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NEUROMECHANICAL CONTROL OF LOCOMOTION IN INTACT AND INCOMPLETE SPINAL CORD INJURED RATS

Anil Kumar Thota
University of Kentucky, anilthota@ieee.org

ABSTRACT OF THESIS

NEUROMECHANICAL CONTROL OF LOCOMOTION IN INTACT AND INCOMPLETE SPINAL CORD INJURED RATS

Rodent models are being extensively used to investigate the effects of traumatic injury and to develop and assess the mechanisms of repair and regeneration. We present quantitative assessment of 2D kinematics of overground walking and for the first time 3D joint angle kinematics of all four limbs during treadmill walking in the intact and in incomplete spinal cord contusion injured (iSCI) adult female Long Evans rats. Phase relationship between joint angles on a cycle-by-cycle basis and interlimb footfalls are assessed using a simple technique. Electromyogram (EMG) data from major flexor and extensor muscles for each of the hindlimb joints and elbow extensor muscles of the forelimbs synchronized to the 3D kinematics is also obtained in intact rats. EMG activity indicates specific relationships of the neural activity to joint angle kinematics. We find that the ankle flexors as well as the hip and elbow extensors maintain constant burst duration with changing cycle duration. Overground walking kinematics provides information on stance width (SW), stride length (SL) and hindfoot rotation (Rot). SW and Rot increased in iSCI rats. Treadmill walking kinematics provides information on joint angle trajectories. In iSCI rats double burst pattern in ankle angle as seen in intact rats is lost and knee extension and range are reduced. Intra and interlimb coordination is impaired. Left-right interlimb coordination and forelimb kinematics are not altered significantly. In iSCI rats, maximum flexion of the knee during swing occurs in phase with the hip as opposed to knee flexion preceeding hip flexion in intact rats. A mild exercise regimen in intact rats over eight weeks does not alter the kinematics.

Keywords:
Gait analysis, Electromyograms, Biomechanics, Coordination, Stance, Joint angles.

Anil Kumar Thota

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NEUROMECHANICAL CONTROL OF LOCOMOTION IN INTACT AND INCOMPLETE SPINAL CORD INJURED RATS

By

Anil Kumar Thota

Prof. Ranu Jung
Director of Thesis

Prof. David Puleo
Director of Graduate studies

May 19, 2004

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Anil Kumar Thota

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NEUROMECHANICAL CONTROL OF LOCOMOTION IN INTACT AND INCOMPLETE SPINAL CORD INJURED RATS

THESIS

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By

Anil Kumar Thota

Lexington, Kentucky

Director: Dr. Ranu Jung, Associate Professor of Center for Biomedical Engineering

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2004

To my

Grand Mother

Bhoolaxmi Thota

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Chapter 1: Introduction

Locomotion

Locomotion is a fundamental and essential feature of most terrestrial multi-cellular animals, including humans. There are several modes of locomotion, such as walking, swimming, crawling, flying etc., but all types of locomotion exhibit rhythmic and alternating movements of the body or parts of the body [1]. Vertebrates ambulate under the torque produced at the joints by contracting the agonist and antagonist skeletal muscles that attach to bones. A complex sequence of rhythmic muscle contractions and coordination of such muscle groups are required to elicit a particular motor behavior. Locomotion is controlled by: (1) supraspinal control, (2) sensory feedback, and (3) spinal central pattern generators (CPG) [2]. The repetitiveness of the motor task allows locomotion to be controlled autonomously at relatively low levels of the nervous system.

Supraspinal control

Although several studies have shown that commands from supraspinal centers are not necessary for producing the basic motor pattern of stepping [3-5], the locomotor movements must be initiated and continually modulated and manipulated to adapt the movements to the environment. The supraspinal centers thus (1) activate the spinal locomotor central pattern generator system and control the overall speed of locomotion, (2) refine locomotor function in response to feedback from proprioceptive receptors, and (3) guide the limb movements in response to visual input.

The primary components of the supraspinal centers are: the motor cortex, the mesencephalic locomotor region (MLR) and the cerebellum. Locomotion is initiated by signals from the MLR descending to the spinal CPG networks via the medial reticular formation. The tonic (non rhythmic) signal from the MLR also controls the speed of locomotion; the more the intensity of the signal the more the speed of locomotion. The cerebellum receives feedback signals via spinocerebellar pathways from the proprioceptive receptors and also an efferent copy from the CPG. These inputs are processed in the cerebellum and the brain stem nuclei for adjustments of the gait pattern and/or position of the limbs. Finally the motor cortex also fine-tunes the locomotor pattern under the guidance of signals from the visual cortex [1].

Sensory feedback

Although the results from several studies support the notion that sensory feedback from proprioceptive receptors is not necessary for central motor rhythm generation [6-8], it is essential for shaping and coordinating the neural activity. Mere pinching of the tail (exteroceptive facilitation) or rubbing the hindlimb skin is sufficient to elicit some rhythmic hindlimb movement in deafferented rats with a complete spinal transection [9-11]. Three roles that sensory feedback plays in locomotion are (1) reinforcement of CPG activation of load-bearing muscles, such as the hindlimb extensor muscles during the stance phase gait; (2) providing temporal information to ensure an appropriate biomechanical state of the moving body part in terms of position, direction of movement and force; (3) facilitating phase transitions in rhythmic movements to ensure that initiation of a particular phase of movement occurs only after the appropriate biomechanical state of the moving part has been achieved [12].

Spinal central pattern generator (CPG)

Neural circuits that produce self-sustaining patterns of rhythmic behavior without external periodic forcing are called central pattern generators (CPG). That is, the CPG is capable of generating rhythmic pattern of activity in the absence of supraspinal commands and phasic sensory input from peripheral receptors. Such CPGs for motor pattern generation are found in both invertebrates and vertebrates [12]. The evidence for the existence of spinal CPGs for locomotion in vertebrates comes from studies in in vitro preparations of the isolated brainstem/spinal cord in the lamprey [13], from in vitro preparations of the isolated brainstem/spinal cord in newborn rats [14], from studies on spinalized cats [3, 4, 15, 16], and studies on human subjects with spinal cord injury [17, 18].

The general organization of the CPGs in all the invertebrates and vertebrates studied seems to be quite similar despite dissimilar locomotor patterns in these species that range from swimming to walking, hopping, and flying [12].

In higher vertebrates the structural and functional organization mechanisms of the CPGs for locomotion are not thoroughly understood. It appears that motor CPGs are not isolated hard-wired network circuits but complexly interconnected networks of

neurons embedded within the overall spinal circuitry [19]. For example, in in vitro preparations of the neonatal rat spinal cord, it is reported that the rhythm-generating network is distributed over the entire lumbar region and extends into the caudal thoracic region [20].

The CPGs function as non-linear oscillators. Graham Brown's "half center" hypothesis, supported by studies in the 1960s, proposes that in mammals the rhythmic motor activity is generated by two mutually inhibiting neuronal pools -- an extensor half center exciting extensor motoneurons and a flexor half center exciting flexor motoneurons. Together the half-centers form a motor CPG. Coordination amongst multiple CPGs may be responsible for eliciting coordinated motor output. Grillner postulated a "shared CPG" theory to explain the development of coordinated locomotor gait in higher vertebrates [21]. This theory was based on the study of the spinal architecture responsible for swimming in the lamprey. According to this theory, distinct spinal CPGs, activated by descending pathways, exist in the spinal cord for selective control of joints or muscle groups. Coordinated movement within a limb is achieved thorough phase-dependent interactions between the different CPGs controlling that limb (e.g., between hip and knee CPGs). Another theory of "shared interneurons" was postulated by Dickinson [22] based on studies of crustaceans. As per this theory, complex movements are configured from pools of interneurons that are functionally reconfigured as required by the task, suggesting that pattern generators should be defined by the behaviors they produce rather than by anatomical boundaries (see recent review [8]). Such reconfiguration of a network of neurons has also been postulated as the mechanism for generation of multiple gait patterns such as walk, trot, and gallop in quadrupeds [23]. In summary, in vertebrates the motor CPG is a fundamental functional unit of spinal circuitry that can act independently to elicit basic rhythmic locomotor output. The CPG motor function can be initiated by supraspinal input and modified by sensory inputs.

Spinal plasticity elicited by locomotor training

The ability of the spinal cord circuitry to reorganize itself (plasticity) is illustrated by the ability of different rehabilitative training paradigms to improve locomotor recovery after spinal cord injury [8, 24-27]. Several recent studies have shown that hindlimb step

locomotor (treadmill) training of cats with completely transected spinal cords (no supraspinal input) can restore weight bearing hindlimb stepping with almost normal kinematics, although some impaired intralimb coordination persists [3, 4, 16]. The effects of the training were maximal when the training was begun one week after the spinal cord transection [28]. The plasticity of the spinal cord is task dependent and therefore the nature of the training paradigm affects the improvement of the locomotion. The spinal transected cats trained for standing can stand better and the cats trained for walking on the treadmill can walk better [28]. Nevertheless, animals that can be trained to perform one task can also be trained to perform another task.

Muir et al. demonstrated that additional sensory input along with the locomotor training accentuates and improves locomotor recovery. Locomotor function was significantly improved in chicks with spinal hemisection after receiving phasic cutaneous stimulation of the foot during swimming [29, 30]. Loading of the limbs also affected the outcomes of the locomotor training. Edgerton and colleagues recently introduced a robotic stepper-motor assist device for locomotor training of rats with complete spinal transections [31, 32]. This device made it possible to selectively load the extensor muscles using a forward force during stance phase [32]. Bilateral loading of the muscles during stance increased the magnitude of the EMG in extensors of the hip and knee and decreased both the stance and step cycle durations. When the loading was applied unilaterally, the stance duration of the loaded limb decreased along with a decrease in the swing duration of the unloaded limb, thus maintaining interlimb coordination.

Based on the locomotor recovery observed in spinal transected cats undergoing treadmill training, a similar approach has been applied in humans with spinal cord injury. Assisted treadmill locomotion with body weight support in human subjects with incomplete spinal injury has reduced or eliminated the need of assistance, reduced the amount of the weight support, and increased walking speed [33-36]. Furthermore, functional electric stimulation (FES) assisted treadmill training in which electrical stimulation of the peroneal nerve elicits a flexion withdrawal reflex has been shown to enhance walking speed and improve the gait pattern. The combined effects of partial

body weight supported treadmill training and FES based sensory reflex enhancement persisted even after termination of the electrical stimulation [25, 27].

Induction of movement by neuromodulators

Neuromodulators are neurotransmitter-like substances, delivered via the bloodstream or synaptic terminals. These can modify the function of the CPGs [37, 38] by facilitating, depressing, or initiating motor activity in neuronal circuits. In experiments performed within a week after transection of the spinal cord of adult cats, the Rossignol group showed that administering the nonadregenic agonist clonidine, elicited stable and full weight-bearing stepping patterns [39-41]. Although the effects of the drugs were temporary (lasting for 5 hours), the animals elicited sufficient flexion and extension movements during swing and stance phases. Administration of pharmacological agents that induce locomotor activity could be utilized to influence spinal plasticity.

Objective and Rationale

The long-term goal of this research is to develop and implement strategies to enhance recovery of locomotor function in human subjects with incomplete Spinal Cord Contusion Injury (iSCI). After spinal cord injury the neural system undergoes reorganization, i.e. plasticity which can occur at multiple spinal levels below and above the lesion as well as supraspinal levels [42]. Recent studies (described above) suggest that certain rehabilitation techniques and rehabilitation environments may promote neural plasticity that results in appropriate functional recovery of locomotion. Stimulation of sensory afferents and delivery of neuromodulators can produce spontaneous locomotion. Thus, a combination therapy to enhance functional recovery after incomplete spinal cord injury could consist of (1) repetitive training of a specific task (for example walking on a treadmill, (2) phasic stimulation of afferent inputs (such as proprioceptive and cutaneous stimulation during treadmill training), (3) repetitive elicitation of spinal reflexes (such as the flexion withdrawal reflex elicited by electrical stimulation of the peroneal nerve), and (4) pharmacological intervention for exciting spinal locomotor pattern generating networks (such as glutamate, GABA, glycine) via the blood stream or directly (intrathecally) to the spinal cord or a combination of one or more of the above techniques.

In order to ascertain whether locomotor recovery is improved by utilizing a therapy, it is essential that functional outcome measures be available. Besides using qualitative measures it would be beneficial to have quantitative measures. The biomechanics of gait can be examined using kinematics. Direct or indirect analysis of the position of the limb segments and joint angle trajectories (hip, knee, ankle etc) can be used to assess the range of motion of several joint angles, with detailed information on intralimb and interlimb coordination. These measures would be useful for identifying abnormalities in gait associated with different neurological disorders [43, 44].

However, kinematic descriptions do not permit distinction between active movements of walking legs and passive effects of joints acting on one another. The study of muscle electrical activity using electromyograms (EMG) is widely used as a suitable means of examining the electrophysiology involved in producing joint movements [45]. Temporal characteristics of muscle activation and cessation can be derived from on-off bursting patterns of EMG activity, while the relative amplitude provides information about muscle recruitment density. Several muscles activate in a specific motor task, each muscle contributing to a subcomponent of that task. Some muscles act as synergists to the same subcomponent of the same task. Nevertheless, measurements of EMG activity alone cannot always clearly indicate variations in leg joint kinematics. Kinematic data in combination with the EMG data can provide an integrated picture of a particular behavior and reflect the interplay between the internal control of muscles and the external application of forces that produce the observed movement of the animal. Thus, kinematics of gait and electromyogram measures of the neural control of the movement could help us understand the control of the neuromuscular system, help us examine the spontaneous recovery of the system after spinal neurotrauma, and help us evaluate the effectiveness of therapeutic approaches to enhance recovery after neurotrauma.

To address our long-term goals of developing appropriate therapeutic approaches for locomotor recovery, in this work we have used a rodent model of incomplete spinal cord injury. There are several advantages of using the rat as an animal model of choice. The thoracic contusion rodent model for incomplete spinal injury is extensively being used at the molecular, cellular, and systems level to

investigate the primary and secondary effects of traumatic injury to the spinal cord and the mechanism of repair and regeneration. Besides complete spinal transection, several methods for incomplete spinal cord injury have been developed. Some of these methods are: hemisection [53, 54], graded contusion injuries that have been well characterized [55-59], and focal chemical lesion of gray matter using kainic acid [60]. In many of the injury models, neural transplants and pharmacological intervention for restoring locomotor function have been utilized e.g.[11, 60-67]. The contusion injury model closely resembles compression type injuries seen in several human spinal injuries [59]. To assess neuromechnaical control of locomotion in the rat kinematic assessment of 2D overground walking and 3D treadmill walking can be utilized along with EMG measures. Qualitative locomotor scores for monitoring recovery after injury can also be utilized.

Specific Aims

Our specific aims are:

1. Determine neuromechanical control of locomotion in the intact rat. 2D kinematics of overground walking and 3D kinematics of treadmill walking are used to describe the normal locomotor gait patterns of rats. The 3D kinematics is also related to EMG assessment of neural activity of flexor and extensor muscles. These data also form a basis set for comparison with the kinematic and EMG patterns in rats with iSCI.
2. Determine the recovery in gait kinematics and electrophysiological correlates of muscle activity during locomotion after incomplete thoracic spinal cord injury.

   We use 2D and 3D kinematics and EMG activity of muscles to describe the recovery of locomotion over 9 to 13 weeks after incomplete thoracic spinal cord injury in adult rats. The degree of motor impairment is also classified using a locomotor rating scale. We test the hypotheses that:

   (a) The gait kinematics and neural control of muscle activity will improve over time post incomplete injury and (b) the recovery measures will be related to the level of injury. We utilize a locomotor score to grade the level of injury.

Thesis Organization

This dissertation describes qualitative and quantitative assessment of the neuromechanics of normal rodent gait and alterations in the neuromechanics after an

incomplete thoracic spinal cord injury. The thesis is divided into four chapters. They are organized as follows:

Chapter 1 introduces the thesis; provides a brief literature review on locomotor pattern generation and our current understanding of spinal cord reorganization following spinal neurotrauma, and presents the specific objectives of the thesis and the rationale for the objectives. The next two chapters are self contained. The chapters begin with a brief introduction and relevant review of the literature followed by detailed description of methods. The results are then reported and concluded with the discussion of the results. At the end of each chapter, a list of relevant references, tables and figures are included. Chapter 2 addresses specific aim 1. In chapter 3, we address specific aim 2. We utilize all the techniques developed in chapter 2 to evaluate the inherent recovery of locomotion in iSCI rats in chapter 3. In chapter 4, we present final concluding remarks and discuss opportunities for future work. The software developed for analyzing the data is presented in Appendix 1. The entire bibliography completes the thesis.

