to actual recovery or compensation. This dissociation between impairment, recovery, and compensation is most important. Many current methods lack in their descriptive abilities of behavior and mechanisms used, therefore making such dissociation difficult or impossible. Differentiation between these states is important for a number of reasons. To begin, the pathways of recovery and compensation often include very different neural circuits. Therefore, treatment plans could vary greatly in terms of target and regimen, depending on which circuitry is involved. Inherent in any attempt at treatment is the targeting of specific damaged neural networks. Treatments have the maximum ability to aid in actual recovery only when they are specific to the correct

neural circuitry. If behavioral measures are unable to pinpoint whether a deficit is representative of basic impairment, recovery, or compensation, the treatment itself will also be undifferentiated and may target incorrect networks. In addition, when using behavioral methods to monitor recovery, it is vital to ascertain whether observed changes are representative of recovery or compensation in order to gauge effectiveness of the employed treatment. Many existing methods fail to adequately describe the behaviors needed to differentiate between the two, and/or fail to address the subject in its entirety.

For the purpose of this study, it was reasoned that the initial deficit or impairment was most likely to be observed in the acute stages following injury. As time progressed the animals developed individual patterns of behavioral change. Patterns that developed over a more chronic period and that were distinctly different from initial deficit/impairment measures, and also varied from sham performance and trajectory, were considered possible representations of compensatory mechanisms. Conversely, measurements of TBI-animal performance taken over the chronic period that were not significantly different from baseline and/or sham trajectory, were considered possible indicators of full or "true" recovery.

Currently, through the continuing development and partnering of video and computer technologies, kinematic analysis methods of detecting subtle gait differences post injury and monitoring motor recovery are gaining popularity. While its use in spinal cord research has been documented and is growing, there remains a large opportunity to incorporate kinematic methods into traumatic brain injury research and greatly improve upon the aforementioned weaknesses of existing measures. While traditional methods of behavioral analysis involve quantifying certain endpoints (e.g. foot slips, swim time, the

use of ledges for compensation), kinematic analysis allows precise measurements of the movement of the subject and the deficit itself. This is made possible through the combination of video recorded behavioral measures, and later computer analysis of joint and muscle movement of the subject during the task. As defined by Muir and Aubrey, kinematic measures are those that describe, quantitatively or otherwise, the movement of the whole body and body segments relative to each other and/or to an external frame of reference (2000). There are many potential benefits of employing such a method. Kinematic analysis as a behavioral assessment tool may be more precise and introduce less subjectivity into measures than traditional methods. If proven effective, it could also preempt the need to address compensation due to its ability to precisely measure the physical changes that constitute the compensatory behavior during initial testing. Furthermore, and perhaps most importantly, use of kinematic analysis programs allows increased dissociation between impairment, recovery, and compensation, through precise description of motor movements. This in turn allows for the possibility of development of more accurate and effective treatments that target the appropriate neural mechanisms. It is these possibilities of new, more effective treatments that could help to progress the field of research.

The purpose of this study was to evaluate the investigative potential of quantitative kinematic methods in traumatic brain injury research, by exploring its effectiveness and advantageous qualities. Differences and/or deficits in locomotion that were previously undetectable with existing methods were explored. The study had several goals.

Broadly, it would be shown that using kinematic analysis as a behavioral assessment tool may be more precise and introduce less subjectivity into measures than traditional methods. Three-dimensional kinematic video analysis can be coupled with pre-existing techniques to provide enhanced precision in data collection, and lead to more accurate and informative conclusions. But most importantly, the author hoped to demonstrate the effectiveness of this method in detecting and characterizing compensatory behaviors using the treadmill and beamwalk tasks. This type of analysis helps to further dissociate the domains of impairment, recovery, and compensation in rat models of traumatic brain injury.

More specifically, expectations for this line of research include exploration of postural changes or deficits following injury, as well as differences in adduction and flexion of limbs evident by variations in measured parameters. Additionally, this study aimed to more clearly define compensatory mechanisms.

Preliminary hypotheses suggested that changes in animals' posture or overall gait in response to injury are used as a means of compensation for injury-related deficits. Initial conclusions from a pilot study included detection of a height differential evident at the hip joint, where the right (ipsilateral) hip is higher than the left (contralateral) hip. This seemed to be accomplished either through elevating the right hip, or lowering the left. Previously, animals tested on a beamwalk apparatus showed compensatory behavior when a ledge was provided. This points to possible difficulty in adducting the limb to its original position pre- insult. This may be observed through analysis of relative paw position as described by Hamers et al. (2006). Kinematic analysis will allow researchers to further validate and visualize these changes, as well as define in more detail the

deficits and the ways in which the animal is compensating for them.  In this way, kinematic analysis can improve upon the limitations of previous measures and allow for more accurate targeting of recovery circuits in the future.

The two tasks the animals performed, the beamwalk and treadmill apparatus, entailed different sensorimotor requirements.  In a sense, the treadmill was a modified open field measure.  The animal was allowed to move freely with only some field constraints.  While in spinal cord injury studies a subjective BBB (Basso Beattie and Bresnahan) score from an open field test or kinematic analysis of a single limb might be sufficient (Fouad et al., 2000), deficits from traumatic brain injury may be more complex and less apparent.  Therefore, complete kinematic analysis in a treadmill protocol where the effects of brain injury are not clearly evident, allows the researcher more detail, breadth, and precision in exploring deficits.

The beamwalk apparatus required that the animal have full range of motion in their limbs, specifically adduction and flexion.  If the animal was not able to adduct or flex the limb fully it tended to drop outside and below the surface of the beam, and the animal stumbled.  Without a ledge the animal is forced to alter its posture and weight distribution so that it relies on the non-impaired limbs.  This shifting of posture and weight is a compensatory behavior that serves to mask the deficit. In turn, the experimenter is blinded from any real improvements in outcome associated with treatments or other mechanisms of rehabilitation (Schallert et al., 2002).  When a ledge is added to the beam the animal no longer compensates by shifting posture or weight, but uses the ledge as a crutch and the deficit is therefore apparent.  By using kinematic analysis to document only successful passes across the beam from injured animals, this

study is in fact targeting and quantifying purely compensatory behaviors without the use of a ledge.

Conclusions drawn from kinematic video analysis could be applied post-injury in a variety of ways. It may be used to identify initial deficits and track rehabilitation, as well as contribute to the planning of surgical procedures used to treat injury in clinical settings. In this way kinematic analysis is not only measuring outcome, but also helping to *improve* outcome, by using data output to directly influence and shape rehabilitative techniques. In human care, postoperative gait analysis serves not only as a measure of treatment outcome, but also as a useful tool in planning ongoing care for the patient (Kay et al., 2000). The use of kinematic analysis protocols may greatly aid in the progression of traumatic brain injury research. In the future it would be advantageous to integrate these techniques into the battery of accepted behavioral measures as they may prove to be invaluable tools for research due to their diagnostic, descriptive, and predictive abilities.

Chapter 2: Specific Aims

*Aim I*

To evaluate and quantify changes in gait parameters following traumatic brain injury in both a restricted open field (i.e., treadmill task) and a beamwalk paradigm in which the demands of sensorimotor activity differ for each.

*Hypothesis*

During acute periods of recovery following injury, alterations to different sub-components of gait were expected. This included changes in swing and stance duration of the hindlimbs, which would reflect partial contralateral hindlimb paresis. In this same period it was hypothesized that the beamwalk task would illustrate deficits in adduction and flexion of the contralateral hindlimb.

*Rationale*

Based on preliminary pilot data, it was believed that the use of kinematic analysis in conjunction with the two tasks could reveal traumatic brain injury induced deficits such as changes in adduction and flexion, and swing and stance duration. In the past, various sensorimotor measures have shown deficits in hindlimb adduction and flexion post-TBI using techniques such as inking/foot-print analysis and the beamwalk (e.g., Schallert et al. 2002; Baskin et al., 2004). However, no corresponding deficits have been documented in open field tests. Kinematic analysis was believed to be a more sensitive measure that would be able to reveal these deficits in an open field where it was not previously possible.

*Aim II*

To accurately characterize mechanisms of sensorimotor compensation following traumatic brain injury.

*Hypothesis*

Throughout the recovery period we predicted that gait and postural adjustments allowing the animal to compensate for sensorimotor deficits would be quantifiable through 3-D kinematic analysis. It was also hypothesized that there would be evidence of changes in gait parameters on the ipsilateral side of the animal in order to compensate for contralateral adduction and flexion deficits. Potential changes included pattern and coordination of limbs, as well as joint angle measurements accounting for differences in overall limb height and contributing to postural change relative to sham animals.

*Rationale*

Previously, animals have been shown to use compensatory behaviors in a variety of situations including the beamwalk, staircase test, and the rotarod (Shallert et al., 2002; Whishaw et al., 1997; Crapon de Caprona et al., 2004). Although studies often report the presence of compensatory mechanisms, the component behaviors themselves are not accurately measured or described. Other than it's mere presence, there has been little detailed description of compensatory behaviors in either the treadmill or beamwalk tasks. It was expected that these behaviors might be characterized in more detail than previously possible using 3-D kinematic analysis. Through more accurate and precise measurement of these sensorimotor behaviors, it is possible to target either recovery or compensatory networks for treatment and also confirm their effectiveness. In turn, this specificity allows for greater potential of positive therapeutic outcome.

Chapter 3: Background


Three-dimensional kinematic analysis builds on pre-existing, proven behavioral methods, and therefore a review of these measures is in order. Ideally, these tests would be sensitive to injury, detect chronic sparing of tissue, target the impairment, not be overly influenced by repeated testing, and perhaps most importantly, differentiate between mechanisms of compensation and recovery (Schallert, 2002). The majority of research in kinematic analysis of rodent models has focused on spinal cord injury, and has only just recently been recognized for its potential uses in studying recovery mechanisms following traumatic brain injury. Therefore, one must appreciate how kinematic analysis has grown out of these studies, and learn from their application. The most commonly used measures in spinal cord and traumatic brain injury research include the beamwalk or beam balance, catwalk, treadmill, gridwalk, rotarod, forelimb placing tasks involving the cylinder, and footprint analysis. Other methods include adhesive removal, swimming tests, staircase reaching, open field, sensory, and reflexive tests, but will not be touched on here. Some of these procedures are more sensitive to specific types of injury, and each has it's strengths and weaknesses, therefore it is important that the choice of measure reflect consideration of the tasks' sensitivity to injury, location, and severity (Baskin, 2003). It is also important to keep in mind that the studies discussed in reference to each behavioral measure are only a small representation of the literature employing these methods. Focus will be placed on measures evaluating facets of motor function, reflex, and coordination. It is our belief that gait and kinematic

analysis will grow out of, be incorporated with, and improve upon these measures for a number of reasons.

To begin, the beamwalk test has been used extensively to assess vestibulomotor function and fine motor coordination in animal models of traumatic brain injury. This test uses an adverse stimulus such as a bright light or white noise to motivate the animal to traverse the beam in order to reach a darkened goal box (Piot-Grosjean et al., 2001). Length and width of the beam may vary, and recently ledges on either side have been used to detect compensatory behavior (Schallert, 2002). In a modified cylindrical beamwalk task, Crapon de Caprona et al., noted 'tail wrapping' for additional support and use of paws as compensatory mechanisms to overcome imbalance (2004, quotations in original text). Although the beamwalk task was the first motor test utilized in TBI experiments involving rodents, it has since been proven effective and particularly sensitive to injury lateralization, injury severity, and pharmacologic manipulation involving a variety of TBI models (Dixon et al., 1987; Chen at al., 1996). Even though the beamwalk has been proven a reliable measure in many paradigms, several works from Larry Goldstein (1990; 1993; 2003) and others highlight the sensitivity of the beamwalk to training and testing effects, environmental factors, and experimenter interference. These potential weaknesses are important and proper precautions to avoid such variability should be taken. Even so, the beamwalk remains a standard in traumatic brain injury research for it's reliability in measurement and ability to detect certain compensatory mechanisms. The beamwalk task has been used as a behavioral measure in a variety of injury models including Middle Cerebral Artery Occlusion (MCAO) or stroke (Reglodi et al. 2003), varying degrees of traumatic brain injury (Baskin et al.,

2003; Goldstein et al. 1993; Hamm et al., 1992; Kunkel and Bagden, 1993; Schallert et al., 2000; Fujimoto et al., 2004), and spinal cord contusion and transection injuries (Fouad et al., 2000; Goldberger et al., 1990; Hamers et al., 2001; Schallert et al., 2000). The beamwalk has also been utilized in interpreting the impact of pharmacologic agents on the post-injury recovery process (Boyeson et al., 1994).   By coupling the beamwalk apparatus with kinematic video analysis, the researcher can monitor an animal's performance and is able to document compensatory movements or behaviors without the use of a ledge.

Another common measure, the rotarod task, is popular when investigating motor deficits following fluid percussion traumatic brain injury. Performance at both mild and moderate injury levels was found to be significantly impaired in the rotarod task, proving the method to be a sensitive index of injury-induced motor dysfunction, whereas other methods (e.g. beam balance, beamwalk) only detect dysfunction at more severe injury levels (Hamm et al., 1994). When comparing rotarod to beam balance and beamwalk at the moderate injury level, rotarod exhibits a slower rate of recovery than the others, which is perhaps indicative of its increased sensitivity to deficits (Hamm et al., 1994). Therefore, Hamm et al. concluded the rotarod task to be a more sensitive and efficient index for assessing motor impairment following fluid percussion injury (1994).  Still, all measures (i.e. rotarod, beamwalk, and beam balance) were found to be reliable and valid measures of outcome following experimental TBI (Hamm et al., 1994).  It has been suggested that the task be recorded for further video analysis (Mattiasson et al, 200).  Indeed, by using 3-D kinematic analysis the experimenter would be able to gain a more informative look at the motor mechanisms and specific muscle sets being used to perform

the task. This could reveal mechanisms of compensation even when the animal seems to be performing well (such as in the un-ledged beamwalk task), in turn increasing the sensitivity of the measure.

Two additional behavioral measures often used in traumatic brain injury research include the grid walk and spontaneous forelimb use (SFL) tasks. The gridwalk consists of an animal walking across an elevated wire surface with uniform openings that the animal must avoid (Baskin et al., 2003[a]). It is among the simplest of the vestibulomotor tests involving basic locomotion (Fujimoto et al., 2004). Advantages to this task include the ability to assess each limb individually (both fore- and hindlimb), and also to assess the difference in limb performance ipsilateral and contralateral to injury (Baskin et al., 2003[a]). It should be noted that due to the uniformity of holes in the grid, animals may also have the opportunity to use methods of compensation that are not easily detectable (Baskin et al., 2003[a]). Therefore, the task loses sensitivity through repeated exposure. The grid walk is also often used in investigations of spinal cord injury, although slightly modified. Often the grid walk takes on the form of a grid 'runway', but is still an accurate test of limb placement and therefore substantial motor control (Kunkel-Bagden et al., (1993).

Spontaneous Forelimb Use tasks (SFL) have been used for a variety of purposes including assessing forelimb dominance, limb use asymmetry, and exploring sensorimotor deficits (see Baskin et al., 2003[a]; Schallert et al., 2000; Schallert et al., 2002; Soblosky et al., 1997; Starkey et al., 2005). The limb use asymmetry test (commonly known as the "cylinder test" or "rearing test") was originally developed by Schallert et al. to measure forelimb use during vertical exploration. The cylindrical shape

of the chamber encourages vertical exploration of the walls with the forelimbs and offers high inter-rater reliability (Schallert et al., 2002). The choice of which forelimb to use for weight bearing is thought to be indicative of which forelimb is more functional and in turn preferred by the animal (Soblosky et al., 2001). SFL tasks have been shown to be sensitive and produce robust effects that last throughout testing periods, as in the Starkey et al. study of pyramidotomy lesions (2005). Limitations to grid walk and SFL tasks involve subjective ratings by experimenters and room for human error. These tasks also tend to quantify endpoint measures, providing no information about the underlying motor function of the animals or possible compensatory behaviors. For instance, in the SFL task the only 'compensation' detectable is the difference in the use or placement of the limbs (e.g., switching of dominant limb, increased or decreased use). The investigator is not given any information about the component behaviors and deficits that make up the net movement, which together create a need for such compensation. Likewise, the rotarod task may be too simplistic and unable to break down behaviors into component parts. Kinematic analysis allows for the fractionation and study of these component behaviors resulting in a more detailed look at deficits and the need for and production of compensatory mechanisms.

Perhaps the methods of most relevance to the purpose of integrating kinematic analysis into traumatic brain injury research are open-field tests, treadmill walking, and footprint analysis. All play some role and have been used in the past in identifying the posture of the animal, rotation and placement of limbs, as well as coordination and general motor abilities pre- and post- injury.

Open field tests allow the observation of voluntary movement by the animal and are scored using qualitative scales such as the BBB. Most often used in spinal cord injury research, rated parameters of this method include joint movements, the ability for weight support, foot placement, limb coordination, and gait stability (Basso et al., 1995). Proper protocol requires two separate raters, but scores still rely on visual determination of the quality of locomotion and can lead to incorrect interpretations by the untrained eye (Leblond et al., 2003). In addition, it is important to note that animals with a proper pattern of locomotion on other tasks such as the treadmill may still look paraplegic in an open-field situation (Leblond et al., 2003). Even though this procedure can be videotaped for later analysis, errors of rater interpretation are still possible. Unlike spinal cord injured animals that may easily present as paraplegic in the open-field, deficits from traumatic brain injury tend to be more subtle. While animals with TBI may look normal in the open field, deficits and compensation can be detected on various tasks such as the beamwalk and by using kinematic analysis of the treadmill (Schallert et al., 2002; Goldstein & Davis, 1990; Baskin et al., 2003[b]). This study aims to help characterize these subtle gait deficits on a variety of parameters including adduction and flexion of limbs, as well as overall posture and coordination.

The treadmill is one of the most common behavioral methods for studying gait analysis in both brain injury and spinal cord injury paradigms. Treadmills are used for aiding in behavioral assessment and also as a tool in rehabilitation. Varying speeds may be used as gait changes accordingly. For example, at relatively slow speeds animals demonstrate rhythmic and stable stepping, while step period and step displacement changes in a predictable manner as treadmill speed increases (Broton et al., 1996). It has

been found that quadruped gaits may also be divided into two categories that vary with speed: symmetric and asymmetric (Thota et al, 2005). In symmetric gaits (e.g. walk, trot, and pace) there is strict alternation of the two limbs of the same girdle, while in asymmetric gaits (e.g. canter, gallop) the relative phase between limbs in the same girdle may vary (Thota et al, 2005). Kinematic parameters of forelimbs have been found to be independent of speed and gait in the rat, while fundamental changes occur in the hindlimb kinematics as evident by changes from symmetrical to in-phase gaits (Fischer et al., 2002). Even with these varied gait patterns it remains possible to impose a walking speed on the animal where the step cycle is constant (Leblond et al., 2003). In treadmill procedures, food and water rewards are sometimes provided, as well as the use of nocioceptive stimuli in order to obtain reliable locomotion (Bouet et al., 2004; Goldberger et al., 1990).

Briefly, traditional footprint analysis is a modification of an open-field in which an animal's paws are inked and the tracks are then studied after having walked across an open field or performed on a treadmill. Measurements are made of step length, foot length and other parameters. Unfortunately, this method can often be messy, indirect and inaccurate in several areas. Yu et al. (2000) helped to pinpoint the drawbacks of this method. Those shortcomings included variability in the footprints due to differing amounts of developer or ink applied, and the inability to control the weight borne and force exerted on each limb. Additionally, on some of the prints edges of the heel and toes were unclear and part of the foot was missing, making the measurements subjective and possibly inaccurate. Yu et al., found video gait analysis to be a superior measure and in the same year, Marijan Bervar developed a similar method of static footprint video

analysis (Yu et al., 2000; Bervar, 2000). The video gait analysis procedure designed by Yu et al., was tested in spinal cord injury paradigms and consists of digitizing and analyzing video frame by frame on a personal computer in order to assess seven different parameters of gait. Those parameters included walking speed, step length and step length ratio, tail height and ankle angles among others (2000). In the Bervar method, video analysis was used to assess functional loss following injury to the rat sciatic nerve while the animal stood or rested on a flat transparent surface (2000). Static footprint video analysis was easier to perform than footprint video analysis during walking but still capitalized on the advantages of video versus ink methods. There were fewer non-measurable footprints, higher accuracy, better replication, and more precise quantification of the degree of functional loss (Bervar, 2000).

Perhaps the most similar measure to that of 3-D video kinematic analysis is the CatWalk. Originally developed in an effort to improve upon qualitative and semi-quantitative data collected in open field assessments such as the Basso Beattie Bresnahan scale (BBB) and foot print analysis, it is a wonderful example of early methods of gait analysis in spinal cord injury research and it's protocol encourages collection of quantitative data on selected gait parameters (Hamers et al., 2001). Currently, the CatWalk is used primarily in spinal cord injury paradigms. However, these same gait parameters and measurements are valuable in traumatic brain injury research. This study aims to demonstrate how the precise and accurate collection of these parameters is made possible through the integration of 3-D kinematic analysis.

In 2006, Hamers et al. concluded that the use of the CatWalk allowed for more detailed analysis of various aspects of locomotion, including those that were previously

undetectable. Others believe combining coordination data harvested from the CatWalk and integrating it with commonly used and verified tests such as the BBB, would yield a new standard of reliable and sensitive assessment of locomotor function following spinal injury (Koopmans et al., 2005). Similarly, integrating data from kinematic analysis with commonly used qualitative scales will also result in new levels of precision and sensitivity in measurement. Additional gait parameters can also be explored above and beyond the capabilities of the CatWalk, such as joint angles, limb height, and postural changes.

To date, results of SCI studies have indicated differences in limb coordination, stride length, stance and swing duration, and base of support (Goldberger et al., 1990; Hamers et al., 2001; Kooopmans et al., 2005; Leblond et al., 2003). Most recently, Hamers et al. summarized observed differences in various gait parameters using the CatWalk apparatus (2006). Changes were found in gait "coupling" and in the regularity index (RI) of stride (Leblond et al., 2003; Hamers et al., 2001). Differences were also found in relative paw position. For example, most rodents tend to place their hindlimb paw in the previous position of the forelimb paw, however after injury some changes to this pattern are exhibited (Hamers et al., 2006). While the use of 3-D kinematic analysis in TBI may find some of these same differences as well as others in additional measures, other findings are not expected to be so robust. For instance, in this study we hope to detect differences in joint position (e.g. height, lateral movement), joint angles, limb adduction and/or flexion (changes in relative paw position), and changes in animal posture. Deficits in overall coordination, duration of cycle phases (i.e., swing and stance), and stride length are expected to be more subtle than in spinal cord injury

models. There has also been a call in SCI research for methods to further identify compensatory mechanisms (Goldberger et al., 1990). Research designs may fail to maximize benefits through testing of gross rather than specific behaviors (Goldberger et al., 1990). These concerns extend to traumatic brain injury research, and can be heeded through the use of kinematic video analysis which may be more precise, objective, and able to target specific mechanisms of behavior and movement rather than gross outcomes.

Detecting compensation and distinguishing it from recovery has long been the goal of many studies. If not the aim of a study it is usually considered before reaching conclusive results. For instance, after finding deficits in the lack of coordination between the anterior and posterior parts of the body and gait instability, Xerri et al. went on to add that it was conceivable that any observed functional recovery was a product of increased reliance on behavioral strategies (2004). That same year, Baskin et al. noted compensatory tail movements in mice on the beamwalk task (Baskin et al, 2004). Perhaps Schallert et al. said it best, "Improved end-point scores in tests of complex motor performance might sometimes be accounted for by compensatory postural adjustments…. It is therefore necessary to monitor carefully the kinematics of the behavior and to ensure that the animal does not engage in compensatory motor 'tricks', or that the tests are designed to reveal these tricks" (2000). This is exactly our goal of integrating kinematic video analysis into research. By doing so we may be able to better recognize these compensatory 'tricks' through improved test design, characterize the component behaviors giving rise to these deficits or compensatory mechanisms, and be better able to monitor the overall kinematics of behavior throughout recovery.

The introduction of 3-D kinematic video analysis should allow for more accurate measurement of footprint and gait parameters, as well as greater potential for dissociation between impairment, recovery, and compensation.  This dissociation is important in order to identify the correct neural mechanisms that underlie each stage, and therefore develop the most accurate treatments targeting appropriate circuits.  Specifically, kinematic or gait analysis (both will be used interchangeably from herein), involve the quantification of locomotor behavior in terms of limb movement rythmicity, forelimb/hindlimb coordination, and changes in posture, limb height and joint angles (Broton et al., 1996). The step cycle for each limb is divided into stance and swing phases based on ground contact with the foot (Halbertsma, 1983).  The "swing" portion of the cycle begins with lift-off (LO) of the limb and continues until it makes contact with the floor (touch down; TD) once again.   The "stance" phase begins as the foot strikes the floor, ends with lift-off of the limb (Barbeau & Rossignol, 1987; Perry, 1999) and involves both weight acceptance and single limb support.  In our research, the entire step cycle will be defined as LO to LO, and each joint will be analyzed in relation to the step cycle of its own specific limb, with markers pinpointing the placement of the hip, knee, ankle, shoulder, elbow, and wrist joints. Two additional reference markers will pinpoint the midline of the animal between the shoulder and hip joints.

It is the combination of kinematic analysis with the treadmill and beamwalk apparatus that could potentially yield the most fruitful results.  In the past, the ledged beamwalk was among the first methods to demonstrate compensatory behavioral mechanisms not visible in previous un-ledged trials.  Using 3-D video and kinematic analysis of un-ledged beamwalk trials may allow the researcher to observe behavioral

compensation without necessitating the use of a ledge.  Likewise, coupling kinematic analysis with the treadmill task allows observation and measurement of basic motor function pre- and post- injury.  There has also been much success in SCI research with the use of the CatWalk—a similar apparatus although it measures slightly different parameters.  The use of 3-D kinematic analysis opens the door to studying previously undetected differences in brain injured animals, and could provide more objective, accurate, and precise measures of gait parameters. In addition, it is an important tool that may be used to monitor recovery of function, and further differentiate impairment, recovery, and compensation.

Chapter 4: Preliminary  Protocol and Results

The goal of the present investigation is to confirm, replicate, and expand upon previous findings with a larger sample and more varied tasks.  The procedure and statistical analysis of the pilot study varied slightly from the present protocol.

In the pilot study, data was collected using four male Sprague-Dawley rats over an eight-week period following moderate, severe, or sham fluid-percussion injury to the right cortical hemisphere.  The rats were trained on the treadmill for two weeks prior to baseline testing, and post-injury each was trained once a week for skill maintenance. Every two weeks following surgery the animals were tested and video recorded on a treadmill at a speed of 25- 30 cm/sec, using Peak Motus® three dimensional kinematic analysis equipment.  Each animal was marked with twelve reflectors in identical location to those indicated in Figure 3a-b, excluding mid-scapula and sacrum markers. Prior to testing each animal had been briefly sedated using isoflourane and then shaved in preparation for tattooing.  Each joint that was to be tracked by computer was tattooed after further anaesthetizing the animal, in order to ensure consistent marker placement over the eight-week testing period.  These reflectors were then tracked throughout the animal's performance and later digitized and analyzed to document differences in a number of gait parameters. (See the *Methods* section for a more detailed description of surgical procedures, and digitization techniques). Statistical analysis consisted of creating a limb-specific average step cycle for each animal at each timepoint.  Post-injury datasets were compared to those at baseline within animals, and moderate and severe animal data

was compared between animals to shams.  Statistical significance was determined using standard deviation measures and 95% confidence intervals.

Pilot data revealed a number of findings.  To begin, we quantitatively documented changes in contralateral hindlimb parameters following traumatic brain injury, as well as possible compensatory mechanisms.  Over the eight-week testing period the moderate and severe animal both exhibited significant changes in contralateral hip height.  The sham animal data illustrated no such effect.  However, one of the severe animals also demonstrated no effect over time, perhaps resulting from individual pattern variation or error within the measurement system (most likely due to inconsistent gait production or lack of equipment re-calibration).

It was hypothesized that over time the ipsilateral hindlimb compensates for some deficit on the contralateral side.  In fact, two animals in the pilot study developed uneven hip height or a hip "differential" within the hindlimb girdle, resulting in the ipsilateral hip being held higher over time.  It is interesting to note that this hip differential was obtained in a different way in each animal, either by the lowering of the left (contralateral) hip over time, or the raising of the right (ipsilateral).

One would expect that if such a hip differential does exist, it would be accounted for in hip and knee angle changes, or adduction and flexion deficits.  However, in the pilot study no significant changes in these parameters were found.  This was thought to be the consequence of measurement error and could be easily resolved with the fine-tuning of the experimental protocol and computer analysis techniques.  For example, in the pilot study there was no sacral reflector.  Therefore the hip angle was designated as the angle between the respective forelimb shoulder, through the hip marker and on to the

knee. As a result, any movement of the forelimb could have confounded the hip angle measurement. In the follow-up study the hip angle was designated as the angle between the sacrum, hip, and knee so as to avoid this measurement error. Adduction measurements were also confounded in the pilot study due to camera movement between weeks. This was prevented in the current study by re-calibrating the camera equipment each testing day. Following these improvements to the protocol, significant changes in angle measures, adduction, and flexion were expected in order to account for changes in posture and hip height.

Several other findings from the pilot investigation involved gait speed and forelimb movement. First, the pilot data showed a gradual slowing of gait in TBI animals resulting in fewer step cycles over identical time intervals as the weeks progressed. This could have been due to repeated exposure to the apparatus and/or practice effects. Differences in overall left hindlimb gait were also observed when the moderate animal was compared to the sham, particularly in the swing phase of the step cycle. However, no significant changes were evident in the height or movement of forelimbs over time. One exception was the severe animal, whose left shoulder dropped significantly in post-injury week 2. Although no deficits were evident in the pilot study in some of the previously mentioned areas (e.g. adduction/flexion, angle measurements, forelimb movement), they were once again probed in the current follow-up study.