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Chapter 2: Neuromechanical control of locomotion in intact rats

Introduction

On thoracic spinal cord injury, the descending supraspinal and propriospinal control of and interaction with spinal neural circuitry caudal to the lesion is interrupted. This occurs, not only because of the break in communication but because of a cascade of deleterious events at the cellular and molecular level that have both immediate and long term consequences [1-3]. Depending on the site and severity of the injury several physiological control systems are affected, one of them being the locomotor control system. The initiation and control of rhythmic locomotor activity as well as balance and posture are affected even when the subject is stationary. With incomplete spinal cord injury recovery of motor function can occur and it likely relies on the ability of the nervous system to reorganize its circuitry through multi-site plasticity [4]. While the mechanisms of recovery are still unknown, functional recovery appears to be an activity-dependent process that can be influenced by appropriate locomotor training [5-11].

Rodent models are extensively being used at the molecular, cellular, and systems level to investigate the effects of the traumatic injury and to develop and assess the mechanisms of repair and regeneration [12-19]. A thoracic contusion injury results in an inability of the animal to balance, weight-support, and achieve appropriate movement of the hindlimbs. In order to characterize the effects of injury on motor deficits and ascertain whether locomotor recovery is improved by utilizing a therapy, it is essential that functional recovery outcome measures be available. Functional recovery can be assessed using behavioral scoring (endpoint measures), kinematics, kinetics, and electrophysiological measurements [20-24]. Kinematics allows us to examine the biomechanics of gait. 2D kinematics of overground walking in the rat has been utilized to assess stance widths, stride lengths, hindlimb rotation, footfall patterns and weight bearing capacity (e.g. [20, 24-32]. Although, fewer studies exist, kinematics to assess the position of the limb segments and joint angle trajectories has also been performed. Measures of the range of motion of joint angles, intralimb and interlimb coordination can be useful for identifying abnormalities in gait associated with different neurological

disorders [33, 34]. Documentation of the hindlimb angles (hip, knee, ankle and hip height) during treadmill walking and running using external markers has been performed for qualitative and to some extent quantitative assessment of gait [26, 27, 35-40]. Forelimb-hindlimb coordination values for the wrist and ankle joints have also been reported [26]. X-ray cinematography, which cannot be used routinely but is the most accurate, has been used to document hindlimb joint angle trajectories during treadmill walking [35, 41]. It has also been used to determine the kinematics of the rat forelimb joint angles during gallop in an activity wheel [42]. Most often, the analyses of the joint angle trajectories have been confined to a 2D analysis of a single limb and have relied on a single camera placed perpendicular to the direction of motion. In order to examine interlimb coordination and movement of limb segments in different directions, as often observed in injured animals, a 3D kinematic approach would be useful. To our knowledge, such a comprehensive evaluation of the kinematic analysis of the ipsilateral forelimb and hindlimb or all four hindlimbs together for treadmill or overground walking has not been reported.

Kinematic descriptions do not permit distinction between active movements of walking legs and passive effects of joints acting on one another. Electromyogram (EMG) recordings can unambiguously indicate the presence of active control of the muscle by the nervous system and such recordings are widely used as a suitable means of examining the electrophysiology involved in producing joint movements [43]. Temporal characteristics can be derived from on-off bursting patterns of EMG activity, while the relative amplitude can provide information about recruitment density. Several investigators have recorded EMG activity of different hindlimb muscles during treadmill walking in the rat e.g. [35, 36, 44-50] while a few investigators have reported forelimb muscle activity during treadmill locomotion [51] and during activity wheel locomotion [42].

Activation patterns of muscles can have both flexor and extensor components depending on whether they are uniarticular or biarticular [36]. Kinematic data in combination with the EMG data can provide an integrated picture of a particular behavior and reflect the interplay between the internal control of muscles and the external application of forces that produce the observed movement of the animal [52,

53]. Only a few studies have recorded EMG activity and kinematics during rodent walking e.g. [35, 37, 50, 54, 55] including a recent study in the mouse [56].

In this work, we present a quantitative assessment of 2D kinematics of overground walking and 3D joint angle kinematics of all four limbs during treadmill walking in the adult female Long Evans rat. These data allow us to examine different parameters of gait and intralimb and interlimb coordination. We present a simple technique to quantitatively assess the pattern of coordination amongst different limbs. We also examine the effects of mild exercise on the kinematics of walking. This latter assessment also allows us to evaluate our ability to obtain reliable kinematic data from multiple recording sessions over a span of several weeks in the same animal. We combine the 3D kinematic joint angle assessment with synchronized EMG data from major flexor and extensor muscles for each of the hindlimb joints and elbow extensor joints of the forelimbs. Data and techniques described here are likely to be useful for quantitative comparisons of gait in rodent models of spinal cord injury.

Materials and Methods

Neuromotor assessment of gait was performed in 26 adult (71±4 days old) young female Long-Evans rats weighing 211±15 gms (Group 1). Kinematics of gait were obtained from 2D overground walking measures and 3D treadmill walking measures. In a group of 9 rats (Group 2), neural activation of different flexor and extensor muscles of the hindlimbs (n=9) and extensor muscles of forelimbs (n=7) was assessed in conjunction with the 3D kinematics using electromyograms (EMGs). In a second subgroup of 6 rats (Group 3), the effects of regular treadmill walking exercise (8 wks) on the overground and treadmill kinematics of gait were assessed. An organizational chart indicating the different neuromotor assessment measures is shown in Fig. 2.1A and detailed methods for data collection and analyses are given below.

2D kinematics (Overground Walking)

To perform the 2D kinematic footprint analysis reflective markers (3M retro-reflective tape (4x4 mm) were stuck on the ball (bottom of the 3rd metatarsal) and heel of the plantar surface of the hindpaws and on the belly (5mm diameter). The animals walked uni-directionally, 5 passes in a straight line and at approximately constant velocity, over a transparent bottom elevated Plexiglas track (91.5 x 12 x 16 cm),

illuminated from below. A 60 Hz camera (Sony® handycam, CCD TRV 85) placed to view the entire track from below was used for video capture (Pinnacle DC30 video capture board). A calibration rectangle (21.7 x 9.75 cm) placed witin the transparent bottom track was used for affine scaling of pixel distances. The captured video was preprocessed using Adobe Premiere, and then exported to kinematic image analysis software (Peak Motus®) for digitizing the x-y positions of the reflective markers. 2-5 complete step cycles were digitized per pass. Vectors drawn between the ball and heel markers with respect to the direction of movement defined the left and right hindfoot rotation angles. The average velocity of the movement of the body during the overground walking was obtained by tracking the belly marker. Post processing of the digitized data was done using custom software written in Matlab® .For each step cycle within a pass, the hindlimb (HL) stance width (SW: perpendicular distance between the left and right ball markers), left (right) stride length (SL(SR): distance between the ball markers of the left(right) hindlimb in two consecutive steps), left (right) stride length normalized by velocity (SLv (SRv)) and the total (left+right) hindlimb foot rotation angle (Rot) were calculated. For the Group 3 rats 2D kinematic data were repeatedly obtained once a week (every 6-7th day) for eight weeks.

3D kinematics (Treadmill walking)

All rats were trained to walk on a single lane treadmill (Columbus Instruments) for 15 minutes/day for 4 days and given a fruit loop reward. 3D-kinematic data were collected on the 5th day. Additionally, the rats, were anesthetized (Sodium Pentobarbital, 30 mg/kg ip) to tattoo the bony processes of the pelvis (anterior rim), hindlimb (head of the greater trochanter, and lateral head of the femoral condyle), and forelimb (greater tubercle, and lateral epicondyle). As described by Gruner et al. [35]. the hindlimb hip angle is formed by the anterior rim of the pelvis, the greater trochanter and the lateral head of femoral condyle, the knee angle by the greater trochanter, the lateral head of femoral condyle and lateral malleolus, and the ankle angle by the lateral head of femoral condyle, lateral malleolus and the fifth metatarsal head. For the forelimb, the shoulder angle is formed by the greater trochanter, the greater tubercle, and the lateral epicondyle and the elbow angle is formed by the greater tubercle, the lateral epicondyle and the fifth metacarpal. The tattoo marks improved accuracy and

repeatability of reflective marker placement for longitudinal studies and reduced inter-investigator variability.

The 3D kinematic data were collected using Peak Motus®, while the rats were running on the single lane treadmill on all four limbs without any body weight support. Cone shaped markers made out of 5 mm diameter circles of self adhesive infrared reflective tape (3M) were placed on the pre-tattooed spots on the pelvis, hindlimbs and forelimbs as well as the lateral malleolus and fifth meta-tarsal of the hindlimbs and the fifth metacarpal of the forelimb in order to determine the hip, knee, ankle, shoulder, and elbow joint angles. One marker was also placed on the treadmill belt to calculate the velocity of the treadmill.

The Peak Motus® motion analysis video capture system consisted of four black and white, CCD, genlocked digital cameras placed at approximately ten feet from the treadmill, such that any given reflective marker on the rat was visible in two of the cameras. Two infrared lights attached on either side of each camera illuminated the retro-reflective markers. Video from the cameras was input to an acquisition station composed of four SMPTE (Society of Motion Picture Television Engineers) time code generators, an Event and Video Control Unit (EVCU) and four VCR's, interfaced with a workstation running the Peak Motus® software. The EVCU not only works as a video switcher box but also handles user input events to synchronize external analog data. The functional block diagram for the data acquisition system is shown in Fig. 2.1B.

Prior to data collection, calibration of 3D space was performed using a customized rectangular calibration cube (Fig. 2.1C). The calibration cube consists of 44 points distributed among 10 rods. The points span the 3D space in which the rat walks on the treadmill. 3D kinematic data were collected in 5-10 minute long sessions with the treadmill running at speeds ranging from 18 to 21m/min (30 cm/s to 35cm/s) ensuring that the animal was able to run comfortably at a constant pace without sliding on the treadmill.

From the video captured, the reflective markers were tracked and digitized offline for each of the four camera views. Fig. 2.2 A-F shows six frames during a gait cycle. From the digitized data, the hip, knee, ankle, shoulder and elbow joint angle trajectories and the limb segment positions were calculated for 5-20 cycles. The video frame, in

which the rat's hindlimb (forelimb) toe touched the treadmill belt, was marked as a touch-down (TD) event (Fig. 2.2A for right hindlimb TD). Similarly, a lift-off (LO) event was marked when the rat's hindlimb (forelimb) toe lifted off the treadmill belt (Fig. 2.2D for right hindlimb LO). For each limb, the swing duration (LO to TD), the stance duration (TD to LO), and step cycle duration (TD to TD) were calculated on a cycle-by-cycle basis. In addition, for each joint angle, for each limb, the maximum flexion in swing (SW Min°) and maximum extension in stance (ST Max°) and a range of movement (ST Max° – SW Min°) were calculated on a cycle-by-cycle basis. The joint angle values at touch down (TD Val°) and lift off (LO Val°) were also determined. For each rat an average joint angle trajectory was obtained by averaging 3-22 (average 9) gait cycles (normalized to 100%).

Joint angle-angle plots (hip vs. knee, knee vs. ankle, and shoulder vs. elbow) were used for graphical qualitative assessment of intralimb coordination. Quantitative assessment of intralimb coordination was obtained using a phase relationship measure assessed from the time instant at which the maximum flexion of the hip/ knee/ ankle/ shoulder/ elbow joint occurred during the swing phase of each step cycle of the limb (LO to TD). Thus, we denoted the moments of time of maximum flexion during the swing phase of each hind limb step cycle for the hip joint angle for each cycle as τhi, i= 0, 1, 2, … N, for the knee joint angle as τki, i = 0, 1, 2, …., N, and for the ankle joint angle as τaj, j = 0, 1, 2, …., N. Similarly we defined the maximum flexion during the swing phase of each forelimb step cycle for the shoulder joint angle as τsi, i= 0, 1, 2, … N and elbow joint angle as τei, i = 0, 1, 2, …., N. Then the phase of the knee with respect to the hip in the ith cycle is given by equation (1), of the ankle to the hip by equation (2), and of the elbow to the shoulder by equation (3) [57]

$$\phi(\tau hk_i) = \frac{(\tau h_i - \tau k_i)}{(\tau h_{i+1} - \tau h_i)}, \quad \tau k_i < \tau h_i < \tau k_{i+1} \cdots\cdots\cdots\cdots (1)$$

$$\phi(\tau ha_i) = \frac{(\tau h_i - \tau a_i)}{(\tau h_{i+1} - \tau h_i)}, \quad \tau a_i < \tau h_i < \tau a_{i+1} \cdots\cdots\cdots\cdots (2)$$

$$\phi(\tau se_i) = \frac{(\tau s_i - \tau e_i)}{(\tau s_{i+1} - \tau s_i)}, \quad \tau s_i < \tau e_i < \tau s_{i+1} \cdots\cdots\cdots\cdots (3)$$

Defined in this way, the phase varied between 0 and 1 or 0 and –1 and was defined at discrete moments of time.

Angle-Angle plots between the forelimb and hindlimb joint angles provide qualitative information on interlimb coordination. Quantitative assessment of forelimb-hindlimb coordination can also be performed to assess whether there is one-to-one correspondence between the forelimbs and the hindlimbs and to ascertain the relative phase of each limb with respect to another within a gait cycle. Thus, if we denote the time instants of touch down of the right hindlimb as τrhi, i= 0, 1, 2, … N and similarly we denote the times of touch down of the ipsilateral forelimb as τrfk, k = 0, 1, 2, …., M. Then, the phase of the kth cycle of the right forelimb with respect to the right hindlimb was calculated as [58;57]

$$\phi\left(\tau rflh_k\right)=\frac{\left(\tau rf_k-\tau rh_i\right)}{\left(\tau rh_{i+1}-\tau rh_i\right)},\quad \tau rh_i<\tau rf_k<\tau rh_{i+1}$$

Defined in this way, the phase varied between 0 and 1 and was defined at discrete moments of time. It is possible in this scenario to have more than one forelimb step cycle for each hindlimb step cycle or for no forelimb step cycle for a given hindlimb step cycle.

Neural measures (EMG)

In the nine Group 2 rats, after acquiring the initial kinematic data, upto fourteen bipolar EMG electrodes were implanted under aseptic conditions and surgical anesthesia (Sodium Pentobarbital, 40 mg/Kg i.p., additional doses as needed), bilaterally in six muscles per hindlimb and one muscle per forelimb. These muscles were the iliopsoas (IL; hip flexor), biceps femoris (BF; hip extensor but also knee flexor), semimemberanosus (SM; knee flexor but also hip extensor), vastus lateralis (VL; knee extensor), tibialis anterior (TA; ankle flexor), gastrocnemius medialis (GM; ankle extensor but also knee flexor) and the triceps brachialis (TB; elbow extensor). The bipolar EMG electrodes were fabricated from Teflon-insulated multi-stranded fine wires (AS-633 Cooner Wire) and a tiny restraining disc (~2.3mm diameter mylar sheet). Each of the bipolar electrodes was designed such that it entrapped the muscle belly between the mylar disc attached to the distal end of the electrode and a proximally placed knot. After the EMG electrodes were implanted in the muscles and checked for proper

placement and viability by back-stimulation the wires were routed sub-cutaneously to a 30 pin nano-series custom head connector (Omnetic Corporation®) affixed to the skull surface using dental acrylic and anchored self-tapping screws. A ground wire connected to the head connector was subcutaneously routed and sutured to the muscles on the back.

EMG data along with the 3D kinematics was collected in this group of rats 3-5 days after recovery from implant surgery. The reflective markers were applied as explained above and the head connector connected to an overhead shielded cable system that was in turn connected to a bank of 14 differential AC amplifiers (A-M Systems; Model 1700; amplified and band-pass filtered (100Hz-1KHz). The animals were able to move on the treadmill unrestrained and unaffected by the weight of the cables over head. The EMG signals were manually synced to the video data on all four videos through a sync pulse generated by the EVCU.

The EMG cycle duration (CD; time between consecutive burst onsets), burst duration (BD; duration between onset and termination of neural activity), burst proportion (BP=BD/CD) of neural activation was assessed for each muscle for each gait cycle. Additionally, the phase of the gait cycle at which the flexor and extensor activity were initiated and terminated was also determined.

Statistical Analysis

Mean ± standard error of the mean (SEM) values for all of the measured variables is reported. 2D data were averaged for all passes per rat per trial day. 3D kinematic and EMG data were averaged on a cycle by cycle basis. To assess the effects of mild exercise on kinematics, one-way repeated measures ANOVA were utilized to compare the control 2D kinematic data with that for each of the 8 weeks of exercise and to compare the control 3D kinematic data prior to exercise (week 0) with that after 1, 5 and 8 weeks of exercise. Values of $p < 0.05$ were considered significant.

Results

2D kinematics

2D overground kinematic analysis was performed on all rats. The stance width which is functionally defined as the base of support was 2.24$\pm$0.07 cm and the left and right stride lengths were 17.61$\pm$0.33 cm and 17.57$\pm$0.33 cm respectively. The rats

walked at a velocity between 33–72 cm/sec (52.55±1.99 cm/sec). The stride lengths were linearly related to the velocity (correlation coefficient =0.63, 0.71 for R-Strd and L-Strd respectively). The normalized R-Strd/Vel and L-Strd/Vel were 0.351±0.012 and 0.356±0.011 respectively. Left and right stride lengths were almost equal, indicating that the weight was being transferred equally between the left and right hindlimb. The low standard error of the velocity and the almost equal normalized stride lengths for the left and right hindlimbs indicate that the rats walked at a relatively constant speed. The combined (left + right) hindlimb foot rotation angle was 37.13±1.38°. The values of the different 2D kinematic measures in the Group 3 rats (Table 2.3) did not show statistically significant differences over the eight weeks of treadmill walking.

3D kinematics:

Fig. 2.3 A-H is illustrative of the 3D kinematic data obtained during treadmill walking from each rat. In Fig. 2.3A stick figure representations of the hindlimb during 5 consecutive gait cycles (out of 34 cycles) illustrate the position of the right hindlimb at regular intervals of time projected in 2D space. The limb segments shown are pelvis to hip, hip to knee, knee to ankle and ankle to toe (top to bottom) while the rat is walking on a treadmill in the forward direction (left to right). The limb segments move opposite to the direction of movement in the stance phase (indicated by the long arrow pointing left) and along the direction of movement during swing (short arrow pointing right). Similar to the right hindlimb stick figure, in Fig. 2.3G the right forelimb stick figure shows the shoulder to elbow and elbow to wrist joint segments (top to bottom) during walking.

The right hindlimb trajectories for the hip, knee, and ankle joints are plotted in Figs. 2.3B, C, D respectively and for the right forelimb shoulder, and elbow joints in Fig. 2.3E, F respectively along with TD (vertical solid line) and LO (vertical dashed line) event markers. Maximum (max) angles and minimum (min) angles are the maximum extension and maximum flexion angles. The portion of the angle trajectory between a TD and its corresponding LO is when the HL/FL is in contact with the treadmill belt (stance phase). Similarly, the portion of the angle trajectory between LO and TD is, when the HL/FL is in the air (swing phase, shorter than stance phase). Fig. 2.3H illustrates the footfall pattern for all four limbs corresponding to the gait cycles shown in

3A-G. The filled spaces indicate the stance duration and the empty spaces indicate the swing duration.

The joint angle data was divided into individual cycles (from TD to TD for each individual limb) and each cycle normalized to 100%. Each normalized cycle is represented by 201 data points. All the normalized cycles in each rat were averaged to represent a typical angle trajectory for that rat. The left column of Fig. 2.4 A-E shows the averaged joint angle trajectories from 6 gait cycles with the standard deviation curves for a typical walking trial in one rat while the right column illustrates the mean$\pm$1 SEM of the averaged trajectories (3-22 cycles per rat) from all 26 rats. The average LO occurrence is also indicated. The average cycle period for all rats in the study was 423$\pm$8 msec.

From the hip, ankle and elbow angle trajectories shown in Fig. 2.3B, D, F and Fig. 2.4A, C, E we note that maximum flexion occurs during the swing phase while maximum extension occurs during the stance phase. In the knee and shoulder angle trajectories (Fig. 2.3C, E and Fig. 2.4B, D), both maximum flexion and maximum extension occur during swing phase. In addition, the switch from extension to flexion in ankle and elbow angles is faster and occurs close to LO, whereas for the knee angle the switch from the flexion to extension is faster. Comparatively, the hip and shoulder angles show gradual transitions. The ankle angle shows a prominent double burst pattern unlike the elbow and knee angles, which exhibit a less prominent double burst. The variability in the angular measurements is higher around lift off (similar trend in individual rats not illustrated). Table 2.1 presents the mean (n=26) minimum angle during swing, maximum angle during stance, the range of movement of the joint, and the angular values at lift off and touch down for hip, knee, ankle, shoulder and elbow joints.

Both intralimb and interlimb coordination can be affected by spinal cord injury. Interventional therapy, such as treadmill training in the incomplete spinal cord injured subject is often attempted to improve coordination. Fig. 2.5A-C show the intralimb coordination pattern in the form of joint angle trajectories plotted against one another for the right hindlimb while Fig. 2.5D shows the intralimb coordination pattern for the right forelimb. The averaged$\pm$1 SEM values (n=26) are presented in these 2-D phase plane

angle-angle plots. For clarity, only the positive values of the standard deviation of the joint angles values are illustrated. Intralimb coordination contours for the normal rat are in general all bean shaped except for the hip vs. ankle contour (Fig. 2.5C), which has a distorted “figure 8” shape, due to the double burst pattern in the ankle angle. The other subtleties are in the knee vs. ankle contour (Fig. 2.5B) that has a well-formed tail due to the extension of the knee angle coinciding with the double burst of the ankle angle.

The average interlimb joint angle contour patterns are illustrated in 2-D phase planes in Fig. 2.6A-G. Right and left (interlimb) coordination contours exhibit “figure 8” patterns (Fig. 2.6A, D, E) and butterfly patterns (Fig. 2.6B, C). The hip, elbow and shoulder left-right coordination contours are “figure 8” patterns, since the angles are asymmetric. The knee and ankle right-left coordination contours show butterfly patterns, as both are asymmetric and also have double burst segments, with the ankle having a prominent double burst. The ipsilateral forelimb-hindlimb interlimb coordination contour patterns are shown in Fig. 2.6F, G. These have a triangular shape. The shoulder trajectory shows a faster up-rise than the hip from maximum flexion to maximum extension and reaches maximum extension before the hip joint angle (see Fig. 2.3). This may be indicative of the dominant propulsive force provided by the scapula during walking [41]. The elbow vs. knee (Fig. 2.6G) coordination contour pattern is shaped like an equilateral triangle indicative of the out of phase relationship. For example, during swing the knee goes from maximum flexion to extension while the elbow goes from maximum extension to flexion.

As described in the methods section, quantitative assessments of the intra- and interlimb phase relationships at discrete points during a gait cycle were determined. The relative phase values between the intra- and interlimb joint angles for the same 5 cycles shown in Fig. 2.3 are shown in Fig. 2.7A, B under normal walking conditions. There is a 1:1 correspondence between the forelimbs and hindlimbs. Table 2.2 gives the average values obtained from all 26 rats. In Fig. 2.7A the open triangle and square markers represent the phase of the knee and ankle joint angles with respect to the hip joint angle respectively. The open circle marker represents the phase of the elbow joint angle with respect to the shoulder joint angle. The maximum flexion during swing for the knee leads that for the ankle, which in turn leads that for the hip very slightly while the

maximum flexion for the elbow during swing lags that for the shoulder. The lead and lag values remain fairly constant from cycle-to- cycle. In Fig. 2.7B the filled circle represents the left forelimb (FL_L) touch down, the filled triangle the right hindlimb (HL_R) touch down and the filled square the right forelimb (FL_R) touch down with respect to the left hindlimb (HL_L) touch down respectively. This pattern ($FL_L \rightarrow HL_R \rightarrow FL_R \rightarrow HL_L$) represents a walk pattern ([31]. This procedure can also be used to determine presence of abnormal gait patterns in which multiple steps of one limb occur compared to another. Fig. 2.7C, D shows such a gait pattern for a rat not included in the study. In this case two forelimb steps occur per hindlimb step in every alternate gait cycle (Fig. 2.7D). The footfall pattern observed during the treadmill waking is: right HL followed by right FL, left HL and then left FL. Table 2.3 lists the SWMin°, STMax°, Range°, LOVal°, and TDVal° after 1, 5, and 8 weeks in the Group 3 rats. There were no significant differences detected in these values over time.

Neural Measures (EMG)

The typical neural activity recorded in the flexor and extensor muscles of the hip, knee, and ankle joint and the corresponding hindlimb joint angles and the extensor activity in the elbow joint along with the corresponding shoulder and elbow joint angle trajectories for 5 gait cycles of the same animal is illustrated in Fig. 2.8A-D. The raw neural activity is superimposed with the full wave rectified moving averaged (MA) envelope. The touch down event marker (vertical solid line) and lift off event marker (vertical dotted line) demarcate the gait cycle phase in which the neural activity occurs. Fig. 2.9 presents the average (+ SEM) neural activity and joint angle trajectory values from multiple rats. The neural activity occurs in bursts and has a typical relationship to the stance and swing phases of the normalized gait cycle. Table 2.4 gives the mean burst duration and burst proportion of the activity. As shown in Figs 2.10A-C, the burst duration of the ankle extensors, GM, and the knee extensor during stance, VLII, linearly increase with cycle duration ($y=0.7161x- 0.0377$, $R^2= 0.55$ for GM and $y=0.4964x+0.0244$, $R^2 = 0.40$ for VLII), but not for the hip extensor, BF ($y=0.4964x+0.0244$, $R^2 = 0.02$). The ankle flexor, TA, remains more or less constant irrespective of cycle duration ($y=0.0269x+0.0775$, $R^2 = 0.02$) while the knee flexor, SM, and the hip flexor, IL, burst durations have a weak linear relationship with cycle duration

(y=0.2932x-0.0477, R^2= 0.39 for SM and y=0.3262x-0.0039, R^2 =0.26 for IL). Burst duration of the elbow extensor, TB, (not shown) also does not change with cycle duration (y=0.047x+0.1795, R^2 =0.004 for TB). The relative phases of the bursts of neural activity of the forelimb and hindlimb muscles during one and a half gait cycle are illustrated in Fig. 2.10D. Only VLII burst duration during stance is shown. The grey shaded area represents the stance phase of the gait cycle. The textured bars illustrate the duration of flexor neural activity, while the empty bars represent the extensor activity. The data from Figs 2.8, 2.9 and 2.10 D indicate that the flexor activity in all muscles precedes lift off. It usually shows a sharp rise in amplitude followed by a quick fall and occupies about 20-30% of the gait cycle. Overall, the extensor activity occupies about 50-60% of the gait cycle. The hip extensor (BF) is activated at touch down and shuts of prior to lift off. Typically, the knee extensor (VL) has a small burst before touch down during swing (VLI) followed by a larger amplitude and duration burst during stance (VLII) (Fig. 2.8B). There is a brief silence between the VLI and VLII bursts and the burst during stance initiates with a slight delay after touch down. The ankle extensor (GM) activity is initiated before touch down and shuts off before lift off. The elbow extensor is activated slightly after touch down. Despite extensor activity during stance, the knee continues to flex, because of the activity in GM, which causes an ankle extension and knee flexion as well as that of BF which causes hip extension and knee flexion. The hip and ankle extensors (BF, GM) are primarily shut off during swing. Co-contraction of antagonist muscles for the same joint is not observed and a delay exists between the termination of extensor activity and initiation of flexor activity and vice-versa. There is however, a brief overlap in contraction of the hip flexor and ankle extensors late in swing. The ankle extensors allow the foot to drop for touch down even before the entire limb has extended. While not illustrated, the neural activity in the contralateral limb muscles was 180 degrees out of phase and followed the interlimbs phase relationships observed in the kinematics.

Discussion

In the present study, we have presented detailed quantitative assessment of 2D and 3D kinematics during locomotion in the Long-Evans rat. To our knowledge, this is the first study presenting detailed intralimb and interlimb coordination information for 3D

kinematics of joint angle movement in all four limbs simultaneously. We have also related the neural activity of the flexor and extensor muscles to the kinematics. Additionally, we present a simple and effective way of assessing phase relationships and interlimb coordination on a cycle-by-cycle basis.

Several methods have been suggested for assessing locomotor ability of rodents after spinal cord injury [28, 59-61]. Of the quantitative measures suggested, continuous kinematic measures have the advantage of providing detailed information [21]. Both 2D and 3D kinematics can be utilized. During overground walking in a linear track, we found that the rats walked at a velocity regarded at the upper end of the walk pattern [24, 54, 55]. The stride length was linearly related to the velocity (mean velocity 52.55 cm/sec). This is in agreement with data on female Wistar rats walking at speeds greater than 30 cm/sec [24]. After spinal cord injury, the animals often walk at a lower speed and hence it would be useful to normalize the stride length to the velocity of walking when making comparisons in those animals. The mean stride length (about 17.5 cm) in our Long Evans rats was longer than that reported for Wistar rats [24, 28] and Lewis rats (Metz) but similar to that reported for Sprague-Dawley rats [31] and by Kunkel-Bagden (species not specified) [25]. Perhaps, the differences are a reflection of the linear relationship between stride length and speed of walking. In order to improve their balance after spinal cord injury the animals may increase their base of support, and hence this is a useful measure to assess function. We found the base of support in intact animals to be similar to that of the Lewis and Wistar rats with similar weights [20, 62] but smaller than that for the Sprague-Dawley rats [31] of slightly higher weight. We also measured the hindfoot angle of rotation, which has been suggested as a measure for assessing foot placement by Kunkel-Bagden. It is not a measure that has been utilized extensively, but our preliminary data from another study found consistent increases in the hindfoot angle of rotation after spinal cord injury [63]. Thus, while the base of support may become narrower as the animal can bear more weight on its hindlimbs during the recovery process, the angle of rotation may or may not change. Combined use of these measures would help in differential diagnosis and assessment of therapeutic efficacy.

Using 3D kinematics of all four limbs, we were able to get a detailed description of the intralimb and interlimb coordination including specific information on phase relationships of activity amongst joints and footfall patterns. Quadruped gaits can be divided into two main categories, symmetric and asymmetric. Symmetric gaits such as walk, trot, and pace show strict alternation of the two limbs of the same girdle. In asymmetric gaits (e.g. gallop), the relative phase (fraction of gait cycle between ground contact of one foot and ground contact of another foot) of limbs of a girdle can very from 0 to 0.4 [64]. Cohen and Gans examined gait and footfall patterns, forelimb coordination, and sequence of muscle activity in white rats conditioned to run in an activity wheel [42]. They observed both symmetric (walk and trot) and asymmetric (canter and gallop) patterns with the trot being the most common. The speeds during these gaits varied from 40 cm/sec to 130 cm/sec. Gruner et al. examined treadmill walking in Long Evans rats and reported the presence of a walk pattern at a maximum speed of 30 cm/sec [35]. Cheng et al. have reported rarely seeing a walk ("alternate") but usually seeing a transverse gallop ("cruciate") or a rotary gallop ("rotary") pattern during spontaneous running along a runway [31]. On the other hand, Muir and Wishaw report the common gait for rats running on a runway to be the trot with temporal overlap between ground contact of diagonal limbs at lower speeds (30-50 cm/s) and no overlap at faster speeds (50 –85 cm/s) [65, 66]. Thus, depending on the motor task and speed different types of the gait pattern can be observed. We found all of our rats to have an "alternate" symmetric walk pattern, similar to the sequence described by others for rodents [28, 31, 51] and as the generic walk pattern for quadrupeds [64]. Interlimb coordination has previously been assessed using cross-correlation and vector-coding methods [26, 27, 67]. Rather than using a continuous measure, we present a simple quantitative method based on discrete events by which to assess interlimb coordination. Both the frequency of stepping of one limb with respect to another and the interlimb phase relationships within a gait cycle can be assessed. This approach could be used not only to define a regularity index as described Hamers et al. [28] but allow specific patterns of coordination to be defined on a cycle-by-cycle basis e.g. 1:1 forelimb-hindlimb coupling or 2:1 forelimb hindlimb coupling etc. In Fig. 2.7 we illustrate the cyclic changes in forelimb-hindlimb coordination in a rat.