Chapter 5: Methods

*Animals*

Procedures were conducted with adult male Sprague-Dawley rats, obtained from Charles River Laboratories, weighing 250- 300 grams.  While a number of animals were initially trained, the eight most consistent performers were used in the actual protocol in order to ensure the best chances of representative gait and motor activity.  At each time point the animals were weighed in order to monitor growth.  Rats were randomly assigned to sham or moderate injury groups, with the final eight animals consisting of 5 moderate TBI and 3 sham. They were each housed individually in standard cages (17 inch x 8.5 inch x 8 inch) on foam particle (flake) bedding, and maintained at the Miami Project to Cure Paralysis in an air-conditioned colony on a 12:12 hour light/dark cycle and given food ad libitum.  The animals began training on the treadmill and beamwalk tasks several weeks before commencing the study to ensure mastery of apparatus and acclimate the animals to frequent handling.  All experimental protocols were submitted to and approved by the University of Miami IACUC prior to experimentation. Three-dimensional data of gait kinematics were obtained from treadmill walking and beamwalk measures.

*Surgery Preparation*

Eight young adult male Sprague-Dawley rats weighing between 250 and 350g were used for this experiment (Moderate TBI, n= 3, Sham, n= 5). All animal procedures followed the National Institute of Health 'Guide for Care and Use of Laboratory

Animals' and were approved by the Institutional Animal Care and Use Committee. Animals were anesthetized 24 h prior to surgery with 3% halothane, 30% oxygen, and a balance of nitrous oxide. Rats were placed in a stereotaxic frame where a 4.8 mm craniotomy was made overlying the right parietal cortex, 3.8 mm posterior to bregma and 2.5 mm lateral to the midline (Paxinos & Watson, 1982). A plastic injury tube was next placed over the exposed intact dura and bonded by adhesive. Dental acrylic was used to affix the injury tube to the skull. The scalp was then sutured closed and the animal was allowed to recover before being returned to the home cage.

After fasting overnight, a FP device was used to produce experimental TBI via the injury tube. Intubated anesthetized rats (70% nitrous oxide, 1.5% halothane, and 30% oxygen) were subjected to a moderate TBI (1.8–2.2 atm). To induce injury, a pendulum was allowed to strike one end of the FP cylinder, causing a rapid, high-pressure injection of saline into the closed cranial cavity of the animal attached to the other end of the cylinder. A pressure transducer (Powerlab, AD Instruments) measured and recorded the force of the injury. Prior to TBI, the tail artery is cannulated to monitor arterial blood pressure and blood gases. Brain temperature was indirectly monitored with a thermistor probe inserted into the left temporalis muscle. Temporalis muscle and rectal temperature were maintained at normothermic (37°C) levels before and 30 min after TBI. Sham-operated controls underwent all surgical procedures except for the actual injury. Immediately following trauma or sham procedures the wounds were sutured, and the animals were placed in their cages after waking (as described in Rodriquez-Paez et al., 2005).

*Histopathology*

Animals were sacrificed 3-4 months following TBI. They were anesthetized and perfused transcardially with .9% saline at a pressure of 100-120 mmHg for 1-2min. This was followed by fixative for 25 min. Finally, the brain was removed and stored for possible future reference.

*Treadmill Task*

Three-dimensional kinematic data was collected in 2-5 minute long sessions at a speed of 8-19m/min, depending on the animal's ability. This speed was steady yet sufficiently brisk to hinder any exploratory behavior by the animal. Testing sessions included a baseline, and two post-injury sessions at week 1 and week 6. Prior to baseline testing the rats were trained daily on a single lane modular treadmill (Columbus Instruments, Columbus, OH) until mastery of the tasks was apparent. Between testing sessions at week 1 and week 6 post-injury, the animals were trained bi-weekly for skill maintenance.

Fourteen reflectors, measuring 6mm and 8mm (manufactured by Vicon Peak[®]) were placed on each animal during testing to mark specific joint/body locations. The joints specified included the hip, knee, ankle, shoulder, elbow, and wrist. Additionally, the midpoint between the shoulders (mid-scapula) and hips (sacrum) along the midline of the animal were also marked. The reflectors were fastened to the animal using "glue dots" found at the local craft store. This method did not harm the animals, and does not leave any residue behind. The animals were sedated using isoflourane and the forelimbs, hindlimbs, and back were shaved to prepare for marker placement. In order to ensure

accurate location of markers, the animals were placed over a foam block in an upright standing position. The bony processes were palpated and marked with permanent marker. The legs were also manually moved back and forth to verify marker location. After identifying the joint positions they were permanently tattooed to ensure accurate and consistent location of the reflectors over time. However, some ink fading did occur and therefore needed to be retouched on a regular basis. This retouching procedure was performed a day or two prior to testing under isoflourane anesthesia.

*Beamwalk Task*

This procedure employed the same tattoo and marker placement techniques detailed above. Testing was conducted at a baseline, and 1 and 6 weeks post-injury. During initial training several trials were needed over two to three weeks to accurately shape behavior. During the beamwalk task the animal was placed on one end of the beam and encouraged to walk across the span to a darkened goal box on the other end. Each trial was recorded for later video analysis using the digitization and analysis techniques mentioned shortly. Only trials in which the animals made a successful pass were digitized. This served two purposes: 1.) to preserve the integrity and reduce variability of kinematic measures, and 2.) to focus on purely compensatory mechanisms. Using trials that included footfaults would have only confirmed and documented known deficits. Meanwhile, in order to make a successful pass injured animals must use compensatory mechanisms of some kind, or have fully recovered. By analyzing only the successful crossings, it was then possible to target and quantify the compensatory behaviors of interest.

*Computer Analysis*

Computer software used for this experiment was the Peak Motus® 8.0 motion analysis detection system. The set up includes four black and white, gen-locked, CCD cameras, each equipped with an infra-red light ring around each lens, which help to pick up light from the reflective markers on each animal. Each camera is placed about 4-5 feet from the behavioral apparatus, arranged so that each marker (or all of the markers on one side of the animal) are visible in at least two of the cameras (see Figure 1). The video from each camera is then fed into four time code generators (SMPTE, Society of Motion Picture Television Engineers), four VCR's recording for back-up purposes, and an Event and Video Control Unit. All of these are then interfaced with a desktop workstation featuring Peak Motus® software allowing for digitization and analysis of the collected video.

The video and camera system was calibrated before collecting data at each time point, and for each individual day of testing in order to avoid confounding effects of interim camera movement. This was achieved using a rectangular calibration platform consisting of eleven vertical rods, each with 5 reflective markers along their surface. The platform spanned the 3D space in which the animals performed on both tasks. The markers were digitized in each of the four camera angles allowing calibration in three directional planes (i.e. *X, Y,* and *Z; Y* being vertical).

After capturing video during treadmill and beamwalk trials, the reflective markers were tracked and digitized offline from the four different camera views (see Figure 2). In addition, the point in time that each limb lifted off and touched down was labeled (event

markers). Lift off was denoted in the forelimb and hindlimb by the frame in which the final toe left the treadmill or beamwalk. Touch down was noted in the first frame that the paw again touched the walking surface. These lift-off and touch-down markers were used to identify the stance and swing phases of the step cycle. The raw data collected after digitization was then scaled and filtered. A myriad of data sets are automatically produced by the computer, including 3-D transformed coordinates and 3-D angles. After digitization was complete each trial was averaged, and normalized (201 points) using sub-trial cycles (demarcated by lift-off and touch-down markers). For the treadmill task, the data was normalized over within subject step cycles. However, in the beamwalk task two separate video segments were digitized and then the data was averaged and normalized across these two segments according to sub-trial cycles. These analyses created an average step cycle for each animal on each task, at each time point. Sub-trial cycle averages were computed for each limb, beginning with the lift-off and ending with the touch-down markers of that specific limb. Hence, the step-cycle was individualized for each, allowing for limb specific data collection and analysis. It should be noted that the normalized and averaged step-cycle representations generated by Peak Motus® were compared with cycle averages based on point-by-point data, and found to be comparable. Therefore all statistical analysis based on the normalized and averaged cycle data is assumed to be valid.

*Statistical Analysis*

Comparisons within each animal, and between animal groups were conducted via repeated measures analysis of variance, over one baseline (pre-morbid), and two post-

injury time points. Differences in joint height, angle measures, adduction of limbs, and

overall placement of markers and animal posture were explored (See Figures 3*a-b* for

marker and angle placement). In addition, gait parameters of moderate animals were

compared to sham controls.  Statistical packages used included StatView©, and SPSS®,

and Microsoft Excel®.  However, it was hypothesized that the animals would display

individual patterns of compensatory behavior, therefore rendering most group statistics

inappropriate. This was based on previous studies having conservative sample sizes (i.e.,

≤ 30) and main analyses consisting of phase relationships, correlations, cross-

correlations, and identifying component behaviors (e.g., toe spread) rather than whole

body dynamics across groups (Thota et al., 2005; Hamm et al., 1994; Bervar, 2000).

Therefore, this study chose to also investigate individual patterns of gait parameter

change and compensatory behavior.  More detailed descriptions of analyses are provided

below.

To begin, repeated measures ANOVA's were conducted to identify statistically

significant differences in joint height (i.e., bilateral markers at the hip, knee, ankle,

shoulder, elbow, wrist, and also the mid hip and mid shoulder). This comparison was

made using the maximum values of each marker over the normalized step cycle.  Two

separate analyses were conducted for the treadmill and beamwalk tasks.  This same

process was used to elucidate changes in joint angles by comparing the average

maximum angle values over time, and also within and between groups.

Changes in limb adduction were based on the absolute horizontal distance (z-

dimension) between ankle and mid hip markers.  Average distances were calculated from

normalized and averaged cycle data for the left and right hindlimb for each animal, at

each time point.  Adduction measures were then compared using repeated measures ANOVA.

Through further analysis it became clear that, as hypothesized, group statistics could not provide adequate descriptions of individual change (deficit, compensation, and recovery) over time.  Therefore, more qualitative descriptions were gathered using several methods.  First*, substantial differences,* as determined by the distinct differences in the standard deviations of the normalized data points over time were identified within and between animals on all height and angle measures.  The patterns of these changes were then studied within single step cycles (intra-cycle change), within animals, within injury groups, between injury groups, within and between tasks, and over time.

Intra-cycle change was qualitatively categorized as either being present throughout the cycle, or within several cycle portions labeled as early half, late half, mid cycle, end movement, or no change.  It was also noted whether movement returned to/towards baseline (RTB) at weeks 1 and 6.  In addition, general increases and decreases observed across both height and angle measures were compared quantitatively using chi square analyses with nominal variables between groups and tasks.

Differences in swing and stance duration and limb coordination were also explored. The average percent time spent in swing and stance phases was calculated over time for each animal. To investigate limb coordination the pattern of LO and TD of each limb was documented via graphic illustration and compared to normal step sequence patterns at baseline.

On the beamwalk task, latency to cross the beam was calculated using the same trials from which limb height and angle data were procured.  Two complete passes for

each animal, without footfault, were used at each timepoint. As trials were edited to include the animal passing from one defined point on the beamwalk to another, the time length of each trial was used as the latency value. These values were then analyzed using repeated measures ANOVA's to compare differences in latency between animals and over time. Footfault analysis differed from latency measures in that all of the attempted passes of an animal were considered. Footfaults were defined as the number of times the animal's paw fell below the plane of the beam, in addition to instances in which 3 or more toes fell to the side of the beam (e.g., the animal held on with only one toe, and in fact the foot may have been completely to the side except for that one digit). The footfault index accounted for the proportion of steps that were faults. The formula assumes that footfaults and true steps are exclusive identities. All footfaults are attempted steps, but all "steps" are non-footfaults. Therefore, the resulting formula is equal to the (# of footfaults - # of true steps)/ # of attempted steps. For an animal with four attempted steps, all being footfaults, the resulting index would equal 1 or 100%.

Chapter 6: Results

*Method of Analysis*

Three-dimensional animations of rodent performance were created on both the treadmill and beamwalk task. Qualitative observations were then gathered from these animations. Next, statistical analyses were performed on all measures and assessed as to whether they supported or contradicted qualitative findings, or provided novel information. For each task, sham animal performance is discussed followed by that of the TBI animals, and concludes with a discussion of observed change (if applicable) across injury groups.

*Treadmill Task*

*Sham Behavior*

Qualitative observation of 3-D kinematic animations on the treadmill revealed several patterns of movement. To begin, sham animals adopted an increasingly upright stance during treadmill locomotion, exhibiting greater height (i.e., distance from the treadmill) for the hip, mid-hip and mid-shoulder markers over time. However, the mid hip rose above and beyond that of the mid shoulder creating a forward slope from the hindlimbs to the forelimbs. In relation to changes in joint angles, sham animals demonstrated symmetrical hip angle change, although the direction of change (i.e., increase or decrease) varied between animals. These hip angle changes were also maintained throughout the step cycle. It is important to remember that hip angle measures are representative of each animal's hindlimb flexion ability. Therefore,

increases in hip angle indicate a decrease in flexion, while a decrease in the hip angle represents an increase in flexion.  Finally, the hindlimb stance of sham animals also appeared wider at baseline than at later time points, indicating an increase in adduction over time.  This change appeared to be bilateral.

These findings, and several additional patterns were confirmed through statistical analysis.  Across groups, hip height and mid hip height increased over the six week testing period ($F(2, 10) = 17.482$, p < ,001; $F(2, 10) = 16.548$, $p < .001$, respectively).  Although there was no statistical difference between moderate and sham groups, sham within-group means revealed an increase over time for hip (Baseline- $M = 6.338$, $SD = .456$; Week 1- $M = 7.089$, $SD = .279$; Week 6- $M = 7.726$, $SD = .454$) and mid hip markers (Baseline- $M = 6.623$, $SD = .682$; Week 1- $M = 7.420$, $SD = .450$; Week 6- $M = 8.207$, $SD = .756$).  An increase across groups in knee height and knee angle supported the above findings ($F(2, 12) = 11.579$, $p < .01$; $F(2, 10) = 3.914$, $p = .056$, respectively).  The mid shoulder marker also increased across groups during the testing period ($F(2, 12) = 10.919$, $p < .01$) but was not significantly different between groups.  Even so, within-group means for sham animals once again revealed an increase across time from Week 1 to Week 6, and overall from Baseline to Week 6 (Baseline- $M = 6.792$, $SD = .493$; Week 1- $M = 6.568$, $SD = .220$; Week 6- $M = 7.230$, $SD = .270$).  When the means for mid hip and mid shoulder are compared, a resulting "slope" at Week 1 and Week 6 from mid hip to mid shoulder is evident (see Figure 4).

The symmetrical nature of sham hip angle change is apparent when the pattern of means is explored for both contralateral and ipsilateral hindlimbs.  In congruence with qualitative observations, statistical analysis showed no significant difference between

sides ($F(1, 5) = .796$, $p = .4132$). In addition, the direction and magnitude of change at each timepoint is similar for each hindlimb in that the angles increase from Baseline to Week 1 (Baseline- contralateral, $M = 139.102$, $SD = 18.857$, ipsilateral, $M = 136.035$, $SD = 14.506$; Week 1- contralateral, $M = 141.636$, $SD = 6.129$, ipsilateral, $M = 141.718$, $SD = 2.582$), and then decrease from Week 1 to Week 6 (contralateral- $M = 135.739$, $SD = 27.553$, ipsilateral- $M = 132.358$, $SD = 4.385$), with the net change indicating a decrease in the bilateral limbs. This relationship is perhaps best represented in Figure 5.

Symmetrical increases in adduction were also confirmed by further analysis. Across injury groups, hindlimb adduction increases ($F(2, 10) = 4.328$, $p = .044$) from Week 1 to Week 6 ($t(15) = 2.115$, $p = .05$) and overall from Baseline to Week 6 ($t(15) = 2.380$, $p = .031$). Means for the sham group indicate an increase over the testing period (Baseline- $M = 2.18$, $SD = .858$, Week 1- $M = 2.019$, $SD = .790$, Week 6- $M = 1.449$, $SD = .693$) as illustrated in Figure 6. Although differences in adduction between injury groups were non-significant (power $= .263$), testing more animals resulting in higher power would allow for further investigation of group change.

In addition to qualitatively observed patterns, within animal statistical analysis of swing/stance phase duration highlighted two of three sham animals demonstrating an increase in swing phase duration over time ($F(2, 14) = 8.273$, $p < .01$; $F(2, 2) = 13.286$, $p = .07$, *trend*). As the swing phase increases, the time the animals spends in stance (in contact with the treadmill) decreases. This is most likely due to practice effects.

*TBI Behavior*

TBI animals exhibited different patterns of movement than shams. While they also adopted a more upright stance over time, mid hip height did not exceed that of the mid shoulder to a large degree resulting in a more level presentation from hindlimb to forelimb. In addition, hip angle changes for TBI animals were asymmetrical. For example, while the contralateral hip angle may have increased, the ipsilateral angle decreased, and vice versa. This was true for all five TBI animals. Their ability to adduct also appeared asymmetrical, with opposite sides of the animal exhibiting different patterns of movement.

As mentioned previously, hip, mid hip, and mid shoulder height measures across injury groups increased over the six week testing period ($F(2, 10) = 17.482$, $p < .001$; $F(2, 10) = 16.548$, $p < .001$; ($F(2, 12) = 10.818$, $p < .01$, respectively). While no significant difference between groups was noted, TBI group means for hip (Baseline- $M = 6.202$, $SD = .672$; Week 1- $M = 6.784$, $SD = .405$; Week 6- $M = 7.422$, $SD = .502$), mid hip (Baseline- $M = 6.605$, $SD = .492$; Week 1- $M = 7.052$, $SD = .277$; Week 6- $M = 7.720$, $SD = .488$), and mid shoulder height showed an increase over time (Baseline- $M = 6.288$, $SD = .317$; Week 1- $M = 6.414$, $SD = .146$; Week 6- $M = 7.084$, $SD = .567$). Although comparison of mid hip and mid shoulder height means for sham animals revealed a slope from the rear of the animal to the front, this relationship is much less pronounced in TBI animals (see Figure 7). In fact, comparison of TBI and sham postural trends reveals a smaller difference (or ratio) between mid hip and mid shoulder markers in TBI animals at two of the three timepoints, corresponding to a lesser degree of slope (see Figure 8).

The asymmetrical nature of hip angle change in TBI animals was confirmed through comparison of mean and standard deviation curves using the Peak Motus statistical analysis program. This measure was explored on an individual animal basis and evident qualitatively in all five injured animals. Two animals exhibited a decrease in the contralateral hip angle, and an increase in the ipsilateral angle over time. This type of pattern is illustrated in Figure 9*a-b*. The three remaining TBI animals demonstrated the opposite pattern, in that the contralateral angle increased and the ipsilateral decreased (see Figure 9*a-b*). This last pattern is most consistent with a loss of flexive ability in the contralateral limb due to injury.

Adduction patterns of TBI animals were variable, both when comparing between and within individuals. Overall, two of five TBI animals exhibited an increase in adduction over time similar to that of shams ($F(2, 6) = 158.745$, $p < .001$; $F(2, 10) = 131.563$, $p < .001$), while another two of five showed decreases over time ($F(2, 2) = 19.912$, $p = .048$; $F(2, 6) = 9.665$, $p = .013$), and one of five showed no significant change. In terms of within animal measures, several demonstrated movement different from shams in that adduction was asymmetrical, instead of exhibiting similar movement on both sides of the body. One animal in particular showed contralateral increases (Baseline- $M = 3.057$, $SD = .302$); Week 1- $M = 2.741$, $SD = .217$; Week 6- $M = 2.414$, $SD = .153$) and ipsilateral decreases (Baseline- $M = 1.053$, $SD = .263$; Week 1- $M = 2.099$, $SD = .353$; Week 6- $M = 2.883$, $SD = .238$) in adduction over every timepoint (resulting net change was a decrease in adduction). Here it is important to note that decreases in means indicate an increase in adduction and vice versa.

Similar to sham findings, within animal analysis of swing/stance phase duration highlighted three of five TBI animals demonstrating an increase in swing phase duration over time at every interval explored (i.e., Baseline to Week 1, Week 1 to Week 6, and overall from Baseline to Week 6; $F(2, 4) = 12.053$, $p = .02$; $F(2, 3) = 49.00$, $p < .01$; $F(2, 12) = 12.123$, $p < .01$). Again, this was assumed to be evident of practice effects.

*Beamwalk Task*

*Sham Behavior*

Animals on the beamwalk appeared to display different patterns of movement than those on the treadmill. Changes in movement also seemed more discrete, rendering fewer qualitative findings following review of the beamwalk animations. However, in regards to shams, it did appear that the animals maintained level body positioning from hindlimbs through the forelimbs, in contrast to the "slope" effect evident with increased practice on the treadmill. Changes in angle measures, adduction, and swing/stance duration were not discernable via qualitative observation, however statistical analysis helped shed light on additional patterns.

In across-groups analysis, shoulder height and mid shoulder height increased significantly over time ($F(2, 12) = 4.327$, $p = .039$; $F(2, 12) = 8.270$, $p < .01$, respectively), but there was so significant change in hip or mid hip markers. Further comparison of sham group means showed a slight elevation of the mid hip (Baseline- $M = 6.203$, $SD = .202$; Week 1- $M = 6.694$, $SD = .098$; Week 6- $M = 6.719$, $SD = .009$), and confirmed the increase in mid shoulder height (Baseline- $M = 6.189$, $SD = .185$; Week 1- $M = 6.430$, $SD = .186$; Week 6- $M = 6.669$, $SD = .064$). When taken together, the level

nature of the midline is evident and lends support to qualitative observations (see Figure 10).

Quantitative study revealed several other kinematic relationships. While there were no significant differences in adduction across groups, within animal analysis showed two of three sham animals exhibited similar patterns of change. Each animal increased in adduction from Baseline to Week 1, then decreased from Week 1 to Week 6. Although, for each animal the overall change remained an increase from Baseline to Week 6 ($F(2, 10) = 6.531$, $p = .015$; $F(2, 4) = 35.559$, $p < .01$). The initial decrease in adduction was interpreted as a surgical recovery-related deficit, which returned to a normal/expected projection (i.e., an increase) by Week 6.

*TBI Behavior*

Observation of injured animals suggests that they held their bodies closer to the beam with their hindlimbs hunched or not fully extended. This gave the appearance of the hip markers decreasing in relation to the shoulders. Different post-injury strategies also emerged, with some animals demonstrating a drop in the contralateral hip and others in the ipsilateral hindlimb. Qualitative observation of adduction and swing duration were unclear, but elucidated via statistical analysis.

Quantitative analysis of the "hunched" hindlimb observation revealed the hip (Baseline- $M = 6.842$, $SD = .361$; Week 1- $M = 6.548$, $SD = .682$; Week 6- $M = 6.839$, $SD = .520$) and mid hip markers (Baseline- $M = 7.167$, $SD = .287$; Week 1- $M = 6.888$, SD = .345; Week 6- $M = 7.258$, $SD = .390$) of TBI animals did decrease (albeit non-significantly) at Week 1. However, this change was small in comparison to significant

increases in shoulder and mid shoulder markers ($F(2, 12) = 4.327$, $p = .039$; $F(2, 12) =$ 8.270, $p < .01$, respectively), thus resulting in the possible qualitative observation of the hindlimbs dropping when in reality they were not (Figure 11). Interestingly, statistical analysis also revealed that the hindlimbs of TBI animals were held more upright than shams at baseline. And although TBI animals demonstrated little change, the result remained a group effect for injury severity, in that moderate hip and mid hip height were greater than that of shams ($F(1, 5) = 7.458$, $p = .041$; $F(1, 5) = 8.063$, $p = .036$, respectively). This difference between groups at baseline is not easily explained, however it is not thought to be due to animal size, as there was no significant difference in weight between groups at any timepoint ($F(1, 6) = .451$, $p = .5270$). Significant differences between groups on any measure at baseline are likely due to marker placement and must be taken into account when making further interpretations.

Adduction measures for TBI animals were variable. Three of five animals increased in adduction over time ($F(2, 6) = 22.822$, $p < .01$; $F(2, 4) = 7.031$, $p = .049$; $F(2, 6) = 27.545$, $p < .001$), however these changes were achieved through different mechanisms. Some of those animals who increased in adduction overall, also decreased during certain intervals, and none demonstrated significant increases consistently throughout the time period. Adduction was also explored in terms of individual hindlimb change but results were varied and no recognizable pattern resulted. More informative measures may be drawn from the footfault analysis below.

*Between Group Interactions*

Analysis of measures collected during the beamwalk task also revealed several

significant changes across time and between injury groups (Time x Injury).  Perhaps most

striking, were the Time x Injury interactions for measures of knee, ankle, elbow, and mid

hip height.  Although they are trends, the similarities between the interactions on

different measures point to reliable and consistent patterns of change, and warrant further

investigation.   Week 1 values appeared to be the main sources of variation in the

interactions, as they seemed discrepant when compared to expected patterns of change

for each injury group.

To begin, Time x Injury interactions for knee height ($F(2, 12) = 3.301$, $p = .072$)

and ankle height ($F(2, 12) = 3.233$, $p = .075$) demonstrated similar patterns of change,

with injured animals decreasing from Baseline to Week 1, then increasing from Week 1

to Week 6.  However, the overall change (from Baseline to Week 6) remained negative.

In contrast, sham animals increased in knee and ankle height from Baseline to Week 1,

and decreased from Week 1 to Week 6.  Still, net change for shams remained negative.

These relationships are most easily understood by examining Figure 12*a,b*.  Change

related to Week 1 deficits and surgical recovery clearly elicited different adaptive

mechanisms.  Similar disparities between Week 1 changes are evident in the Time x

Injury interactions for elbow height ($F(2, 10) = 3.213$, $p = .084$; see Figure 12c) and mid

hip height ($F(2, 10) = 3.465$, $p = .072$; see Figure 13).

In angle measures and those relating to flexion of the hindlimbs, there was also a

Time x Injury effect for hip angle ($F(2, 10) = 4.673$, $p = .037$; see Figure 14).  There were

no differences between injury groups except at Week 1, in which there was a trend for sham hip angles greater than moderates (Week 1- $t(3) = -2.595$, $p = .081$).

Analysis of swing/stance phase duration revealed a significant decrease in swing duration across groups over time ($F(2, 12) = 5.539$, $p = .020$), and also a significant interaction effect for Time x Injury ($F(2, 12) = 5.089$, $p = .025$) in which TBI animals exhibited longer swing durations at Baseline than shams ($t(5) = 3.235$, $p = .023$). Additional main effects analyses showed a significant decrease in swing duration over time for TBI animals ($F(2, 8) = 9.167$, $p <.01$), but no change for shams ($F(2, 2) = 3.344$, $p = .140$). It is possible that because TBI animals began with a longer swing duration, subsequent decreases over time were more evident. Nonetheless, such decreases are interesting because a decrease in swing duration means a longer stance phase. The animals were encouraged to move continuously across the beam but often moved at a slower rate. Hence, it is possible that moderate animals were unable to swiftly traverse the apparatus following injury and therefore spent more time in contact (stance) with the beam. This hypothesis is further explored in terms of footfaults and latency to traverse the beam.

*Additional Measures*

Across groups, footfault analysis revealed significantly more contralateral footfaults than ipsilateral ($F(1, 24) = 6.723$, $p = .016$), and a greater proportion of faults at Week 1 ($M = .101$, $SD = .182$) than Baseline or Week 6 ($M = .064$, $SD = .16$; $M = .080$, $SD = .206$, respectively). A time x Injury interaction ($F(2, 48) = 5.061$, $p = .01$) demonstrated TBI animals experienced most footfaults at Week 1, which was an

expected indicator of initial deficit following injury. Likewise, measures at following weeks may be considered valid representations of recovery or compensatory mechanisms. There was also a significant Side x Injury interaction in which TBI animal footfaults were more equally distributed on both sides, while shams were almost exclusively contralateral ($F(1, 24) = 5.783$, $p = .024$).

Analysis of latency to cross the beam revealed no significant differences between injury groups ($F(1, 14) = 3.055$, $p = .102$). However, *across* injury groups there was a significant increase in latency over time ($F(2, 28) = 7.453$, $p < .01$). In addition, analysis of latency measures indicated a Time x Injury interaction ($F(2, 28) = 3.450$, $p = .046$) in which TBI and sham groups differed at Baseline ($t(5) = -3.139$, $p = .026$). These findings are surprising as one would guess that latency to cross the beam would become shorter over time due to practice effects. It is possible that individual differences among animals may have distorted this pattern. Reproduction of these results with a larger sample would be helpful for more conclusive results. While this data may seem surprising, one must keep in mind that latency values were taken from the same trials used in kinematic analysis, which were successful passes and devoid of any footfaults. Thus the range of latency was limited. Although this may have hindered the ability to draw conclusions about latency, it was considered necessary by the researcher in order to collect more precise kinematic measurements and target compensatory behaviors.

Data from a previous pilot study had highlighted differences in hip height ("differentials") as potential mechanisms of compensatory behavior on the treadmill apparatus. The current investigation did not replicate these findings. While some changes in hip and mid hip height were evident, they were generally small and

symmetrical.  Instead, changes in hip *angle* were most apparent.   Kinematic analysis also

revealed changes in shoulder and mid shoulder height, as well as distinct differences in

post-injury behaviors between injury groups on the beamwalk.   These findings were

mentioned previously and will be discussed in more detail below.