3D kinematics of all four limbs provides the most comprehensive assessment of locomotor gait. While there have been reports on uniplanar 3D kinematics of the hindlimb, and a few reports on uniplanar 3D kinematics of both the ipsilateral hind and forelimb, to our knowledge, our work is the first to report on a 3D kinematic assessment of gait from all four limbs. Most techniques, including ours, rely on external marker placement [26, 35-37, 68]. The best measures of angular assessment would be provided with a technique such as X-ray cinematography. Kinematics of the rat forelimb joint angles during gallop in an activity wheel have been determined using X-ray cinematography [42]. Recently, X-ray cinematography was used to assess joint movement using uniplanar kinematics (ipsilateral hindlimb and forelimb) in a set of therian mammals and data from Rattus norvegicus is reported [69]. In this study, the definition of the knee and ankle joints is similar to that of ours. Although the authors do not describe what was considered touch-down or lift-off the values are very similar at lift-off (mean knee angle of 64.9° for our study vs. 63° and mean ankle angle 101.5° in our study vs. 99°). Our values at touch down were a little higher (110.9° vs. 79° for knee and 86.3° vs. 81° for ankle). X-ray cinematography is not a viable option for repetitive use. We find that with the skin tattooing and sufficient care in marker placement external markers can be reliably placed and utilized for joint angle measurements and allow safe utilization over longitudinal studies.

Based on our 3D kinematic data we can define typical patterns for the joint angle trajectories (Fig. 2.4). The hip and shoulder joints show monophasic patterns. In contrast, the knee, ankle and elbow joints show a biphasic pattern. During most of the gait cycle the forelimb joint angles move in the opposite direction to the corresponding hindlimb joint angles, i.e. when the hip and knee flex, the shoulder and elbow extend. The extension of the hip joint starts shortly before touch down and lasts till about 60% of the next step cycle. The knee joint flexes slightly before touch down and in general continues to flex during stance. We used the same approach to calculate phase relationships amongst joint angles at discrete markers during gait as that used for examining the sequence of touchdown of the different limbs during a gait cycle. These data indicate that in the hindlimb, maximum flexion during swing occurs first for the knee, then the ankle and then the hip joints. In contrast, in the forelimb, the maximum

shoulder flexion occurs before maximum elbow flexion. The intralimb and interlimb coordination shows typical patterns as presented under results. Changes in these patterns would be indicative of changes in joint angle coordination. Also, from the joint angle trajectories quantitative values at specific phases of the gait cycle can be obtained and statistically compared to determine changes in the kinematics. For example, in our study we do not find any statistically significant changes in several of these quantitative measures over repeated assessments of the kinematics over a period of weeks. These data suggest that mild exercise in itself does not alter the kinematics of gait. Additionally, this indicates that repeated kinematic measures can be obtained successfully.

Several muscles activate in a specific motor task, each muscle contributing to a subcomponent of that task. While several studies have reported EMG activity in pairs of muscles, and even up to 5 muscles per hindlimb (see Introduction), we report here EMG activity patterns from flexors and extensors for each of the hindlimb joints as well as from a forelimb joint. Because we also have the corresponding kinematic data, specific contributions of the flexors and the extensors to the kinematics can easily be assessed. During treadmill locomotion in the intact rat, most muscles of the hindlimb such as the ankle extensor (GM), the ankle dorsiflexor (TA), and the hip flexor (IL) have a single burst of EMG activity within a cycle. Other muscles show multiple bursts but individual bursts within these multiple bursts can be predictably related to flexor or extensor activity. For example, the knee extensor (VL) shows double bursts in which a small amplitude burst is active during swing (VLI) when the knee is extending and the hip is flexing with a second larger burst (VLII) during stance [36]. Still others such as the hip extensor and knee flexor (BF) have a fused double burst pattern that is confined to a single phase of the gait cycle (stance in this case). Thus, both uniarticular muscles such as VL and biarticular muscles such as BF show specific patterns of neural activation corresponding to a given phase of the cycle. BF and GM activity early in stance helps in the extension of the hip and ankle respectively. VLII activity late in stance not only helps prepare for paw lift off but may also help develop the propulsive force.

It has previously been reported that with decreasing cycle duration the burst duration decreases in the extensor muscles active during stance, thus maintaining the

burst proportion (e.g. GM and the VLII) while the burst duration of other flexor muscles active during swing remains unaffected thereby resulting in an increase in burst proportion (e.g. TA, VLI) [36, 44, 50]. We confirm these findings, however we find that unlike the ankle flexors the hip and knee flexors change burst proportion while the hip and elbow extensors do not maintain constant burst proportion. Thus, the responses obtained depend on the joint being controlled and the nature of the muscle being innervated. As previously discussed, these interrelationships of EMG activity with the corresponding kinematics would prove useful in quantitative assessment of altered gait and neural control mechanisms after neurotrauma.

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Table 2.1: Joint angle values at different points in the gait cycle. SWmin- Swing minimum; STMax - Stance Maximum; LOVal – Lift off value, TDVal – Touch down value.; Mean +/- SEM for 26 rats.

	SWMin°	STMax°	Range°	LOVal°	TDVal°
Hip	94.1 ± 1.8	116 ± 2.4	21.9 ± 1.3	111.3 ± 2.4	95.6 ± 1.9
Knee	60.1 ± 1.7	111 ± 1.7	50.9 ± 1.0	64.9 ± 1.5	110.9 ± 1.7
Ankle	55.5 ± 1.9	120.7 ± 2.2	65.1 ± 2.1	101.5 ± 2.4	86.3 ± 1.7
Shoulder	42.8 ± 1.6	80.5 ± 2.3	37.7 ± 1.5	43.1 ± 1.6	80.5 ± 2.3
Elbow	82.8 ± 2.1	142.9 ± 1.8	60.1 ± 1.4	133.5 ± 1.8	101.7 ± 2.6

Table 2.2: Intralimb and interlimb phase relationships. (see text for details), H-hip, K-knee, A-ankle, S-shoulder, E-elbow. In each pairing (e.g. HK) phase values are calculated as lead (positive value) or lag (negative value) of the second joint angle with respect to the first joint angle. HL – Hindlimb, FL- forelimb, subscripts L - left, R - right. All interlimb phase values are with respect to right hindlimb touch down. Mean +/- SEM for 26 rats.

Intralimb Phase		Interlimb Phase	
HK	0.20 ± 0.008	HL_R-HL_L	0.49 ± 0.005
HA	0.12 ± 0.007	HL_R-FL_L	0.85 ± 0.008
SE	-0.17 ± 0.004	HL_R-FL_R	0.35 ± 0.007

Table 2.3: Joint angle values at different points in the gait cycle in rats exercised for 9 weeks. SWmin- Swing minimum; STMax - Stance Maximum; LOVal – Lift off value, TDVal – Touch down value.; Mean +/- SEM for 6 rats.

Week 0	SWMin°	STMax°	Range°	LOVal°	TDVal°
Hip	95.5 ± 4.3	117.5 ± 5.9	21.9 ± 3.2	113.4 ± 5.9	97.5 ± 5.4
Knee	63.1 ± 1.8	113.9 ± 1.9	50.9 ± 1.5	67.6 ± 1.4	113.9 ± 1.9
Ankle	61.1 ± 6.1	123.0 ± 7.1	61.9 ± 4.1	104.2 ± 7.8	93.0 ± 4.1
Shoulder	50.6 ± 2.3	87.8 ± 2.3	37.2 ± 2.1	50.7 ± 2.3	87.8 ± 2.3
Elbow	90.6 ± 3.9	148.4 ± 2.4	57.9 ± 2.6	136.9 ± 2.9	111.5 ± 4.9
Week 1					
Hip	92.2 ± 4.7	117.7 ± 6.2	25.6 ± 3.2	111.5 ± 4.6	93.6 ± 4.7
Knee	64.7 ± 3.9	112 ± 3.8	47.2 ± 2.3	74.0 ± 4.9	111.4 ± 3.9
Ankle	61.5 ± 5.4	135.3 ± 3.6	73.8 ± 4.1	113.9 ± 5.3	94.1 ± 3.8
Shoulder	44.5 ± 3.9	72.5 ± 4.6	28.0 ± 1.5	45.3 ± 3.7	72.3 ± 4.6
Elbow	77.1 ± 5.7	137.9 ± 3.6	60.8 ± 2.8	126.5 ± 4.8	95.5 ± 8.0
Week 5					
Hip	89.3 ± 2.7	109.7 ± 3.2	20.4 ± 1.4	106.7 ± 2.7	92.1 ± 3.0
Knee	65.3 ± 2.7	118.8 ± 4.6	53.5 ± 3.1	74.7 ± 2.5	118.7 ± 4.6
Ankle	65.6 ± 2.2	135.1 ± 5.4	69.5 ± 5.0	121.1 ± 2.5	95.1 ± 6.3
Shoulder	47.6 ± 3.0	78.7 ± 3.3	31.1 ± 1.5	47.7 ± 3.0	78.6 ± 3.3
Elbow	81.6 ± 2.7	140.5 ± 1.8	58.9 ± 1.2	129.0 ± 4.2	97.3 ± 3.9
Week 8					
Hip	86.7 ± 3.0	111.8 ± 2.8	25.2 ± 1.8	105.7 ± 3.2	88.9 ± 3.4
Knee	66.4 ± 4.3	117.6 ± 3.7	51.2 ± 2.4	71.2 ± 4.1	117.5 ± 3.8
Ankle	65.1 ± 2.6	135.1 ± 6.8	69.9 ± 7.9	112.7 ± 5.8	97.7 ± 2.1
Shoulder	46.1 ± 3.1	77.2 ± 4.3	31.2 ± 2.1	46.5 ± 2.9	77.1 ± 4.4
Elbow	76.3 ± 5.8	141.0 ± 4.3	64.7 ± 3.9	123.3 ± 5.5	92.8 ± 6.1

Table 2.4: Burst duration (BD) and Burst Proportion (BP) of different flexor and extensor muscles. See text for abbreviations; VL values for bursts occurring during stance. n=number of animals, Mean +/- SEM.

	Hip		**Knee**		**Ankle**		**Elbow**
Muscle	IL (n=6)	BF (n=5)	SM (n=2)	VL (n=3)	TA (n=6)	GM (n=5)	TB (n=3)
BD(msec)	126.5±14.3	196±16.9	69.5±5.1	221.7±19	88.5±2.1	253±7.2	209.7±11.9
BP(%)	30.4±3.3	48±4.8	17.4±0.6	53.7±1.1	21.3±0.8	60.8±1.8	50.9±2.6

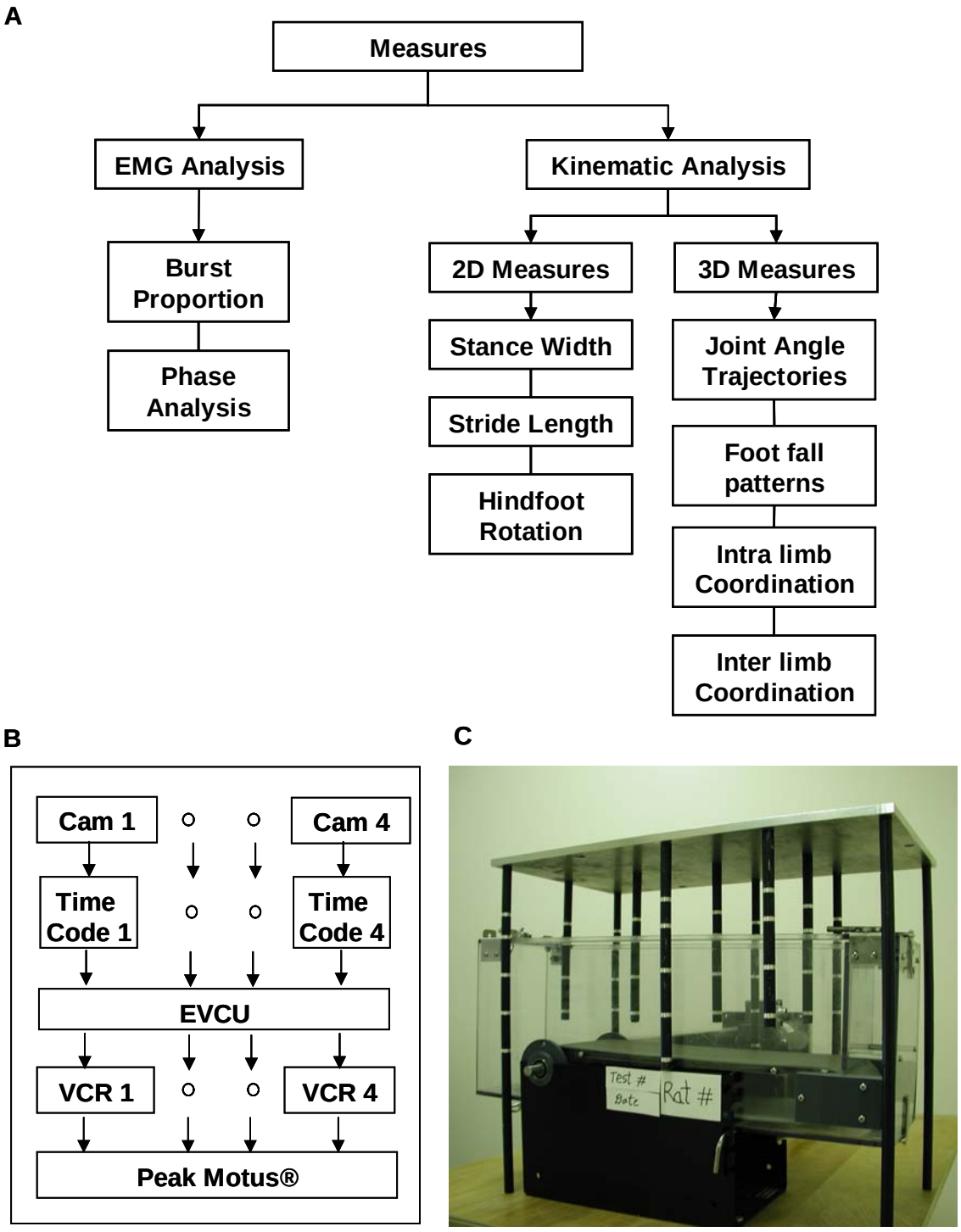

Figure 2.1: Methods and analysis overview: (A) Organization chart for different measures and outcomes for motor assessment. (B) Schematic for 3D/2D kinematic video data acquisition system using Peak Motus® (C) Custom-designed 40 point calibration cube (distributed among 10 rods) used to calibrate 3D space for 3D kinematic analysis.