Chapter 7: Discussion


Preliminary goals of this study included evaluating and quantifying changes in gait parameters following injury on both the treadmill and beamwalk tasks. Results were expected to elucidate kinematic patterns such as alterations in gait reflecting contralateral hindlimb paresis and deficits in adduction and flexion.

It would be an understatement to say that three-dimensional kinematic analysis provided an abundance of quantitative information for evaluation. This method of analysis allowed the researcher to collect information on any measure desired, with the number of individual data points limited only by protocol constraints. Changes in limb and joint height, limb angles (flexion), adduction, and swing and stance phase duration were observed, following documented initial deficits. On the treadmill, sham animals adopted an increasingly upright posture over time that was attributed to a combination of growth and practice effects. Ipsilateral and contralateral hip angle changes were also noted and observed to be symmetrical (either increases or decreases). Adduction of both hindlimbs increased over time as did swing phase duration. These patterns were considered to be representative of normal treadmill locomotion, growth, practice effects, and progression over time. Performance of TBI animals was subsequently compared to these norms.

Injured animals demonstrated changes in hip, mid hip, and shoulder height, which were similar in nature to shams yet less pronounced. For example, sham animals demonstrated an obvious sloping posture from hindlimbs through the forelimbs, while TBI animals exhibited a similar but more subtle pattern. Interestingly, hip angle changes

in TBI animals were asymmetrical, with one hindlimb angle increasing while the other decreased, and vice versa. It is important to remember that increases in hindlimb angles in comparison to the opposite limb may be representative of deficits in flexion. In other words, the animal was unable to bring the limb up towards its body as it did pre-injury. Adduction measures in TBI animals were inconsistent compared to shams, with some animals demonstrating increases over time while others decreased or showed no change. Similar to sham findings, TBI animals spent more time in the swing phase of the step cycle as the weeks progressed, perhaps gaining confidence from repeated exposure to the apparatus.

On the beamwalk apparatus, animals displayed different patterns of movement than those on the treadmill. Changes were more discrete and rendered fewer qualitative findings. Quantitatively, sham animals exhibited increases in forelimb (shoulder and mid shoulder) height, with more minimal increases in the hindlimbs (non significant but evident in group mean differences). This resulted in a level posture from hindlimbs through the forelimbs in contrast to the sloping nature of sham performance on the treadmill. No significant changes in adduction were evident, however the direction of change (increases or decreases) at different timepoints was similar across animals. TBI animals appeared to crouch on the beam during beamwalk performance. This observation was due to the hip and mid hip markers decreasing initially at Week 1, then increasing slightly from Baseline to Week 6. This slight increase was small in comparison to the change in shoulder and mid shoulder markers. This pattern of hindlimb change also differed from that found in shams in which the hip and mid hip markers slightly, yet steadily, increased. TBI animal measures of adduction were

variable.  The lack of consistent information regarding adduction on the beam could be due to the constraints of a successful crossing or confounding variables such as marker placement (discussed shortly).  Similar to latency measures, variability in adduction may have been limited due to the use of trials without footfaults.  Footfault analysis, which was derived from all attempted animal crossings,  confirmed an initial deficit in TBI animals. Footfaults were apparent on both the contralateral and ipsilateral sides, possibly due to both initial deficit (contralateral) and early compensatory behaviors (ipsilateral).

Perhaps most striking, were the effects found following statistical analysis of beamwalk performance.  Four joint height measures (knee, ankle, elbow, and mid hip) and one angle measure (hip angle) demonstrated Time x Injury interactions, indicating the patterns of change for each injury group over time was significantly different from one another.  Upon further investigation, Week 1 values appeared to be the main source of variation between injury groups.  For knee, ankle, and elbow height measures, regardless of whether the resulting change over time was positive or negative, sham animal markers increased and TBI animals decreased in height at the Week 1 timepoint. This finding is truly best illustrated by Figure 12*a-c*.  It is possible that these changes could be due to the animals' variable ability to cross the beam following injury or sham procedure.  Sham animals, having not undergone an injury, adapt patterns of movement in response to surgical recovery and are able to lift the body due to preserved motor functioning.  In contrast, shortly after injury, TBI animals experience deficits in motor functioning, and are unable to lift the body resulting in a lowering of the knees, ankle, and elbows.  Measures of mid hip height, and hip angle also demonstrate these patterns but to a lesser extent. A larger sample size and further research would be helpful in

confirming these findings. Still, the consistency of the interaction effects lends support to the notion of kinematic analysis as a useful tool in dissociating compensatory mechanisms from true recovery. It also appears that in conjunction with kinematic analysis, the beamwalk is a more sensitive measure than the treadmill in distinguishing between injury groups.

In relation to step cycle changes such as swing phase duration, there was no effect for the sham group while there was a significant main effect (decrease) for TBI animals. Therefore, the TBI group was assumed responsible for the majority of the overall variation in this measure. A decrease in swing phase duration indicates an increase in stance time, or the interval in which the animal is in contact with the beam. If this measure is then taken as a marker of skill to perform a task, it could be generalized that the TBI animals were unable to move as swiftly across the beam as shams and needed proportionally more assistance to cross. Indeed, group means for latency in the TBI group increased over the testing period. However, sham animals also increased in latency over time. In general, latency data must be interpreted with caution due to the nature of trials from which it was collected.

Overall, kinematic analysis provided abundant information for analysis on both treadmill and beamwalk performance. Different postures and patterns of movement were evident between motor tasks- each with their own sensorimotor demands- and also between injury groups. The latter part of the first stated aim was to also detect contralateral changes and weakness following injury. Unfortunately, it was difficult to quantify and confidently compare ipsilateral and contralateral limbs on some measures as data could be confounded by initial marker placement. For example, when measuring

adduction, which is confined within the "z" dimension, any misplacement of markers at Baseline within this dimension would have resulted in false readings throughout the testing period. If a contralateral marker was placed too far to the left, then adduction measures could have indicated that contralateral adduction was greater than ipsilateral at all timepoints due to marker placement alone. For this reason, certain measures were analyzed for change over time, not differences between sides. Others, such as limb height and angle measures, were not affected by such confounds but also did not detect contralateral hindlimb paresis or deficit. Small sample size, corresponding lack of power, and imprecision of marker placement could have contributed to the lack of predicted findings on certain measures. For example, power for non-significant main effects such as Side (contralateral vs. ipsilateral) and Injury group were low (.131, .127, respectively). However, other measures such as the significant effects for treadmill joint height across time were accompanied by more than sufficient power (.944 - .998). One must also take into account the dearth of prior research in kinematic assessment of brain injury in rodent models, thus making estimations of effect size and a priori power calculations difficult. Replication and expansion of kinematic studies will be helpful.

Additional aims and hypotheses focused on accurately characterizing and quantifying mechanisms of sensorimotor compensation following injury. Kinematic analysis was proven effective in characterizing component behaviors such as changes in limb height, angles, adduction, and swing phase duration. However, it was difficult to discern larger, more integrative, compensatory mechanisms. It is highly likely with a larger sample size and more effective marker placement that these patterns would become evident.

Kinematic analysis was an extremely sensitive measure and therefore useful in revealing patterns of component behavior that qualitative methods were unable to characterize. However, the detailed output of kinematic analysis also obscured or muddled more general patterns that qualitative methods did define. One might liken kinematic analysis to a high-powered microscope that can focus on individual cells of an organism. It can tell you much about those cells, but not necessarily anything about the larger macroscopic make up of the organism of which they are part. Several possibilities exist to explain discrepancies between qualitative and quantitative findings. First, it is possible that there are no group patterns of recovery or compensation, only individual within-animal mechanisms. Or it may be that qualitative patterns may only be superficial (artifactual or valid) which quantitative methods see beyond. Regardless of which conjecture is true, one must appreciate the diversity of recovery and compensatory mechanisms evident in rodent and human models of brain injury.

In 1995, Robert Newton described distinct motor strategies in the ankle, hip, and step cycle of humans. Each strategy was characterized by particular patterns of muscle activation, sway frequencies, and limits of stability. When the limits of one strategy were reached, the next was employed in somewhat of a hierarchical fashion. This same framework is also possible for rodent models. However, data provided by the present kinematic analysis appears to be a conglomeration of these strategies which have yet to be parceled into hierarchical levels or other clear functional organization. It is evident that additional, perhaps higher order, analysis is needed.

Human studies have also shown that each joint has characteristic patterns of dysfunction, which lead to spasticity, contractures, and deficits in flexion and extension

(Perry, 1999). Motor control deficits may result in primitive motor patterns, which fail to provide complete and essential joint action. Primitive patterns are often incomplete and do not allow the patient to modify output (Perry, 1999). These patterns have yet to be defined in rodent models yet are crucial for describing kinematic change and compensatory strategies.

*Additional Hypotheses*

Various mechanisms of change were hypothesized throughout the study. Further investigation is needed to confirm their accuracy and utility. To begin, the function of observed changes may be inferred by their timing within the step cycle. In this study, the first 1/4 of the step cycle (lift-off to lift-off) was spent in the swing phase. It could be assumed that changes early in the step cycle to a specific limb were responses to either lift-off or swing activity of the specified limb, or touch-down/stance activity of an opposing limb. In contrast, late cycle changes are indicative of stance activity of the specified limb, and lift-off/swing activity of the opposite limb. These hypotheses are supported by earlier work in human populations. As previously mentioned, Perry (1999) described the stance phase as involving two basic tasks: weight acceptance and single limb support. Both flexor and extensor muscle action is required for weight acceptance and must precede joint motion. Therefore, the actions that prepare the limb for stance actually commence in the late swing phase (Perry, 1999). In addition, single limb support begins with lift-off of the opposite limb, and demands control of the rate of limb progression. As one can see, no limb movement is isolated within itself as changes in the opposing limb are inevitable and necessary for movement.

It is interesting to note that over time, the pattern and timing of intra-cycle changes was relatively stable. For example, if an initial change occurred during the swing portion, changes at later weeks also were most likely to again occur in the swing portion. Ideally, patterns could be analyzed for each cycle phase. However even when setting quantifiable limits on certain cycle phases the natural change is not necessarily nicely bound by those limits, and can also vary within those limits.

In order to account for alterations in hip angles found in TBI animals on the treadmill, a mechanism of action coined "hip angle alternation and differential maintenance" was hypothesized. Hip angle change in the TBI animals was bilaterally opposite, so that when one hip angle increased the other decreased, with no observed resultant change in hip height. However, upon further examination it was noted that those hip angle changes occurred at different parts of the step cycle. Taking into account the intra-cycle timing of these changes (i.e. early or late) it appeared that the animals could have been adapting to other kinematic changes at the time while trying to maintain a steady state (no change in hip height). For example, if the contralateral hip decreased early while the ipsilateral hip increased late, a preferred differential is maintained (*see* Fig. 15).

A mechanism termed "absorption" was also hypothesized. It implies that even with quantitative methods, some changes may be hidden, unclear, or nonexistent due to the adaptive responses in other areas (e.g. aforementioned changes in hip angle do not result in hip height changes, therefore the movement would have to be absorbed in the foot angle, plantar rotation, or other unmeasured dimension).

*Limitations*

Throughout the time spent conducting this study, limitations of the protocol, behavioral tasks, and computer analyses were noted as well as obstacles that must be overcome when conducting similar studies. Every attempt was made to avoid these predefined potential obstacles. They included soliciting optimal animal performance, limiting human error or contributed variance, and avoiding confounding effects of camera and equipment movement.

Achieving consistent performance from the animals on both the treadmill and beamwalk task is vital to the success of the experiment. If the rats do not walk continuously for 5-10 step cycles then the data must be exported to an outside program for data analysis. The Peak Motus® program is only able to analyze step cycles that are back to back and cannot excise undesirable material. Therefore, in order to average several step cycles they must be collected together (not "pieced" together). In order to promote optimal performance of the animals each experimenter taking part in the project (in addition to receiving a thorough orientation to the program and equipment) was properly instructed on the techniques of animal handling, training, and testing in relation to each behavioral task. Each animal was trained over many trials several weeks prior to testing to ensure acquisition of the appropriate skills. However, it was subjectively observed that performance and motivation over the 6-week period seemed to vary regardless of prior training. It is possible that some changes thought to be due to practice effects (i.e., postural and adduction change on the treadmill), could be mitigated through more training prior to testing and injury. Reaching a ceiling on performance abilities before testing would allow a more controlled analysis of post-injury behaviors. Setting

strict performance criteria that animals must meet before inclusion and commencement of testing would be helpful. The interaction of animal growth and practice effects must also be considered and is difficult to parse from many of the results. However, statistical analysis showed no differences between injury groups in animal weight over time. Therefore, when comparing measures *between* groups weight gain is considered a controlled variable and does not warrant concern. Caution must be taken nonetheless in interpreting within injury group, and within-animal effects in terms of weight increase.

As in most research, human error is also often a contributing factor to variance. In this project error of this type is limited due to the large computer component. However, there is room for inaccuracies in the placement of reflectors on the animal and later digitization of those points. To reduce error of reflector placement the joints were tattooed in order to maintain correct position throughout the testing period. The reflectors were then centered over those tattoos during the tasks. Once the video was collected each reflector was then digitized in a process requiring experimenter and computer input. Initially, the experimenter identified the position of each reflector and then the computer tracked these markers. Having a mold or cast to hold the animal and ensure correct and consistent marker placement would be ideal. Following conclusion of the study, marker placement was deemed the most likely source of error despite all efforts to circumvent this downfall.

Additional areas of concern included movement of the equipment during testing, particularly the cameras. Any movement could greatly distort the calibration of the 3-D field and the subsequent data analysis. In order to avoid this, the equipment was re-calibrated each testing day and the camera positions were marked on the floor to monitor

and/or correct any accidental movement. Despite taking these precautions, it is important to note that any camera movement should be avoided at all costs and those conducting testing should be very aware of the position of the equipment and their environment.

Despite avid attempts to avoid these obstacles, some limitations remained. They included a small sample size, mildly variable animal performance, difficulty with initial marker placement, and unexplained differences between injury groups at Baseline (i.e., hip and mid hip height of TBI animals on the beamwalk) on a few discrete measures. The differences between injury groups were not due to differences in animal size or weight, and this finding was taken into account and controlled for when drawing conclusions. In order to capitalize in the future on the applications of kinematic analysis for research and allow consolidation and interpretation of the data it provides, several additions and modifications to the protocol would be advantageous. To recap, marker placement MUST be controlled and standardized. This weakness has been shown in previous studies, is believed to be the main source of confound in this investigation, and will continue to plague future works if not done correctly. Any confounding effects have a much larger impact when using a tool to investigate such microscopic components of behavior. Purchase of the Vicon Peak® statistical package is also recommended and allows for on-site analysis, rather than having to export data to other terminals as was done in this study. In addition, having reliable performance from a larger pool of animal subjects would be a great advantage and contribute to overall statistical power and confidence in conclusions. With more subjects, however, comes a greater abundance and complexity of data that could be easily overwhelming. The need for an adequate statistical package becomes more important with added subjects.