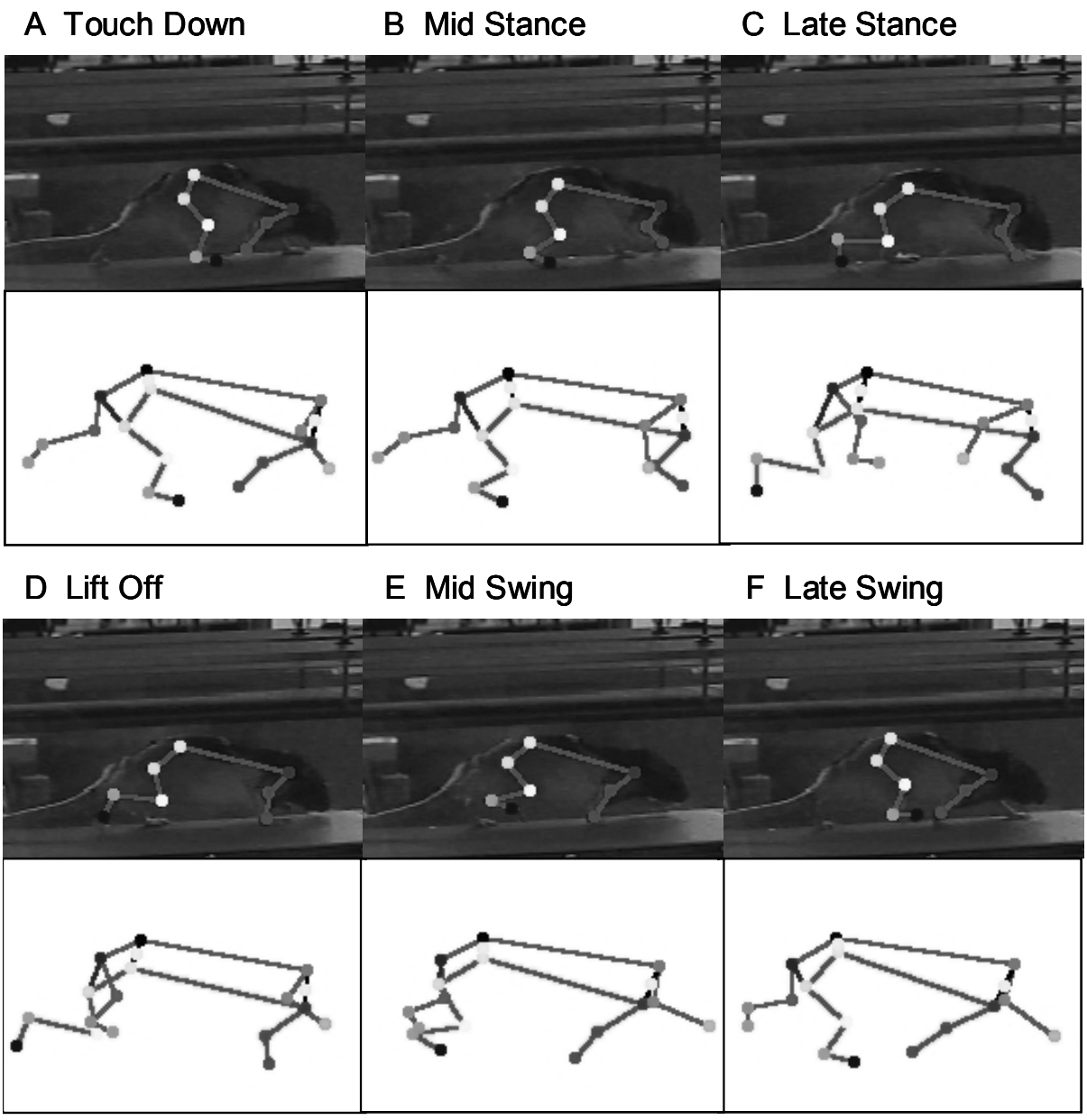

Figure 2. 2: 3D video for gait analysis of treadmill walking. The sagittal views from one camera are overlayed with stick figures connecting the markers (filled circle spots). These views and the associated spatial model illustrate the limb and body positions at different temporal events in one step cycle. Based on the right hindlimb, the panels indicate (A) touch down of the right hindfoot on the treadmill; (B) mid-stance; (C) late (end) stance; (D) lift-off of the toe from the hindlimb; (E) mid-swing; and (F) late (end) swing.

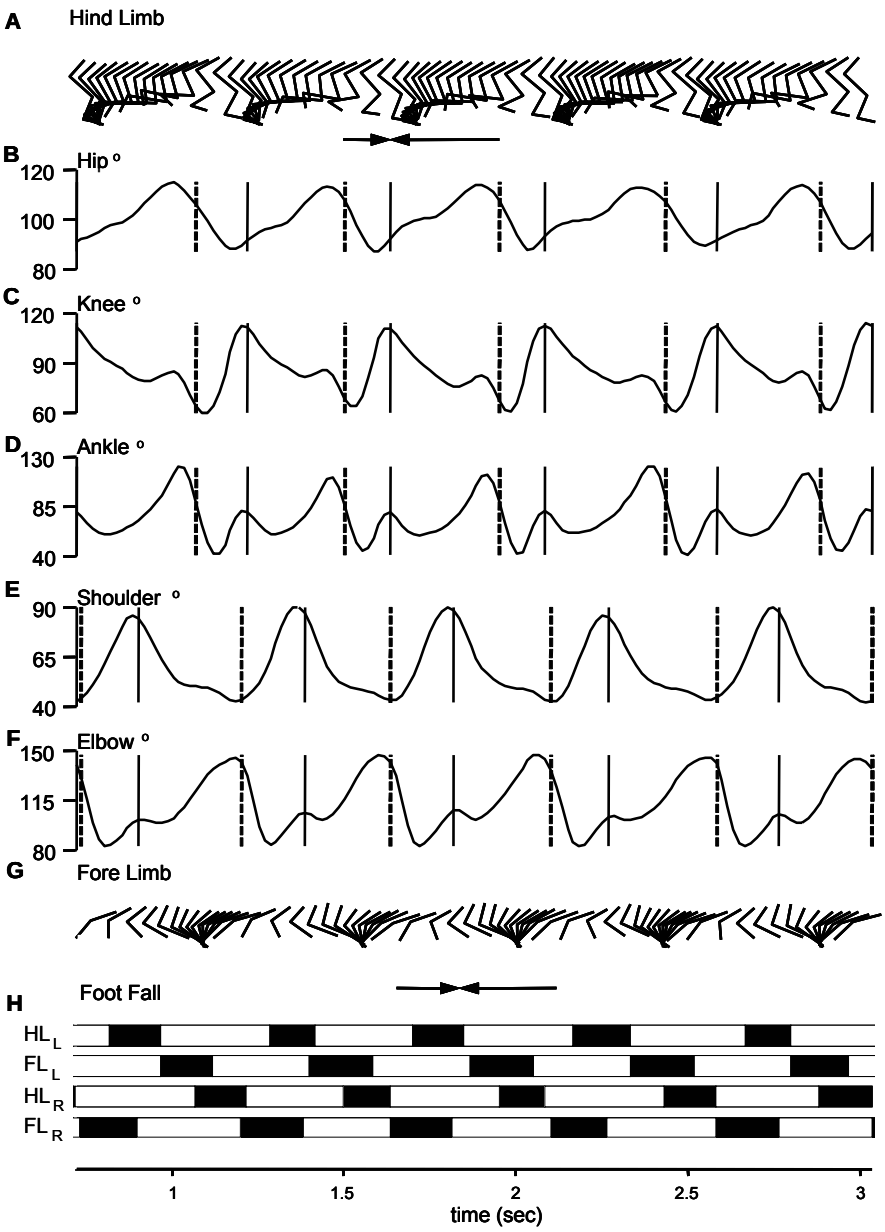

Figure 2.3: Typical 3D kinematic analysis of gait. (A) Stick figure representation of the right hindlimb showing (top to bottom) pelvis to hip, hip to knee, knee to ankle and ankle to toe segments. The short and long arrows show the direction and duration of the swing and stance phases respectively during forward walking (left to right). (B)-(F) Angle trajectories (5 cycles) of hip, knee, ankle, shoulder and elbow along with lift-off (dotted vertical line) and touch-down (solid vertical line) event markers for the right hindlimb. (G) Stick figure representation of the right forelimb showing (top to bottom) shoulder to elbow and elbow to wrist segments. The short and long arrows show the direction and duration of the forelimb swing and stance phases respectively. (H) Foot fall pattern indicating swing (filled rectangles) and stance (empty rectangles) of all four limbs. HL:Hindlimb; FL: Forelimb, L: left, R: right.

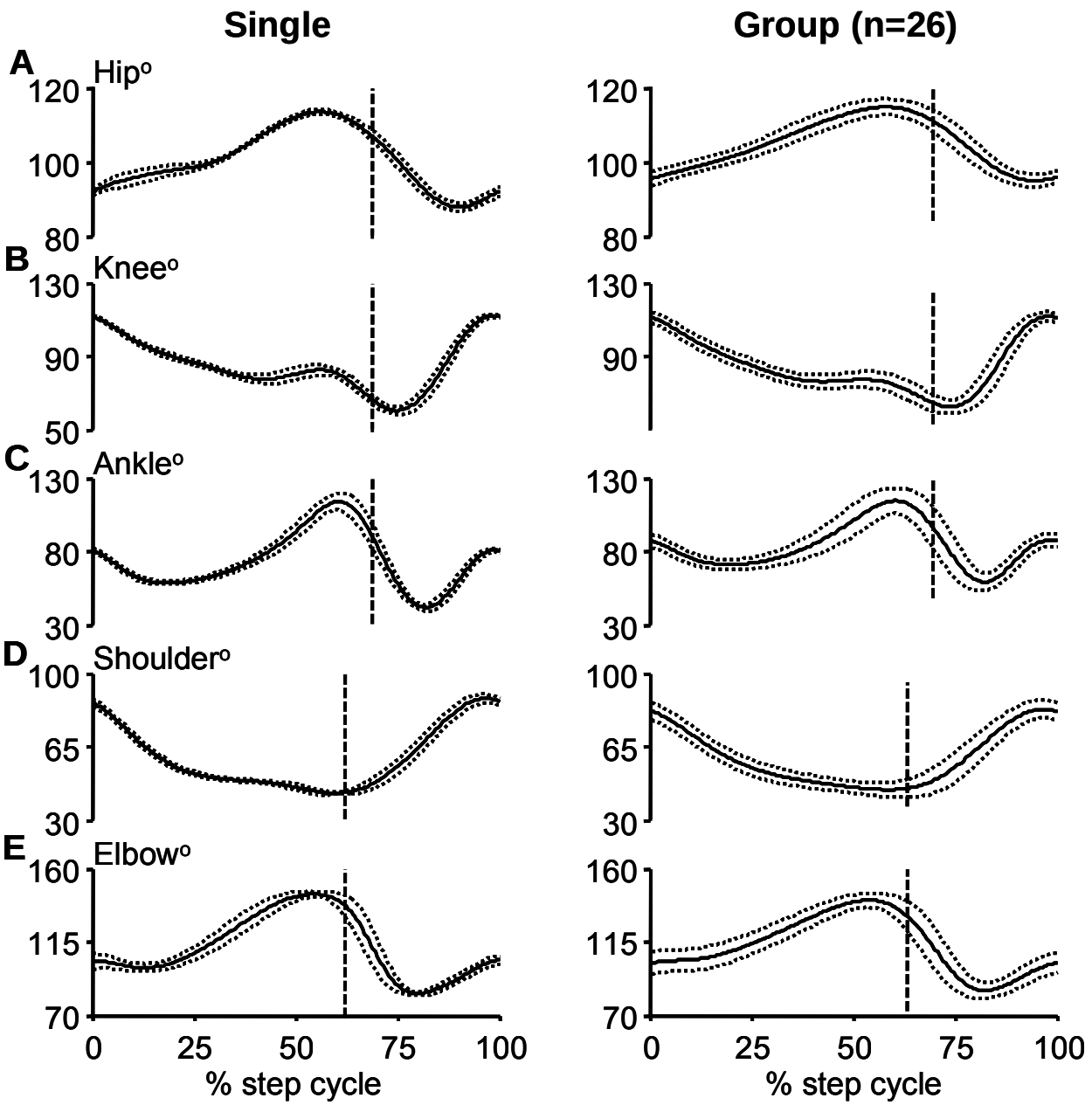

Figure 2.4: Angle trajectories. The left panel illustrates the average (solid line) +/- 1SD (dashed line) angle trajectory of six consecutive gait cycles in one rat from touch-down to touch-down (right hindlimb, same rat as in Fig. 2.3). The right panel illustrates the average +/- 1SEM angle trajectories obtained from a group of 26 rats (5-15 cycles per rat). (See text for further details). (A) Hip, (B) Knee, (C) Ankle (D) Shoulder, and (E) Elbow angle trajectories. Vertical dotted line: Lift-off event marker

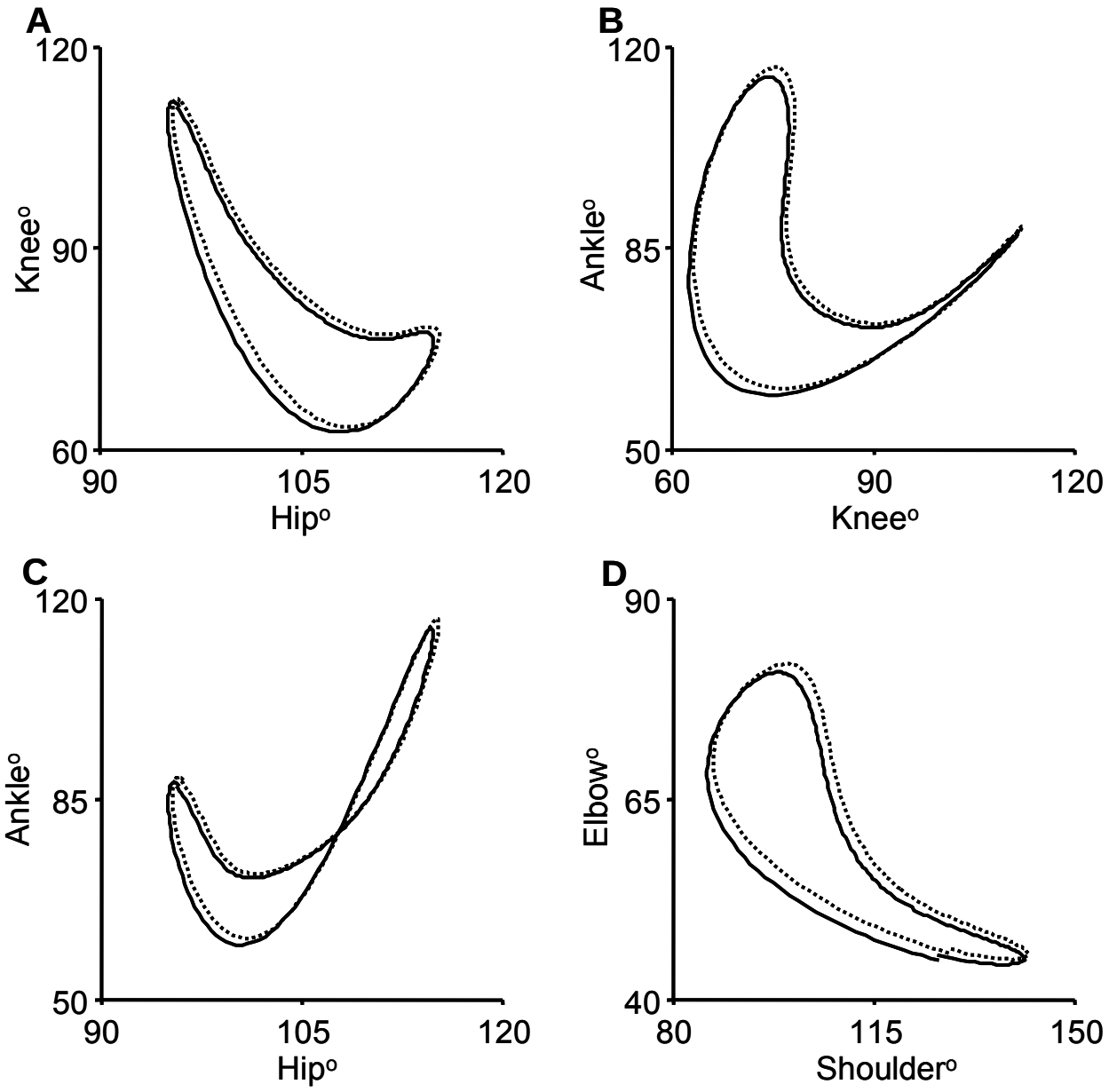

Figure 2.5: Intralimb joint-angle coordination. Angle-angle plots illustrate the coordination between the joints of the same limb during treadmill walking. Data (average (solid line) +/- 1SEM (dashed line) from 26 rats (5-15 cycles) per rat shows the maximum, minimum and range of excursion of each joint. (A) Hip vs. Knee; (B) Knee vs. Ankle; (C) Hip vs. Ankle; (D) Shoulder vs. Elbow.

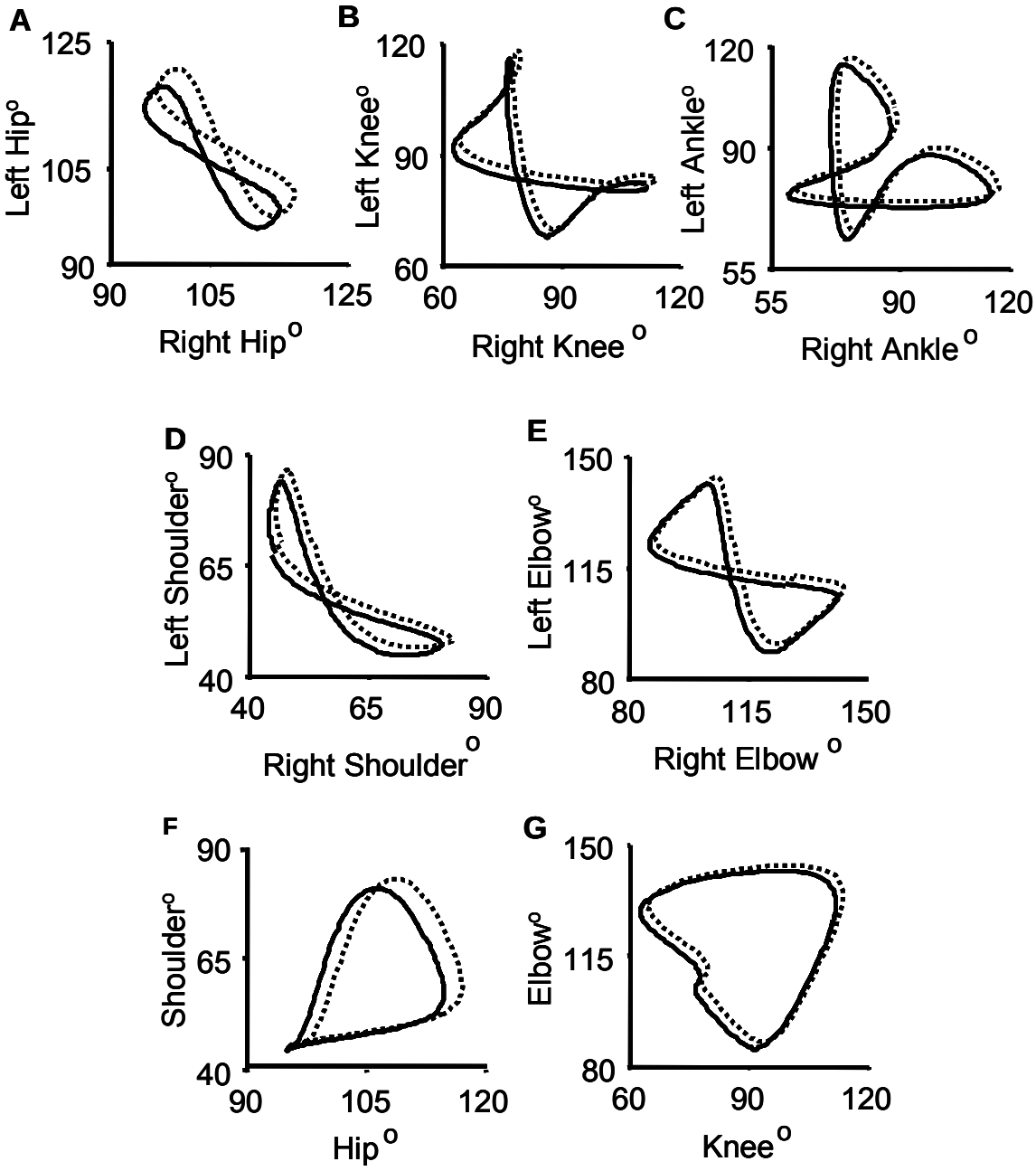

Figure 2.6: Left-right and front-back interlimb joint-angle coordination. Angle-angle plots illustrate the coordination between the joints of different contralateral and ipsilateral limbs during treadmill walking. Data (average (solid line) +/- 1SEM (dashed line) from 26 rats (5-15 cycles) per rat shows the maximum, minimum and range of excursion of each joint and the typical patterns observed. Contralateral joint angles have an approximately 180 degree out of phase relationship. A-E show left-right coordination between the hindlimbs and forelimbs ((A) Hip, (B) Knee, (C) Ankle, (D) Shoulder, (E) Elbow) with a typical almost symmetrical figure-eight pattern. The ankle shows a "butterfly" pattern because of a double-hump in it is joint angle trajectory. The forelimb-hindlimb front-back coordination is seen in (F) hip vs. shoulder and (G) Knee vs. Elbow.

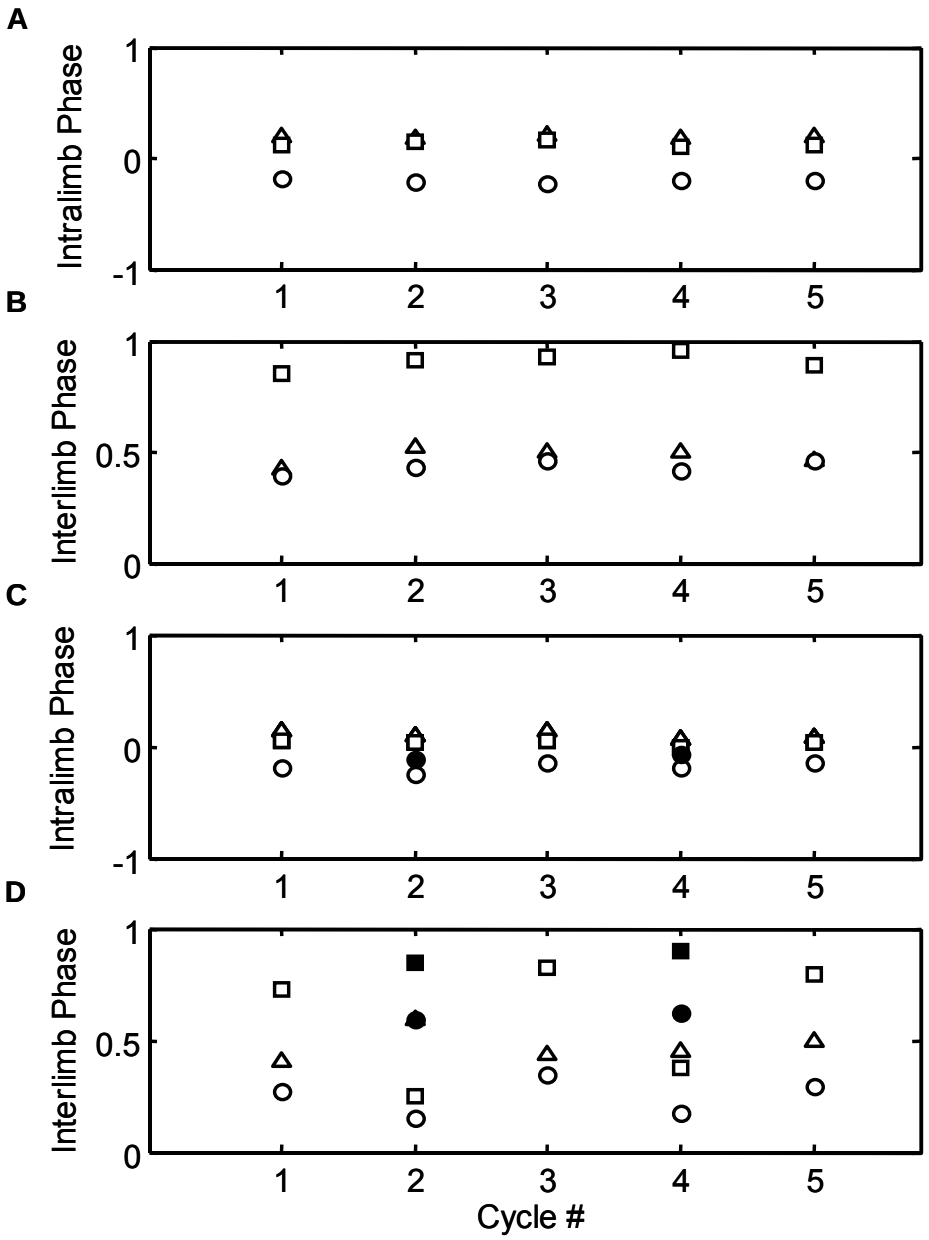

Figure 2.7: Phase relationship between intralimb joint angles and interlimb footfalls with respect to right hindlimb touchdown (see methods for details) for 5 consecutive right hindlimb step cycles. (A, C) Intralimb relative phase: Δ knee to hip; □ ankle to hip; ○ elbow to shoulder. (B, D) Interlimb relative phase: ○,● right hindlimb to right forelimb; Δ,▲ left hindlimb to right hindlimb; □, ■ left forelimb to right hindlimb. This analysis procedure can be used to determine presence of multiple steps of one limb compared to another, thus illustrating interlimb coordination patterns. An example, from a different rat, illustrates the relative intra- and interlimb phase values during two forelimb steps per hindlimb step on alternate gait cycles in panels (C) and (D) respectively. In these panels, the filled symbols represent the presence and relative phase value of the second step.

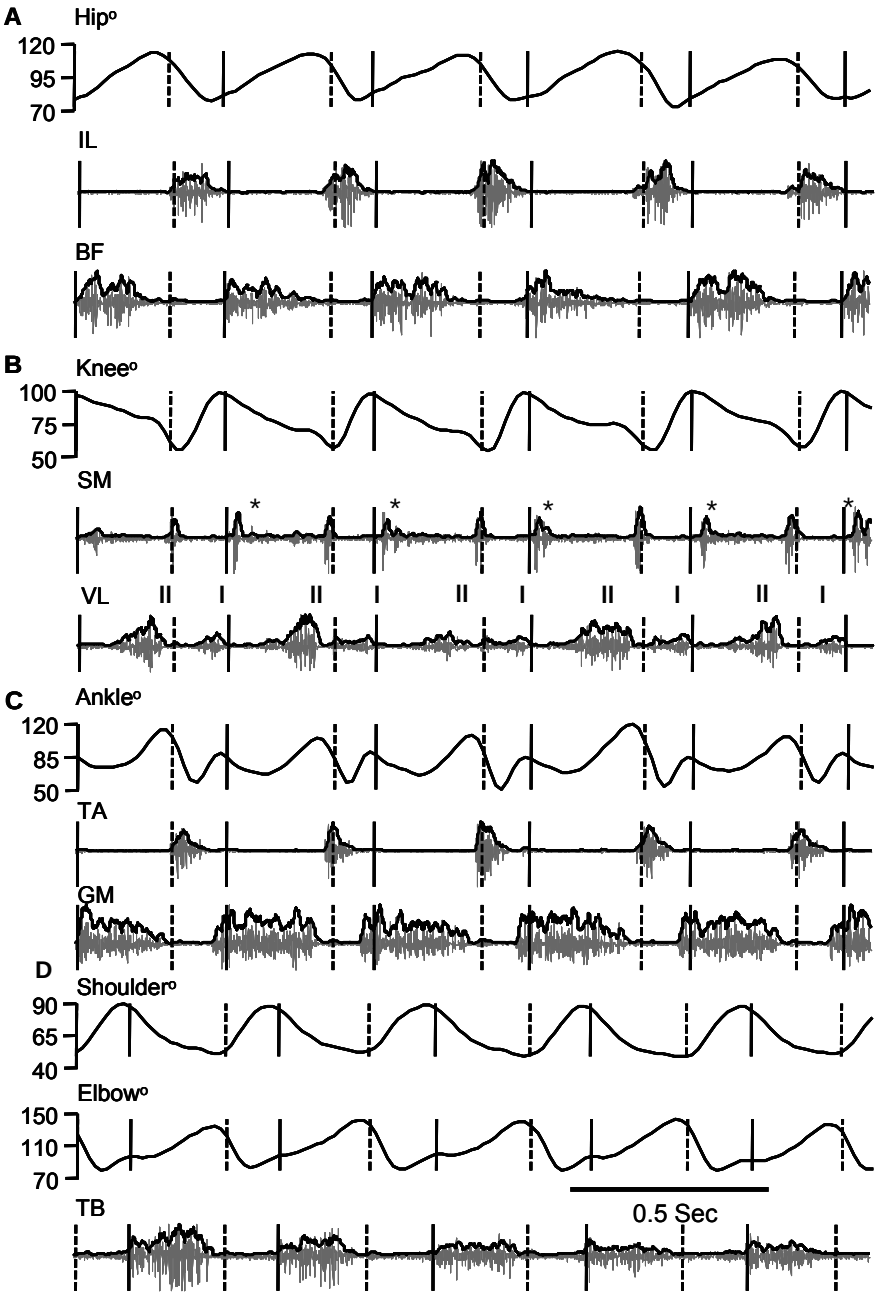

Figure 2.8: Relationship of EMG to joint angle trajectories during treadmill walking. (A-C) illustrate the typical flexor (IL, SM, TA) and extensor (BF, VL, GM) muscle activity, superimposed with the full wave rectified moving averaged (MA) envelope recorded from right hindlimb muscles of the hip (A), knee (B), and ankle (C) along with the corresponding synchronized hindlimb joint angles during five consecutive gait cycles of treadmill walking. (D) illustrates the extensor (TB) activity in the right elbow joint along with the corresponding shoulder and elbow joint angle trajectories. * indicates recording artifacts in SM. The two bursts in VL during swing and stance are distinguished as VLI and VLII respectively. IL-iliacus, BF-biceps femoris, SM-semimembranosous, VL-vastus lateralis, TA –tibialis anterior, GM – gastrocnemius medialis, TB – triceps brachialis. Vertical dotted line: lift-off marker, solid vertical line: touch-down marker.

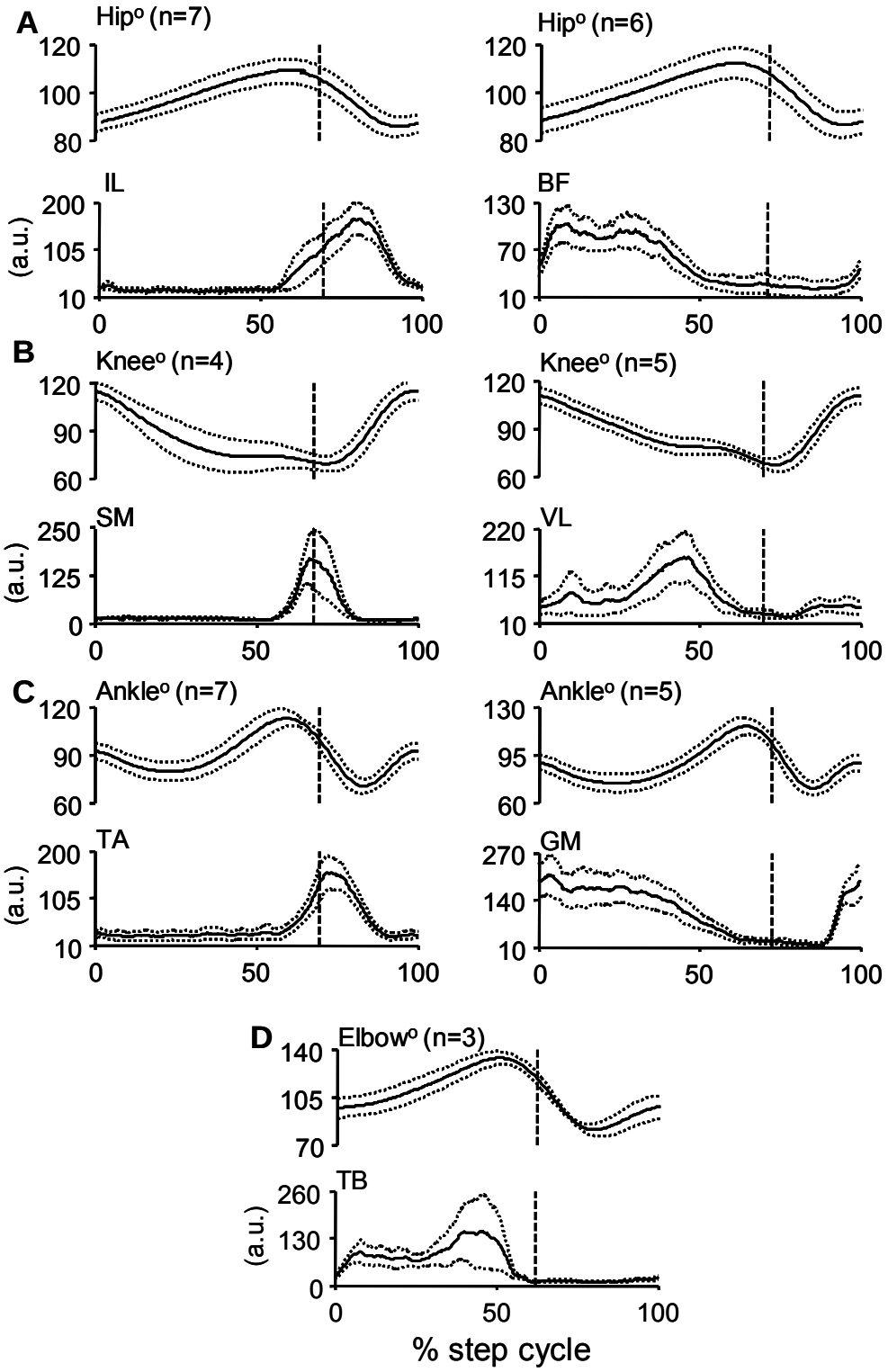

Figure 2.9: Average EMG and joint angle trajectories. The average +/- 1 SEM of the moving averaged EMG and corresponding joint angle trajectories during treadmill walking were obtained from multiple rats. n=number of animals averaged.