Even with the aforementioned limitations, three-dimensional kinematic analysis remains a potentially viable tool for more clearly characterizing individual differences in sensorimotor recovery and compensation following TBI in rat models, than previous methods to date. It is the author's opinion that there are specific sensorimotor systems involved in recovery and compensation for animal groups, while within those systems there are more individualized patterns of change. Kinematic analysis may help to identify those broad systems, as well as individual differences depending on the goals of research. In the past, many authors have advocated for greater use of kinematic analysis in efforts to overcome the shortcomings of endpoint measures, and to properly monitor and characterize post-injury behaviors. Indeed, this study has effectively demonstrated the ability of kinematic analysis to document changes in a myriad of component behaviors, as well as characterize initial injury and more chronic behaviors involved in compensation or recovery. However, it has also become apparent that no single measure, including kinematic analysis, is sufficient. It is the integration of kinematic analysis with other current methods that has the greatest potential to improve the accuracy, precision, and overall quality of information gathered from traumatic brain injury research. Kinematic analysis may serve to compliment current measures by refining data pertaining to certain points of interest, and can also contribute novel information. In turn, by advancing the methods of data collection, kinematic analysis when used in conjunction with other measures, has the greatest opportunity to contribute to the progression of the field.

References

Barbeau, H., & Rossignol, S. (1987). Recovery of locomotion after chronic spinalization in the adult cat. *Brain Research, 412*, 84-95.

[a]Baskin, Y.K., Dietrich, W.D., & Green, E.J. (2003). Two effective behavioral tasks for evaluating sensorimotor dysfunction following traumatic brain injury in mice. *Journal of Neuroscience Methods, 129,* 87-93.

[b]Baskin, Y.K., McDonald, J., Karkera, M., Dietrich, W.D., & Green, E.J. (2003). *Modification of beamwalk task for distinguishing between recovery and compensation of sensorimotor deficits in the mouse following TBI.* Unpublished poster presentation, University of Miami.

Baskin, Y.K., Merkel, E., McDonald, J., & Green, E.J. (2004). *Compensatory behavioral strategies in beamwalking following controlled cortical impact injury in mice.* Unpublished poster presentation, University of Miami.

Basso, D.M., Beattie, M.S., & Bresnahan, J.C. (1995). A sensitive and reliable locomotor rating scale for open field testing in rats. *Journal of Neurotrauma, 12*(1), 1-21.

Bervar, M. (2000). Video analysis of standing- an alternative footprint analysis to assess functional loss following injury to the rat sciatic nerve. *Journal of Neuroscience Methods 102,* 190-116.

Bouet, V., Borel, L., Harley, F., Gahery, Y., & Lacour, M. (2004). Kinematics of treadmill locomotion in rats conceived, born, and reared in a hypergravity field (2g) Adaptation to 1g. *Behavioural Brain Research, 150,* 207-216.

Boyeson, M.G., Jones. J.L., & Harmon, R.L. (1994). Sparing of motor function after cortical injury, a new perspective on underlying mechanisms. *Arch Neurol. 51,* 405-414.

Bramlett, H.M., Green, E.J., & Dietrich, D.W. (1997). Hippocampally dependent and independent chronic spatial navigational deficits following parasagittal fluid percussion brain injury in the rat. *Brain Research 762,* 195-202.

Broton, J.G., Nikolic, Z., Suys, S., & Calancie, B. (1996). Kinematic analysis of limb position during quadrupedal locomotion in rats. *Journal of Neurotrauma, 13*, 409-416.

Chen, Y., Constantini, S., Trembovler, V., Weinstock, M., & Shohami, E. (1996). An experimental model of closed head injury in mice: pathophysiology, histopathology, and cognitive deficits. *Journal of Neurotrauma, 13*(10), 557-568.

Crapon de Caprona, M.D., Beisel, K.W., Nichols, D.H., & Fritzch, B. (2004). *Brain Research Bulletin, 64*, 289-301.

Dietrich, W.D., Alonso, O., Busto, R., Globus, M.Y.-T., & Ginsberg, M.D. (1994). Post-traumatic brain hypothermia reduces histopathological damage following concussive brain injury in the rat. *Acta Neuropathologica, 87*, 250-258.

Dixon, C.E., Lyeth, B.G., Povlischock, J.T., Findling, R.L., Hamm, R.J., Marmarou, A., Young, H.F., et al. (1987). A fluid percussion model of experimental brain injury in the rat. *Journal of Neurosurgery, 67*(1), 110-119.

Fisher, M.S., Schilling, N., Manuela, S., Haarhaus, D., & Hartmut, W. (2002). Basic limb kinematics of small therian mammals. *Journal of Experiemntal Biology, 205*, 1315-1338.

Fouad, K., Metz, G.A., Merkler, D., Dietz, V., & Schwab, M.E. (2000). Treadmill training in incomplete spinal cord injured rats. *Behavioural Brain Research, 115*, 107-113.

Fujimoto, S.T., Longhi, L., Saatman, K.E., & McIntosh, T.K. (2004). Motor and cognitive function evaluation following experimental traumatic brain injury. *Neuroscience and Biobehavioral Reviews, 28*, 365-378.

Goldberger, M.E., Bregman, B.S., Vierck, Jr., C.J., & Brown, M. (1990). Criteria for assessing recovery of function after spinal cord injury: Behavioral methods. *Experimental Neurology, 107*, 113-117.

Goldstein, L.B. (1993). Rapid and reliable measurement of lesion parameters for studies of motor recovery after sensorimotor cortex injury in the rat. *Journal of Neuroscience Methods, 48*, 35-42.

Goldstein, L.B. (2003). Model of recovery of locomotor ability after sensorimotor cortex injury in rats. *ILAR Journal, 44*(2), 125-129.

Goldstein, L.B., & Davis, J.N. (1990). Beam-walking in rats: Studies towards developing an animal model of functional recovery after brain injury. *Journal of Neuroscience Methods, 31*, 101-107.

Halbertsma, J.M. (1983). The stride cycle of the cat-the modeling of locomotion by computerized analysis of automatic recordings. *Acta Physiol Scand, 521*, 1-75.

Hamers. F.P., Koopmans, G.C., & Joosten, E.A. (2006). CatWalk-Assisted Gait Analysis in the Assessment of Spinal Cord Injury. *Journal of Neurotrauma, 23*(3/4*)*, 537-548.

Hamers, F.P., Lankhorst, A.J., Van Laar, T.J., Veldhuis, W.B., & Gispen, W.H. (2001). Automated quantitative gait analysis during overground locomotion in the rat: Its application to spinal cord contusion and transection injuries. *Journal of Neurotrauma, 18*(2), 187-201.

Hamm, R.J., Dixon, C.E., Gbadebo, D.M., Singha, A.K., Jenkins, L.W., Lyeth, B.G., et al. (1992). Cognitive deficits following traumatic brain injury produced by controlled cortical impact. *Journal of Neurotrauma, 9*(1), 11-20.

Hamm, R.J., Pike, B.R., O'Dell, D.M., Lyeth, B.G., & Jenkins, L.W. (1994). The rotarod test: An evaluation of its effectiveness in assessing motor deficits following traumatic brain injury. *Journal of Neurotrauma, 11*(2), 187-196.

Kay, R.M., Dennis, S., Rethlefsen, S., Skaggs, D.L., & Tolo, V.T. (2000). Impact of postoperative gait analysis on orthopaedic care. *Clinical Orthopaedic Related Research, 374*, 259-264.

Koopmans, G.C., Deumens, R., Honig, W.M., Hamers, P.T., Steinbusch, H.W., & Joosten, E. A. (2005). The assessment of locomotor function in spinal cord injured rats: The importance of objective analysis of coordination. *Journal of Neurotrauma, 22*(2), 214-225.

Kunkel-Bagden, E., Dai, H.N., Bregman, B.S. (1993). Methods to assess the development and recovery of locomotor function after spinal cord injury in rats. *Experimental Neurology, 119*, 153-164.

Leblond, H., L'Esperance, M., Orsal, D., & Rossignol, S. (2003). Treadmill locomotion in the intact and spinal mouse. *Journal of Neuroscience, 23*(36), 11411-11419.

Mattiasson, G.J., Philips, M.F., Tomasevic, G., Johansson, B.B., Wieloch, T., & McIntosh, T.K. (2000). The rotating pole test: evaluation of its effectiveness in assessing functional motor deficits following experimental head injury in the rat. *Journal of Neuroscience Methods, 95*, 75-82.

Muir, G.D., & Webb, A.A. (2000). Assessment of behavioural recovery following spinal cord injury in rats. *European Journal of Neuroscience, 12*, 3079-3086.

Newton, R.A. (1995). Balance abilities in individuals with moderate and severe traumatic brain injury. *Brain Injury, 9*(5), 445-451.

Paxinos, G. & Watson C. (1982). *The rat brain in stereotaxic coordinates*. New York: Academic Press.

Perry, J. (1999). The use of gait analysis for surgical recommendations in traumatic brain injury. *Journal of Head Trauma Rehabilitation, 14*(2), 116-135.

Piot-Grosjean, O., Wahl, F., Gobbo, O., & Stutzmann, J. (2001). Assessment of sensorimotor and cognitive deficits induced by a moderate traumatic injury in the right parietal cortex of the rat. *Neurology of Disease, 8*, 1082-1093.

Reglodi, D., Tamas, A., & Lengvari, I. (2003). Examination of sensorimotor performance following middle cerebral artery occlusion in rats. *Brain Research Bulletin, 59*(6), 459-466.

Rodriguez-Paez, A.C., Brunschwig, J.P., & Bramlett, H.M. (2005). Light and electron microscopic assessment of progressive atrophy following moderate traumatic brain injury in the rat. *Acta Neuropathologica, 109*, 603-616.

Schallert, T., Fleming, S.M., Leasure, J.L., Tillerson, J.L., & Bland, S.T. (2000). CNS plasticity and assessment of forelimb sensorimotor outcome in unilateral rat models of stroke, cortical ablation, parkinsonism and spinal cord injury. *Neuropharmacology, 39*, 777-787.

Schallert, T., Woodlee, M.T., & Fleming, S.M. (2002). Disentangling multiple types of recovery from brain injury. *Pharmacology of Cerebral Ischemia*, 201-216.

Soblosky, J.S., Colgin, L.L., Chorney-Lane, D., Davidson, J.F., & Carey, M.E. (1997). Ladder beam and camera video recording system for evaluating forelimb and hindlimb deficits after sensorimotor cortex injury in rats. *Journal of Neuroscience Methods, 78*, 75-83.

Starkey, M.L, Barritt, A.W., Yip, P.K, Davies, M., Hamers, F.P., McMahon, S.B., et al. (2005). Assessing behavioural function following a pyramidotomy lesion of the corticospinal tract in adult mice. *Experimental Neurology, 195*(2), 524-539.

Thota, A.K., Watson, S.C., Knapp, E., Thompson, B., & Jung, R. (2005). Neuromechanical control of locomotion in the rat. *Journal of Neurotrauma, 22*(4), 442-465.

Whishaw, I.Q., Woodward, N.C., Miklyaeva, E., & Pellis, S.M. (1997). Analysis of limb use by control rats and unilateral DA-depleted rats in the Montoya staircase test: movements, impairments and compensatory strategies. *Behavioural Brain Research, 89*, 167-177.

Xerri, C., Benelhadj, M., & Harlay, F. (2004). Deficits and recovery of body stabilization during acrobatic locomotion after focal lesion to the somatosensory cortex: a kinematic analysis combined with cortical mapping. *Arch. Ital. Biol., 142*(3), 217-236.

Yu, P., Matloub, H.S., Sanger, J.R., & Narini, P. (2001). Gait analysis in rats with peripheral nerve injury. *Muscle & Nerve, 24*, 231-239.
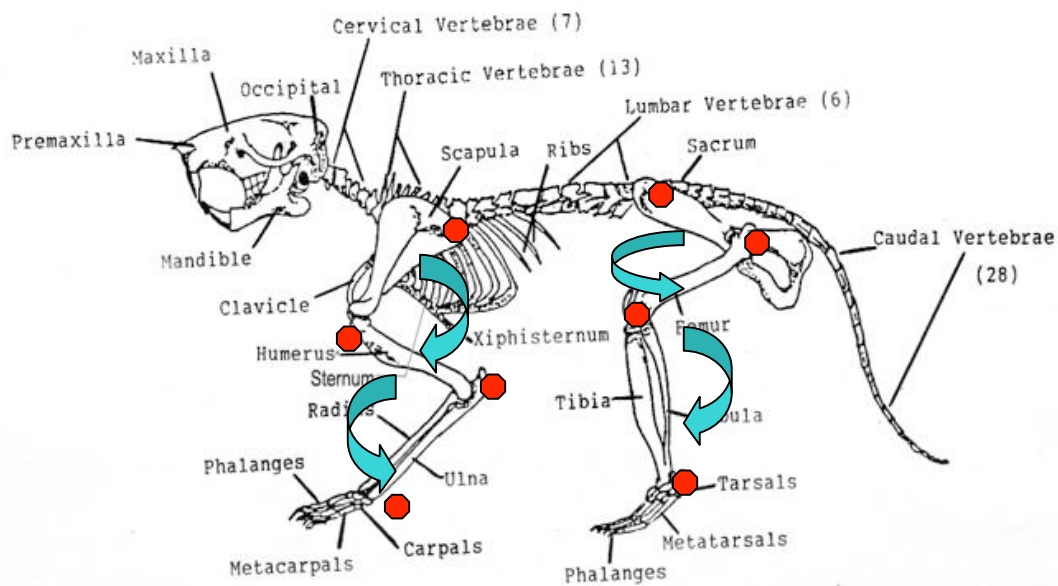
Figures
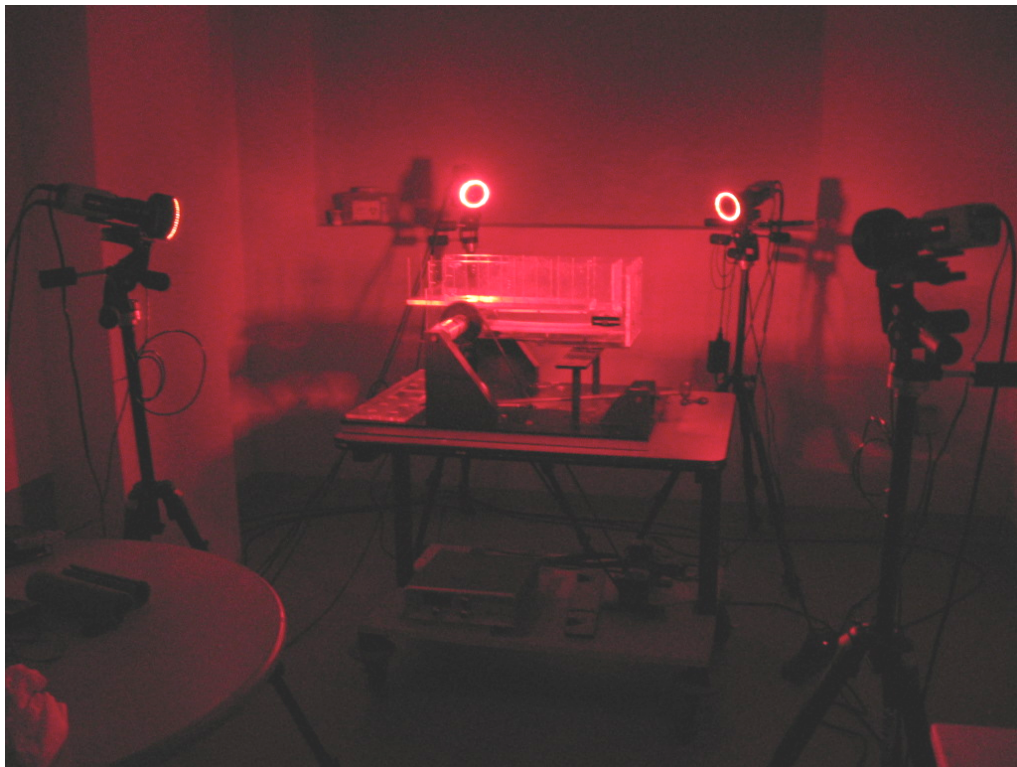