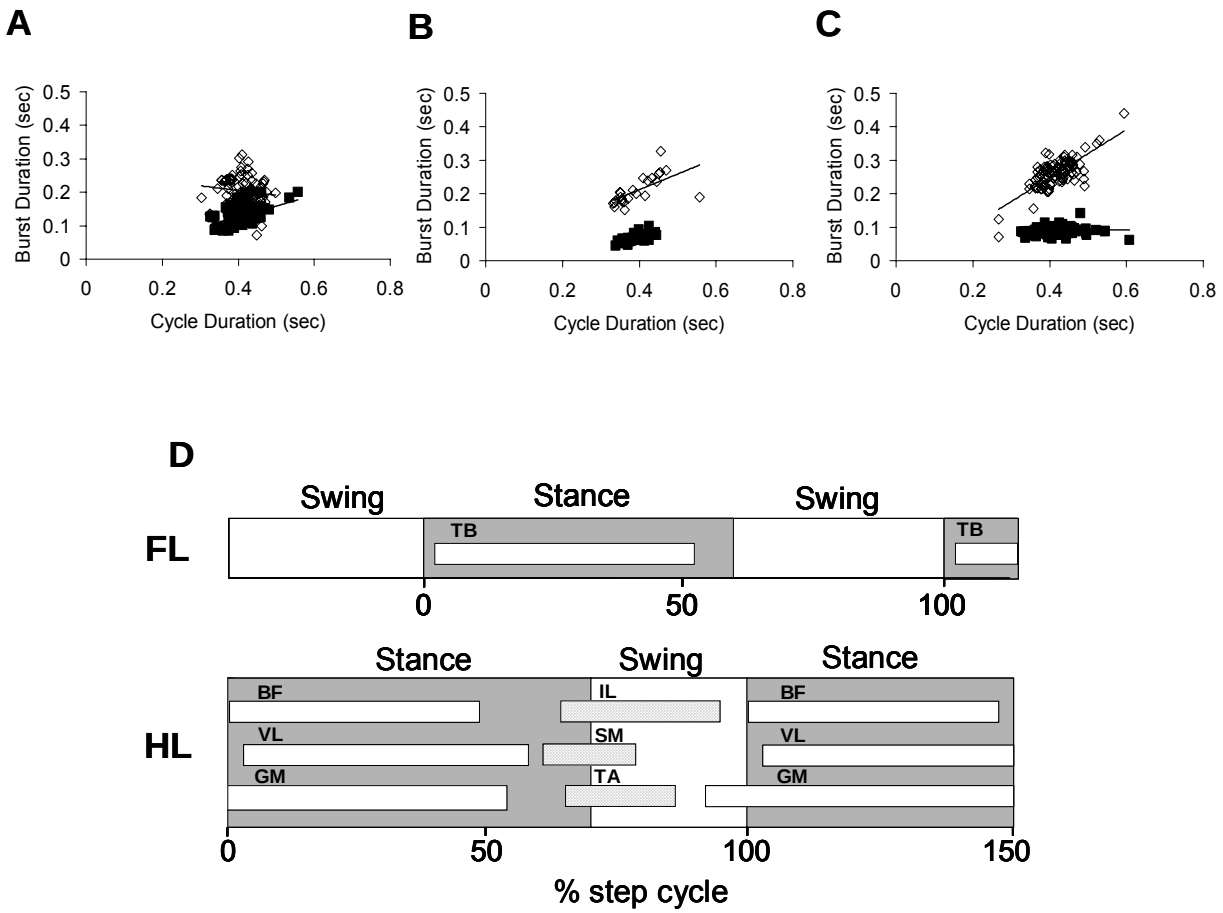

Figure 2.10: Phase relationship between/among forelimb and hindlimb muscle activation. The relationship of burst duration to cycle duration of neural activity per gait cycle is illustrated for the hip extensor (BF) and flexor (IL) in panel A for the knee extensor (SM) and flexor (VLII) in panel B and the ankle extensor (GL) and flexor(TA) in panel C. ◊ indicate extensor burst durations while ■ indicate flexor burst durations. In panel D, the average relative phases of the bursts of neural activity during one and a half gait cycle of the right forelimb (top) and right hindlimb (bottom) are illustrated. Data are presented as % step cycle duration. The grey shaded area represents the stance phase and the white area represents the swing phase of the gait. The textured bars represent the flexor activity while the plain bars represent the extensor activity. The forelimb extensor activation (TB) occurs with a 40% delay relative to ipsilateral hindlimb touchdown. See Figure 2.8 legend for explanation of abbreviations.

Chapter 3: Neuromechanical control of locomotion in incomplete spinal cord injured rats.

Introduction

Majority of traumatic spinal cord injuries involve impairment of motor function. Following primary injury (initial damage of neural and vascular structures), a series of early and delayed deleterious events damage the neuronal tissue further, causing a secondary injury. The secondary injury could persist from days to even weeks [1, 2]. Clinical observations indicate that in the first year following a spinal cord injury classified as incomplete (iSCI), substantial improvements in sensorimotor function may occur, but the pattern of functional recovery differs across a population of individuals. Any functional recovery depends upon the nature of the injury, the primary and secondary injury levels and the early and long-term adaptive response of the neural system to the trauma. The adaptive neural responses are believed to occur at multiple sites in the motor control system [3-6] and involve complex processes that are highly variable and poorly characterized. A very important component of this recovery is likely to be the reorganization of the dynamic interaction between the supraspinal and spinal segmental circuitry for motor control of the musculoskeletal system.

Rodent models are extensively being used at the molecular, cellular, and systems level to investigate the effects of the traumatic injury, to develop mechanisms for repair and regeneration, and to assess the effectiveness of various physical, pharmacological, and combinatorial therapies for the recovery of locomotor function [3, 7-26]. Complete transection, hemisection, contusions, compression, ischemia or crush are all being utilized to induce spinal cord injury. Each of these models allows investigators to focus on one or more aspects of the effects of injury. The most commonly used model for assessing recovery after incomplete spinal cord injury that has been validated against features of human spinal cord injury is the thoracic contusion model [27]. A thoracic contusion injury in the rodent results in an inability of the animal to balance, weight-support, and achieve appropriate movement of the hindlimbs. Appropriate implementation of interventional strategies employed to enhance

motor recovery will critically depend not only on our detailed mechanistic understanding of the post iSCI recovery process but also utilization of reliable outcome measures.

Several qualitative and quantitative tests are currently being used to track functional motor recovery after injury in rodents. These include, the Basso, Beattie and Bresnahan (BBB) locomotor score to assess open-field locomotion, grid walking, narrow/wide beam crossing, thoracolumbar height test, footfall patterns and footprint analysis and other 2D kinematic measures that indicate stance widths, stride lengths and hindlimb rotation [9, 12, 14, 28-37]. Brief utilization of 3D kinematics of gait has also been done [8, 32, 33, 38, 39] to assess the functional locomotor recovery.

After complete spinal transection, untrained rats show paraplegia with almost no rhythmic movement in the hindlimbs. They utilize the forelimbs for 100% of the movements as compared to 50% for normals and cross a given length of the room by taking double the forelimb steps [28, 36, 40]. Placing reflexes can be observed in the spinalized animals and pinching the tail or rubbing the hindlimb skin can elicit some rhythmic hindlimb movement, but interlimb coordination is impaired [40]. Convulsive and synchronous EMG activity can be recorded but the pattern is not well organized and ankle flexor-extensor co-activation can be observed [40].

With incomplete SCI, cats can recover quadruped locomotion after locomotor training of the hindlimbs as long as either the dorsal or ventral and ventrolateral funiculus pathways are preserved. Important deficits remain in fore- and hindlimb coupling [41]. Similarly, in rats the severity of the SCI is related to the ability to regain coordinated forelimb-hindlimb movement as assessed by the BBB score [28]. The animals also show deficit in footfall patterns with wider stance widths and outward hindlimb rotation [12]. EMG patterns to ankle extensors and flexors can also change [42]. Few studies have however systematically analyzed locomotor recovery after an incomplete contusion injury using behavioral measures, 2D kinematics, 3D kinematics and EMGs.

Inthis study, weassessed functional locomotor recovery after incomplete thoracic spinal cord contusion injury in adult rodents. We hypothesized that locomotor scores and functional outcome measures would improve with time because of intrinsic recovery mechanisms. Wealso hypothesized that the degree of recovery would be related to the

level of injury. BBB scores, 2D overground and 3D treadmill measures were used to quantify the intrinsic recovery over time post iSCI. Additionally, we used the BBB score as a surrogate marker for the level of injury and examined the differences in locomotor measures based on the level of injury.

Materials and methods

Studies were performed on 16 adult (73 ± 10 days old) female Long-Evans rats weighing 206 ± 17 gms. Qualitative assessment of locomotor recovery was obtained using the BBB 21 point forced choice locomotor score. Quantitative assessment of locomotor recovery was assessed from 2D kinematic measures of overground walking and 3D kinematic measure of treadmill gait. In some animals neural recovery of muscle activity was assessed using electromyograms (EMG). Data from the sham control group (Group 1) with a laminectomy but no spinal cord injury (n= 6) from Chapter 2 was compared to that from the incomplete spinal cord contusion injury (iSCI) group (Group 2, n=10).. All protocols and surgeries were approved by the University of Kentucky Institutional Animal Care and Use Committee.

Surgical procedures

Chronic EMG Electrode Implantation

In 3/6 sham rats and 3/10 iSCI rats, EMG electrodes were implanted under aseptic surgical conditions as described in detail in Chapter 2. Briefly, the electrodes were implanted bilaterally in six muscles per hindlimb and one muscle per forelimb. These muscles were the iliopsoas (IL; hip flexor), biceps femoris (BF; hip extensor but also knee flexor), semimemberanosus (SM; knee flexor but also hip extensor), vastus lateralis (VL; knee extensor), tibialis anterior (TA; ankle flexor), gastrocnemius medialis (GM; ankle extensor but also knee flexor) and the triceps brachialis (TB; elbow extensor). A reference electrode was inserted in the back under the skin anchored to the adipose tissue. The animals were allowed to recover for 1 week before a laminectomy to perform sham spinal cord injury or an incomplete thoracic contusion injury was performed. Baseline kinematic data (see below) was collected prior to EMG electrode implantation and both EMG and kinematic data were again collected prior to the second surgery.

Thoracic contusion injury

The rats were anesthetized with Pentobarbital (40mg/kg i.p.). During surgery, the body temperature of the rats was maintained at 37ºC using a thermal pad. The spinal cord was exposed through a dorsal laminectomy (T9-T11). The vertebral column was stabilized by clamping the spinous processes two segments rostral and caudal to the injury site. A 10 gm, 2 mm probe of the New York University (NYU) impactor device was dropped from a distance of 12.5 mm at spinal cord level T10 to cause a mild to moderate incomplete spinal cord contusion injury. Before closing the injury site with non-absorbable suture, it was covered with a piece of adipose tissue. The muscles were closed in layers using absorbable suture and the skin closed using non-absorbable suture. In the sham injured rats only the laminectomy procedure was performed. After the injury, the rats were monitored continuously and administered analgesics (Buprenorphine, 0.05 mg/kg, twice a day for three days and as needed thereafter) and antibiotics (Cefazolin, 33.3 mg/kg, twice a day for seven days). The bladder was expressed twice a day until spontaneous micturition recovered, and the animals were monitored for weight loss and sufficient hydration.

Mild exercise

5-12 days post laminectomy the sham injured Group 1 rats began mild treadmill-walking (21m/min) exercise on a rodent treadmill (Columbus Instruments) (15minutes/day, 5 days/week @ 20cms/s) for either 8 (n=3) or 12 (n=3) consecutive weeks. The iSCI group did not receive any treadmill exercise except for the cage activity.

Data Acquisition and Analysis

The BBB Score

Qualitative behavioral assessment of locomotor impairment was performed by two blinded observers observing the spontaneous walking ability of the rats in an open field consisting of a 3.5 ft diameter circular arena with a slightly rough surface that did not provide overt tactile stimulation. The BBB scores were obtained pre-injury, every day after injury until a week post-injury and once a week thereafter.

Kinematic assessment

Quantitative assessment of motor recovery was assessed using 2D and 3D kinematic outcome measures and EMG recordings as described in detail in Chapter 2. 2D kinematic overground testing was done pre-injury and once a week for 9 (or 13) weeks, starting at 2 weeks post-injury for all Group 1 sham rats and Group 2 iSCI rats. 3D treadmill testing was done on all the rats pre-injury and 2-9 (or 13) weeks post-injury. In the iSCI group, 3D data was obtained while the rats received no weight support.

Reflective markers (3M retro-reflective tape) were stuck to the belly, the ball (bottom of the 3rd metatarsal) and the heel of the plantar surface of the hindpaws. A 60-Hz camera was used to capture the video while the rats walked unidirectionally (5 passes) over a transparent bottom elevated plexiglass track (91.5 x 12 x 16 cm) illuminated from below. The markers were digitized offline using Peak Motus®. For each step cycle within a pass, the hindlimb (HL) stance width (SW: distance between the left and right ball markers), left (right) stride length (SL(SR): distance between the ball markers of the left(right) hindlimb in two consecutive steps), left (right) stride length normalized by velocity (SLv (SRv)) and the total (left+right) hindlimb foot rotation angle (Rot) were calculated. The average values for these measures for each pass and across all passes were then obtained.

3D kinematic data during treadmill walking was obtained pre-injury and 2, 6, 9/13 weeks post-injury from all Group 1 sham rats and Group 2 iSCI rats. Video data was captured by four 60-Hz video cameras placed radially around the treadmill while the rats walked on the treadmill with no body weight support. Cone shaped markers made from infrared reflective 3M tape, were placed on joint centers of the hindlimbs and the forelimbs that had been pre-tattooed (see chapter 2). Markers were also placed on the fifth metatarsal of the hindlimb and the treadmill belt. The markers were tracked offline using Peak Motus®. From the digitized data, the hip, knee, ankle, shoulder and elbow internal joint angles were calculated for up to 16 cycles. Prior to data collection, calibration of 3D space was performed using a customized rectangular calibration cube. Post-processing (Matlab®) was used to calculate on a cycle-by-cycle basis, for each of the joint angles, from each limb, the maximum flexion in swing (SWMin^{o}), the maximum extension in stance (STMax^{o}), the range of movement (STMax^{o} - SWMin^{o}), the joint angle value at touch down (TDVal^{o}) and, the joint angle value at lift off (LOVal^{o}). For

each rat an average joint angle trajectory was obtained by averaging 3-22 (average 15) gait cycles (normalized to 100%). Group averaged trajectories were obtained from the above trajectories.

Joint angle-angle plots (hip vs. knee, knee vs. ankle, ankle vs. hip and shoulder vs. elbow) were used for graphical assessment of intralimb coordination. Quantitative assessment of intralimb coordination was performed by calculating the phase of a discrete event (maximum flexion during swing) on a cycle-by-cycle basis. The mean phase values, SD, and coefficient of variation (CV = SD/mean) were calculated. Angle-angle plots between the left and right hindlimb joint angles (hip vs. hip, knee vs. knee and ankle vs. ankle) and left and right forelimb joint angles (shoulder vs. shoulder and elbow vs. elbow) were plotted to assess left-right interlimb coordination. Average angle-angle plots for HL-FL coordination cannot be plotted because 1:1 HL-FL coordination is disrupted after iSCI and different rats have different patterns of HL-FL coordination and hence different joint angle trajectories. Quantitative assessment of left-right and HL-FL interlimb coordination was performed by determining the relative phase of the limb during a gait cycle using limb touch down events (see chapter 2.).