*Figure 1*. Peak Motus® equipment around central treadmill.  This is the actual configuration used during pilot testing.  Notice the infrared light rings around the camera lenses used to illuminate reflectors.
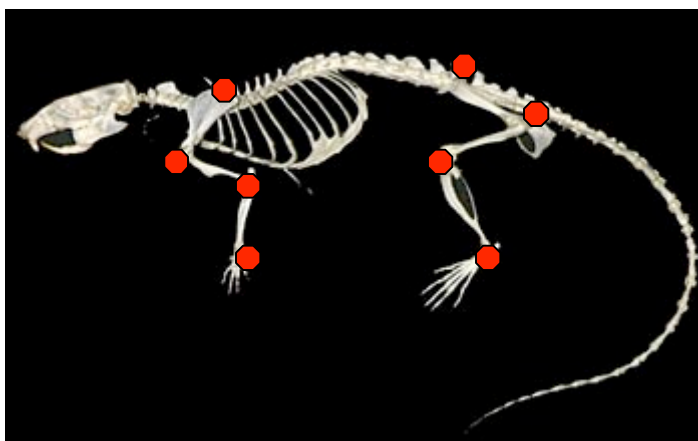
*Figure 2*. Rat on treadmill with joint reflectors post computer digitization (colored dots).

A.)



B.)



*Figures 3a-b*. Each graphic illustrates the placement of reflective markers (red dots) on various joints. Angles to be quantified (e.g., shoulder, elbow, hip, and knee) are also indicated in blue in the top illustration
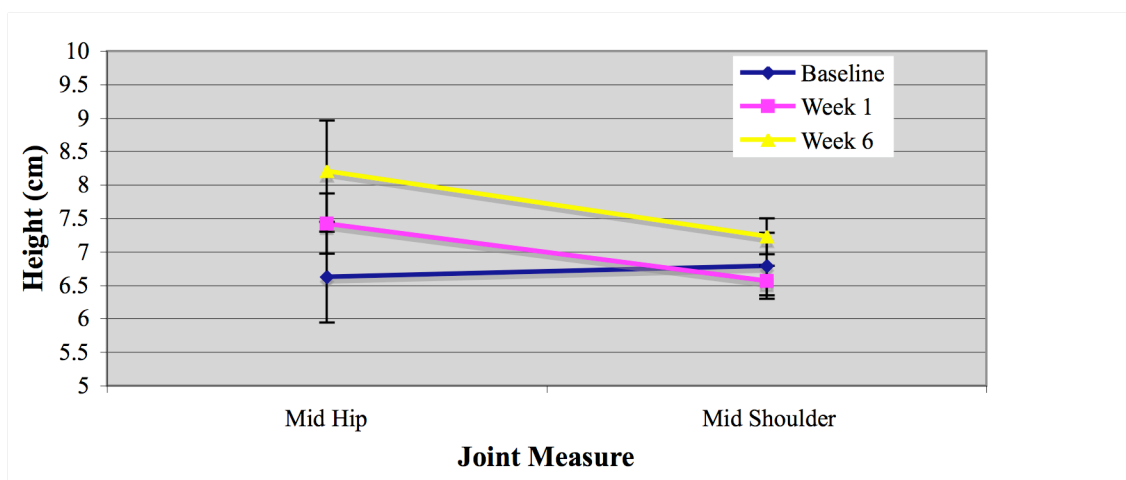
*Figure 4*. Treadmill task: Sham animal group means for mid hip and mid shoulder height. "Slope" effect is evident at Week 1 and Week 6 as the mid hip marker is higher in relation to the mid shoulder; vertical lines depict standard deviations.
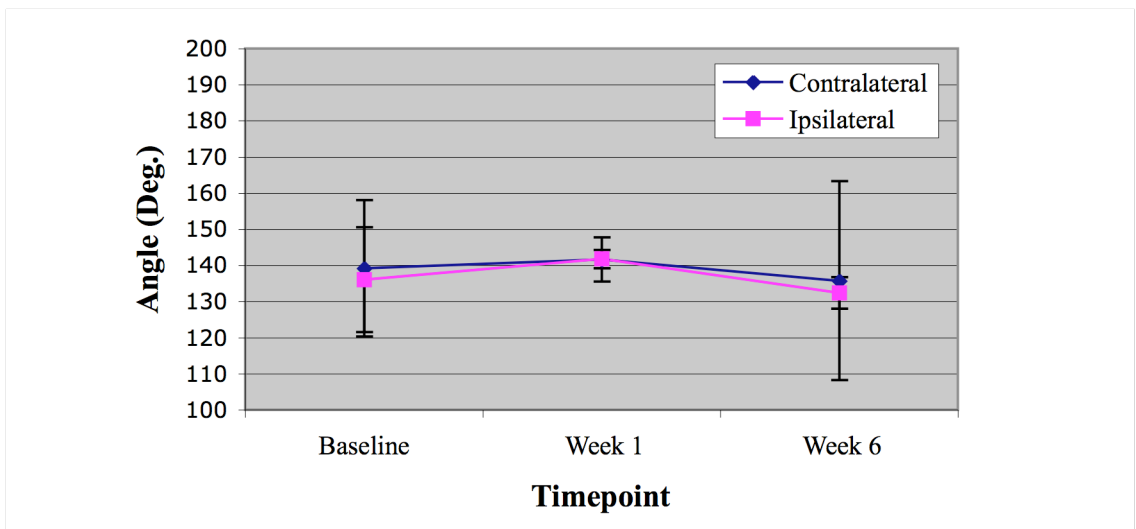
*Figure 5*. Treadmill task: Illustration of symmetrical hip angle change in shams over time via group mean comparisions; vertical lines depict standard deviations.
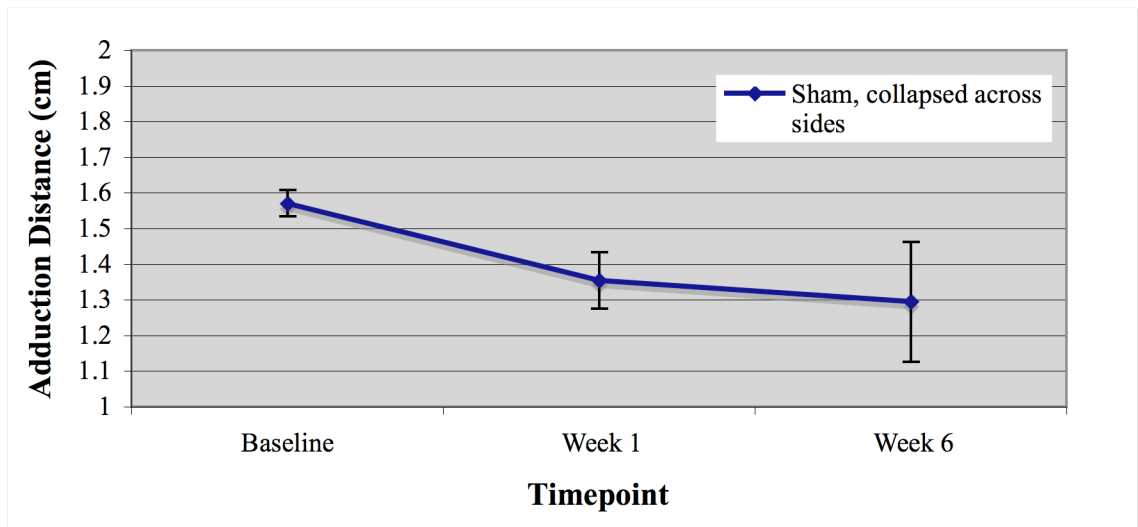
*Figure 6.* Treadmill task: Sham adduction indicated by group means over time, collapsed across contralateral and ipsilateral sides; vertical lines depict standard deviations.
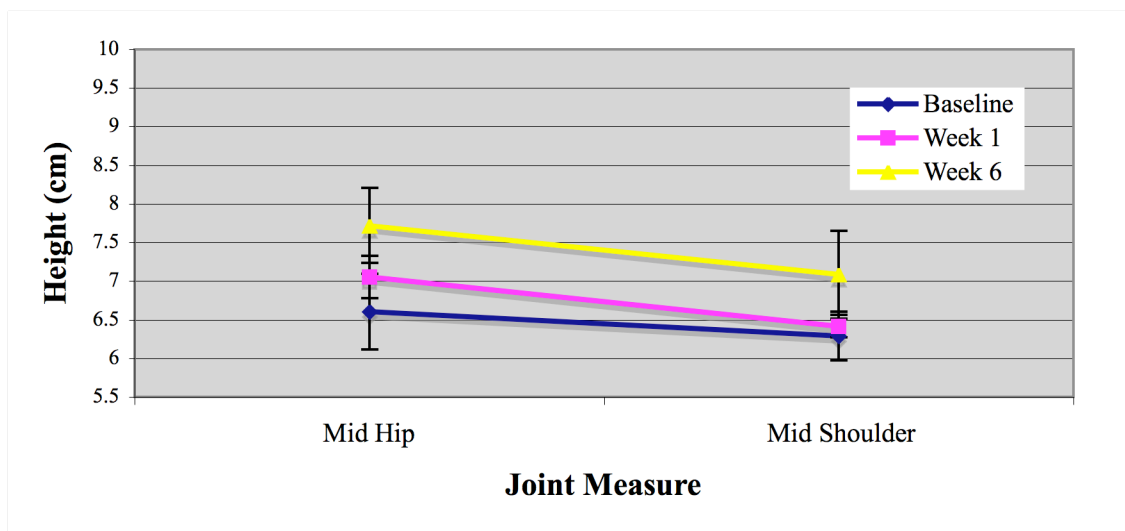
*Figure 7*. Treadmill task: TBI group means for mid hip and mid shoulder height. Note the diminished presence of "slope" from hip to shoulder at Week 6 (in comparison to Figure 4); vertical lines depict standard deviations.
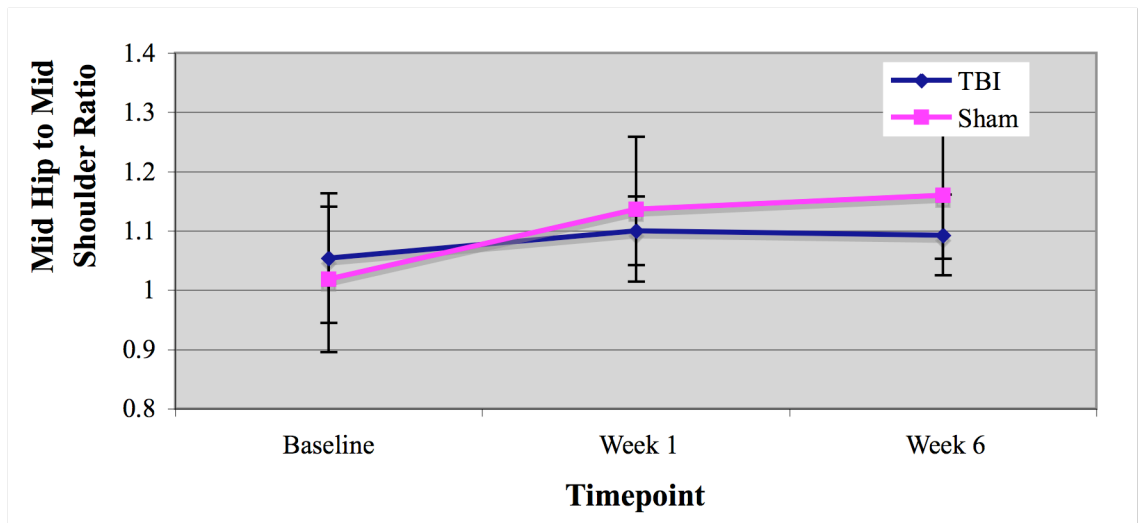
*Figure 8*. Treadmill task: Comparison of "slope" between TBI and sham groups based on the ratio of Mid Hip height to Mid Shoulder height at each time point. The TBI group exhibits smaller differences in height between the two markers (less slope) following injury at Week 1 and Week 6.
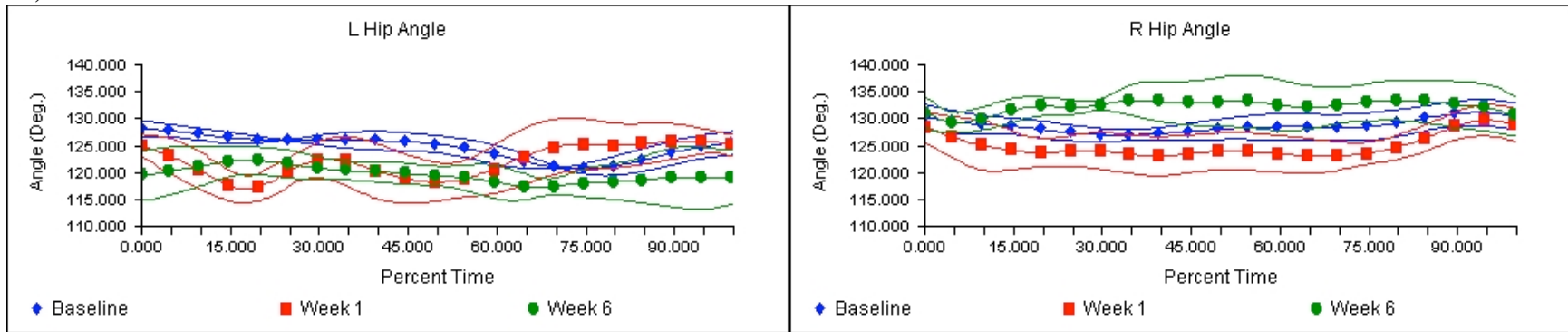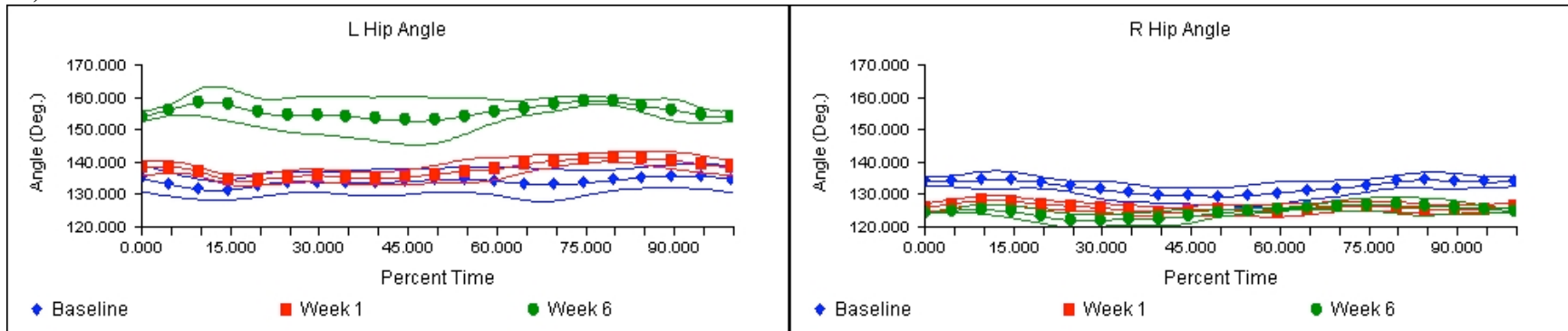
*Figure 9a-b.* Treadmill task: Various patterns of asymmetrical hip angle change in TBI animals over one normalized/averaged step cycle (lift-off to lift-off. *A.)* Individual TBI animal demonstrating contralateral decrease and ipsilateral increase. *B.)* Individual TBI animal demonstrating contralateral increase and ipsilateral decrease. Points represent mean hip angle; outside curves represent standard deviation.
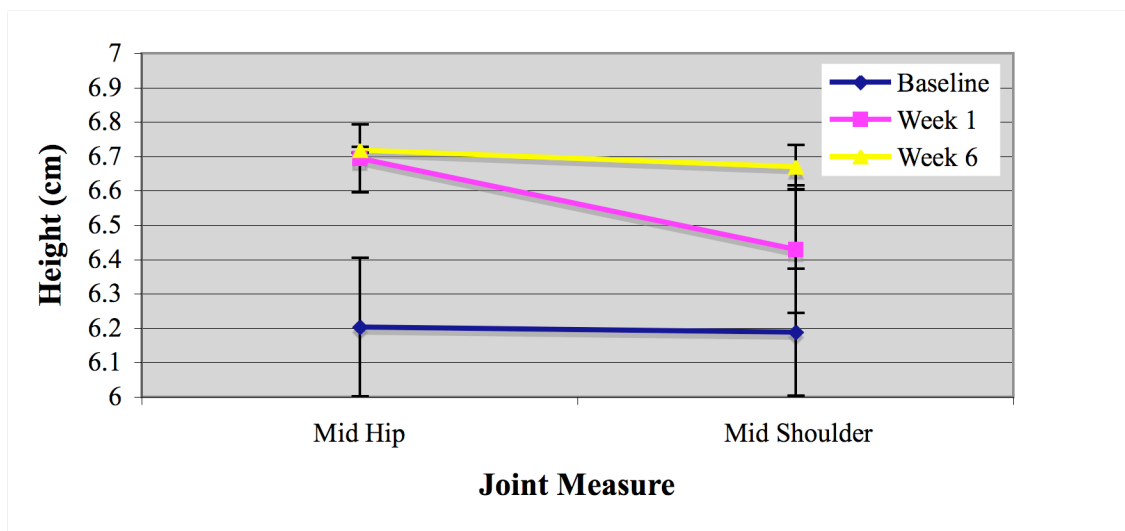
*Figure 10.* Beamwalk: Sham group mean comparison of mid hip and mid shoulder height. Notice the relatively level nature of both markers at Week 6; vertical lines depict standard deviations.
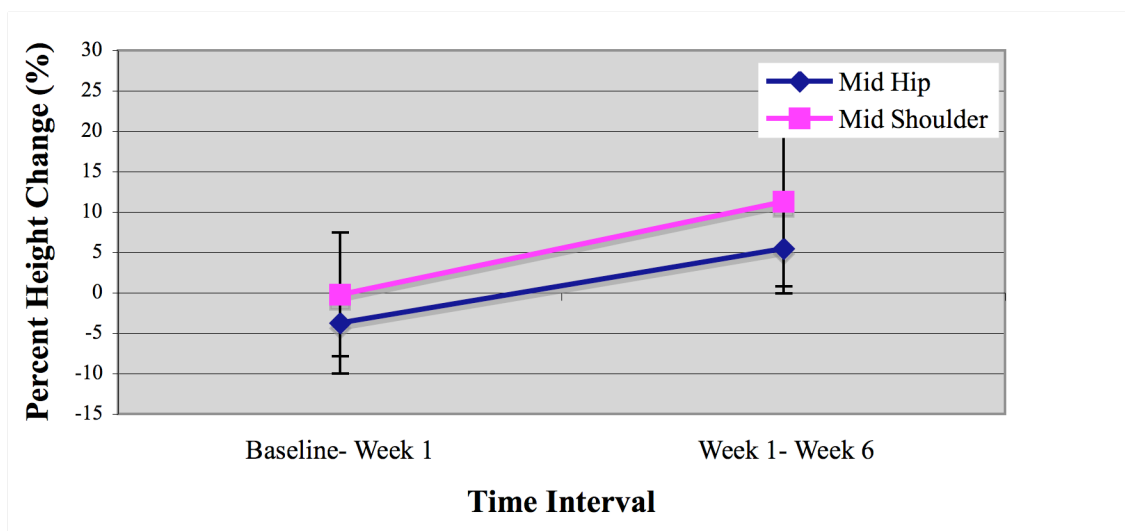
*Figure 11.* Beamwalk: Comparison of percent change in TBI hindlimb (mid hip) and forelimb (mid shoulder) height over the testing period. Notice the greater percent change in forelimbs than hindlimbs at each time interval; vertical lines depict standard deviations.
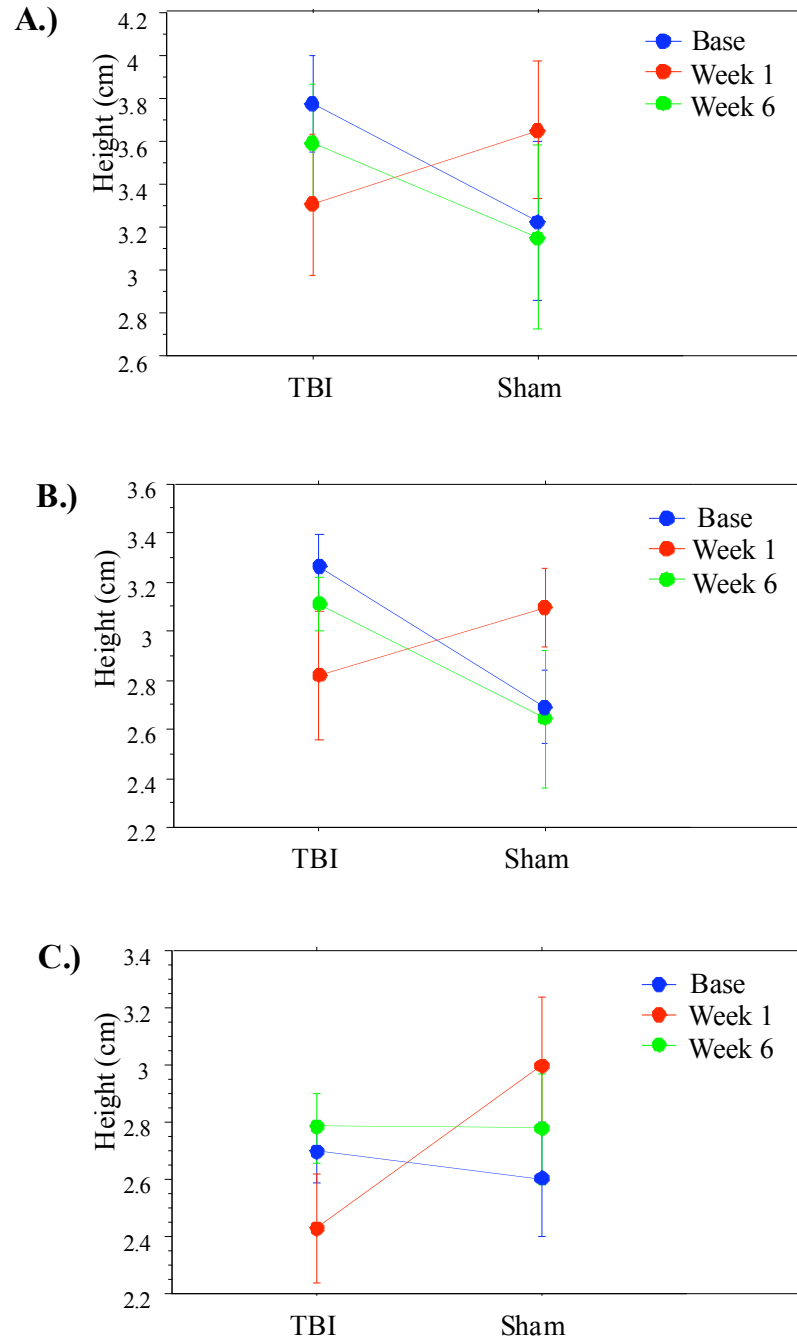
*Figure 12a-c*. Beamwalk interaction effects (trends). Consistent pattern of Week 1 changes (red) demonstrates different compensatory/recovery mechanisms based on injury group. *A.)* Knee height, *B.)* Ankle height, *C.)* Elbow height; Points are group means, vertical lines depict +/-1 standard error.
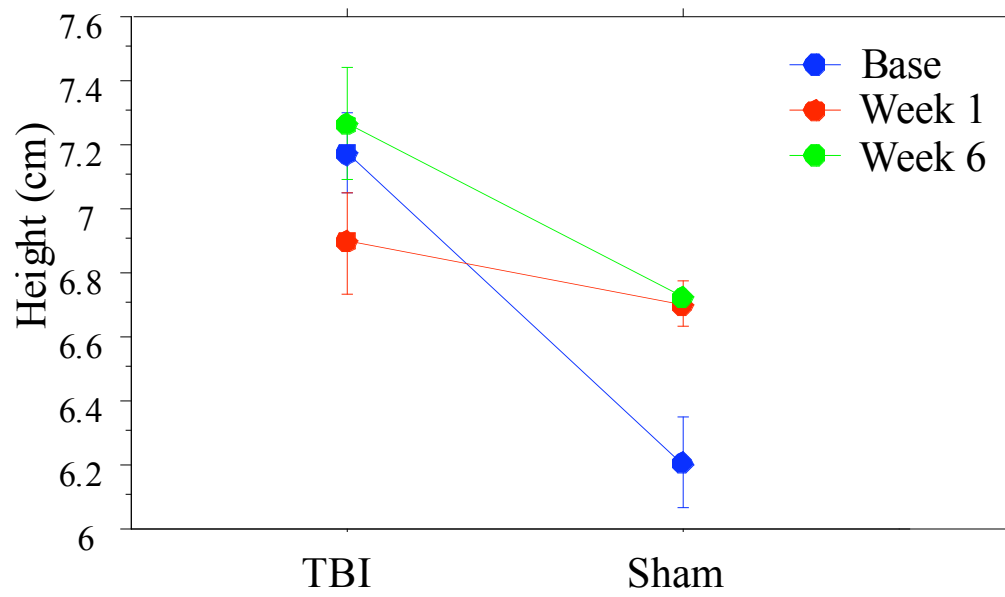
*Figure 13*.  Beamwalk interaction effect.  Mid hip height group means; vertical lines denote +/-1 standard error.
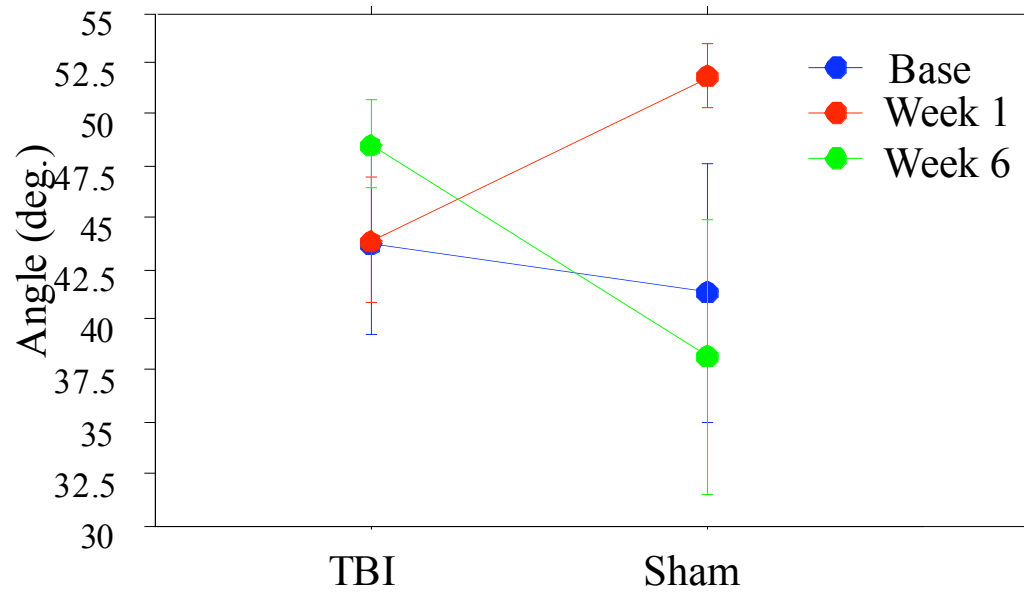
*Figure 14*. Beamwalk interaction effect. Hip angle group means; vertical lines denote +/-
1 standard error.

early cycle hip angle decrease

B

A

A = B- differential

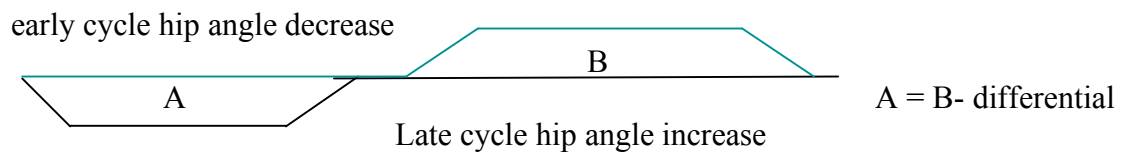Late cycle hip angle increase

*Figure 15*. Hip angle alternation and differential maintenance. Ex: As the contralateral hip angle decreases early, differential A is created.  As this decrease ends, the ipsilateral hip angle increases late, creating differential B which is equal to A.  Therefore, an equal distance or height above the treadmill is maintained throughout the step cycle.