Limb segments, which provide positional information in a 2D projected space, were plotted in rear and sagittal views (Figures 3.2 and 3.3). The rear view of the left and right hindlimb (HL) segments and segments joining the left and right pelvis were plotted for one cycle (HL_{TD} (HL- touch down) to HL_{TD}). The HL segments were: pelvis to hip, hip to knee, knee to ankle, and ankle to toe (top to bottom). Similarly, two rear views of the left and right forelimb (FL) segments and the segment joining left and right shoulder were plotted for one HL_{TD}-HL_{TD} (one or more cycles of FL) and for one FL_{TD}-FL_{TD} (one cycle of FL). The FL segments were: shoulder to elbow, elbow to wrist (top to bottom). The sagittal view of right HL segments during stance and swing were also plotted. In addition, footfall patterns for all four limbs were plotted to assess the relative interlimb swing and stance phase durations and stepping gait pattern.

Statistical Analyses

To assess the influence of time post injury on locomotor recovery a repeated measure Analysis of Variance (ANOVA) was performed on the BBB, 2D kinematic and 3D kinematic measures. Statistical analyses were performed using SAS® software with

the help of STAT Lab at Arizona State University. A Mixed Linear Model was implemented using the restricted/residual maximum likelihood (REML) estimation procedure and the results were considered significant at $p<0.05$. Since there were some missing data points after the injury, procedure "Mixed" was used to handle the unbalanced data. Additionally, factorial repeated measure ANOVA was performed on the data consisting of more than one group to assess the fixed effects of Group, Time and interaction between Group and Time. The simple effects and post hoc comparisons were also obtained. In post hoc comparisons *p* values were adjusted using the "Tukey" adjustment [43].

The BBB locomotor scale is non-linear but it indicates the extent of the injury. To evaluate the effects of the level of injury on the locomotor recovery outcome measures in the iSCI group, the 2D/3D kinematic data for all the weeks was divided into two groups based on the BBB score, i.e. BBB >=14 and BBB <14. A score of 14 or more indicates that the rat has recovered significantly and can place consistent weight-supported plantar steps and elicit consistent FL-HL coordination. One factor ANOVA was utilized to compare the two groups.

Results

Locomotor recovery with time post-injury (The BBB Score)

Open field behavioral testing, BBB, was performed on all the rats and the scores before surgery were 21. Fig. 3.1A shows mean±SEM values at pre-injury and 1-13 weeks post-injury. Sham control rats (■) scored a consistent 21 during the entire study. The BBB score of the Group 2 iSCI rats on Day 1 was 2.5±1.36. All rats showed gradual recovery of the BBB score with time post-injury. 3/10 rats had recovered to a BBB score of 15.3±0.7 on day 7, while 7/10 rats had only recovered to 10.5±0.4. Since the first subgroup of rats had clearly shown a very early recovery that was inconsistent with the rest of the group they were classified as a mild injured subgroup (Group 2a, ●) and their recovery over the following weeks was observed separate from the other moderately injured rats (Group 2b,○). After 2 wks post iSCI there was no significant improvement (p=0.75) in the BBB score of Group 2a rats and attained a plateau at 15±0.5. However,

the BBB score in Group 2b rats recovered significantly ($p \leq 0.05$) until 6 wks post iSCI and there after attained a plateau value of 13.5 ± 0.95.

Locomotor recovery with time post-injury (Kinematic Assessment):

2D Kinematics of overground walking:

Figs. 3.1B-F show the mean±SEM values of the Rot, SW, Velocity, SR and SRv respectively for each of Group 1, Group 2a and Group 2b from pre-injury to 13 weeks post-injury. At all time points pre-injury and post-injury the left stride lengths were similar to the right stride lengths ($p=0.88$) and hence only the right stride lengths were used for the comparisons. In Group 2b, SRv is significantly different until 7 wks post iSCI when compared to pre-injury values. In Group 2a, Rot is significantly different until 5 wks post iSCI and SRv until 7 wks post iSCI. The Group effect between post injury Group 2a and Group 1 is not significant for all the 2D parameters. Group 2b (pre to 13 wks post iSCI) values show a significant effect of interaction with time in all the 2D parameters while Group 2a and Group 1 do not. Two weeks post-injury, Rot, SW, and SRv increased while Velocity and SR decreased significantly for all Group 2a and Group 2b rats compared to their pre-injury values as well as compared to Group 1 sham rats. The foot rotation angle was affected the most and was greater than twice the pre-injury value. Over the 13 wk post-injury, SW and ROT ($p=0.01$, 0.01 respectively) improved significantly from the abnormal values at 2 wks post iSCI.

3D kinematics of treadmill walking:

Figures 3.2 and 3.3 illustrate stick figures of the HL and FL segments (right: pink; left: green) for two representative iSCI rats (Rat 22 and Rat 25) pre-injury and 2, 6, 9 and 13 weeks post iSCI. These representative rats were chosen because although both Rat 22 and Rat 25 started with similar initial BBB scores, Rat 25 showed gradual but faster locomotor recovery than Rat 22 thus exhibiting different patterns of locomotor recovery as indicated by the BBB score. In these figures, column 1-2 illustrate the rear views of the HL and FL segments from HL_{TD}-HL_{TD}. In column 3, the rear view of FL segments from FL_{TD}-FL_{TD} is illustrated. The sagittal view of the right HL segments during stance and swing are illustrated in columns 4 and 5 respectively. A continuous line is traced to track the ankle movement of the hindlimbs (rear view), the toe movement of the hindlimbs (sagittal view) and the wrist movement of the forelimbs (rear

view). In addition, right, left mean BBB scores and the speed of the treadmill at which the animal was walking are provided as surrogate markers for the level of injury. After the injury, Rat 25 had lost its balance control (swaying of hip segments). At the end of 13wks post iSCI, the animal partially regained the balance control by over flexing it's knee and ankle joints by increasing the base of support (stance width). Injury had no effect on the FL rhythm except that the HL-FL coordination was impaired. The flat excursion of the foot at 2 wks post iSCI during the swing and stance phases is a clear indication of the rat's inability to lift the foot and is due to a restricted range of motion (ROM) of the knee. There is a gradual improvement in the foot excursion from 2 wks post iSCI to 13 wks post iSCI. The reduction in the number of frames indicates the shorter swing phase after the injury. Rat 22 (Fig. 3.3) suffered a similar impairment but had greater deficits. It showed a larger base of support, extensive knee and ankle flexion at touch down, larger ankle extension during stance and absence of foot clearance during swing. The locomotor recovery was marginal from 2 wks post iSCI to 13 wks post iSCI.

Footfall patterns of Rat 25 (left panel) and Rat 22 (right panel) are shown in Fig. 3.4 for five consecutive left hindlimb cycles (4 cycles in Rats 22 and 25 at 6 wks post iSCI). In HL, blue and yellow indicate stance and swing phases and in FL, red and black indicate stance and swing phases. Pre-injury patterns show one-to-one FL-HL coordination and a consistency in stance and swing phase durations. After injury, the FL-HL coordination is severely impaired in Rat 22 as compared to Rat 25. Rat 25 patterns indicate that the coordination is improved over time. Rat 22 gradually developed a rhythmic pattern and the left-right coordination improved but the FL-HL coordination is still impaired at 13 wks post iSCI. Although, the durations of stance and swing phases are variable in both rats, the left and right coordination is preserved in HL as well as in FL.

Figs. 3.5 and 3.6 illustrate the average $\pm$ SD curves of Rat 25 (4-16 cycles) and Rat 22 (4-9 cycles) respectively. The iSCI group data shown in Fig 3.7 reflects the patterns of the angle trajectories of Rats 22 and 25. In both rats the angle trajectories are affected by the injury with prominent changes in the range of motion of the knee and ankle extension. In Rat 25, the maximum hip flexion occurred in the stance phase at 2

wks and 6 wks post iSCI as opposed to occurring in the swing phase. Maximum extension of the hindlimb joint angles in intact rats occurs at different times during the gait cycle as seen in the pre-injury trajectories. However, in Rat 22 the maximum extension in hip, knee and ankle occurred very close to each other (at lift off) and this impairment in joint angle coordination remained until 13 wks post iSCI. In addition, in Rat 22, the FL angle trajectories were also altered significantly, probably to compensate for the hindlimb paraplegia and loss of postural control.

Fig. 3.7 illustrates the average (n=7 except at 2-week post iSCI: n=4) of the averaged angle trajectories (see methods section in Chapter 2). 3/7 rats completely dragged their hindlimbs 2 weeks post injury. In those rats that were capable of some weight bearing walking, in both the hindlimbs and forelimbs the lift off event is shifted to 75% of the gait cycle as compared to pre injury occurrence at 65% of the gait cycle. Of all the angles, knee (Fig. 3.7B) and ankle (Fig. 3.7C) joint angles were the most affected by injury. At 2 weeks post iSCI, the hip angle trajectory (Fig. 3.7A) is smoothened as compared to the pre injury trajectory. Later on, the hip angle trajectory slowly recovered to its original shape. The ROM of the knee was drastically decreased after injury and it remained unchanged until the end of the study. The ankle angle lost its double burst pattern and never regained the pattern. The range of ankle excursion also increased and it remained increased until the end of the study. The FL, shoulder (Fig. 3.7D) and elbow (Fig. 3.7E) angle trajectories did not show prominent effects of the injury despite the increased variability in step cycle duration and lack of one-to-one coordination between HL and FL.

3-D analysis parameters for the right side are shown in Fig. 3.8A-E. There is no significant effect of mild exercise on any of the parameters in Group 1. In order to confirm that the pre-injury data of iSCI Group 2 (a & b pooled together) rats fall into the same data pool as Sham injured rats, the pre-injury data of Group 2 was statistically compared to that of Group 1. Results indicate that pre-injury data was not significantly different from Group 1. Therefore, for the Group 2 rats the pre-injury data was compared to post iSCI data points to assess locomotor recovery with time post-injury. All the hip parameter values are not substantially different ($p=0.27 – 0.73$) from pre-injury values and between those at 2, 6, 9 and 13 weeks post iSCI (Fig. 3.8A).

On the other hand, at 2 wks post-injury there is a significant reduction in knee extension, range of motion, and touch-down angle values (Fig. 3.8B) when compared to the pre-injury values. Post-injury there is no significant recovery in knee extension ($p=0.23$), range ($p=0.2$) and touch-down angle values ($p=0.2$) over the 13 weeks post iSCI. The values of these parameters at 13 weeks post iSCI compared to those pre-injury were: 115^0 vs. 73^o for knee extension, 51^0 vs. 25^0 for range of motion and 115^0 vs. 64^0 for touch-down angle values (Fig 3.8B).

At 2 weeks post iSCI, the ankle flexion, extension, range and lift-off angle values are increased and the touch-down values are decreased significantly ($p<0.01$) (Fig 3.8C). Post-injury there is no significant recovery in all the parameters over time. However, at the end of 13 weeks post iSCI, the ankle extension and lift-off value were statistically no different than those of pre-injury ($p=0.38$ and 0.89 respectively). The ankle is still over flexed (60^0 pre-injury vs. 28^0 at 13 weeks post iSCI) with an increased range (67^0 pre-injury vs. 108^0 13 weeks post iSCI) and reduced touch-down angle values (92^0 pre-injury vs. 31^0) along with the loss of the double burst pattern. In FL, shoulder and elbow parameters (Figs. 3.9A, B) did not show prominent impairments with injury and no significant changes except for elbow lift-off value ($p=0.007$) were observed over time.

Qualitative assessment of the coordination was performed using angle-angle plots (see methods in chapter 2). Interlimb HL-FL coordination was not assessed using this method because after injury one-to-one coordination is absent between HL and FL. However, Left-Right interlimb coordination contours for hip, knee, ankle, shoulder and elbow are illustrated in Fig. 3.10A-E respectively. There is a slight disfigurement in hip angle-angle contour at 2 and 6 week post iSCI, but the contour shape is preserved across all weeks post iSCI. The contour shape for knee angle-angle plot is lost for all weeks post iSCI. After injury, the ankle angle-angle contours for all weeks post iSCI are modified from a butterfly pattern pre-injury to a figure-eight pattern. All of these patterns also reflect the changes in the maximum extension, flexion and range of motion that are observable in Figure 3.7 and the bar charts in Fig. 3.8. In FL, shoulder and elbow angle-angle plot contours remained unaltered for all weeks post iSCI and showed consistent figure-eight patterns.

The hindlimb intralimb coordination contours for hip vs. knee (HK), ankle vs. knee (AK), and ankle vs. hip (AH) and the forelimb intralimb coordination contours for elbow vs. shoulder (ES) are illustrated in Fig. 3.11A-D. At 2 weeks post iSCI, the HK, AK and AH contour patterns seen pre-injury were altered. Changes to the pattern continued until 13 weeks post-injury but they did not recover to the patterns pre-injury. At the end of the 13 weeks, the knee angle was still impaired with reduced extension and the ankle angle had increased extension and flexion. In the forelimb the ES angle-angle contour pattern were preserved for all the reported weeks but the variability in the coordination did change.

The group mean $\pm$ SEM intralimb phase values for hip to knee (HKnee), hip to ankle (HAnkle) and shoulder to elbow (SElbow) coordination were calculated as described in Chapter 2. Fig. 3.12A shows the mean phase values. The effect of time on the all pre and post iSCI intralimb phase values is significant. All the mean intralimb phase values are altered significantly ($p<0.004$) at 2 weeks post injury when compared to pre-injury values. The hip and the ankle extensions occur almost in phase. Post iSCI, no significant time effect is observed in intralimb values. At 13 wks post iSCI the SElbow angle values were not significantly ($p=0.65$) different from the pre-injury values. This indicates that SElbow phase abnormalities improved over time. Though the intralimb coordination and FL-HL interlimb coordination is lost, the left to right interlimb coordination is maintained.

The standard deviation of the means of individual rats in a group and coefficient of variation are shown in Fig. 3.12B and Fig. 3.12C respectively. Event though the mean left-right coordination is not significantly altered the SD (and hence variability) in coordination is increased. The changes in SD are reflected in the CV.

Neural measures:

Fig. 3.13 illustrates the raw EMG for the ankle flexor (TA) and ankle extensor (GM), of an iSCI rat pre-injury, 1 and 2 weeks post-injury. The flexor and extensor activities alternate and do not overlap. At 1-week post-iSCI, the duration of the GM activity increased and occupied most of the stance phase. At 2-week post-iSCI, the pattern of activation of the TA is altered from a sharp onset to a gradual onset. Even

though the duration of the GM is, decreased initiation of the extensor activity begins before termination of the flexor (TA) activity thus showing co-contraction of the muscles.

Effects of injury (BBB Vs. 2D kinematics)

The bar charts in Fig 3.14 present comparisons of the 2D kinematic parameters (mean±SEM) compared based on the BBB values. The mean BBB (Fig. 3.14A) for the iSCI Group 2 rats with BBB>=14, BBB<14 and the Group 1 sham rats were 16.18±0.27, 11.72±0.15, and 20.78±0.07 respectively. The rotation angle (Rot; Fig. 3.14B) is significantly lower in Sham control rats (35.2±1.15^{o}) and rats with BBB>=14 (50.6±1.8^{o}) than that of the rats with BBB<14 (63.7±5^{o}). The variability in Rot is higher when BBB<14 than when BBB>=14 or under sham injury. Stance width (Fig. 3.14C) was significantly higher in the BBB<14 (3.97±0.2 cm) than in the sham group (1.92±0.04 cm) and the BBB >=14 group (2.6±0.09 cm). Rats with BBB<14 had lower velocity (37.7±1.12cm/sec; Fig. 3.14D) than rats with BBB >=14 (47.4±1.76 cm/sec) and those with a sham injury (54.3±1.5 cm/sec). Stride length (Fig. 3.14E) was higher in rats with BBB<14 (15.8±0.3 cm) than BBB>=14 (17.3±0.5 cm) and sham injured (17.9±0.3 cm) rats. Since stride length is a function of velocity, we determined stride length / velocity (Fig. 3.14F) which was significantly higher in rats with BBB<14 (0.44±0.02 a.u.) than the rats with BBB>=14 (0.37±0.01 a.u.) and sham injury (0.33±0.01 a.u.).

Discussion

In the present study, we have assessed intrinsic locomotor recovery as a function of both time post iSCI and the level of injury, utilizing 2D and 3D kinematics during locomotion. To our knowledge, this is the first comprehensive study presenting detailed qualitative and quantitative evaluation of locomotor recovery over time. We have also provided the qualitative and quantitative indices for intralimb and interlimb coordination or 3D kinematics of joint angle movement in all four limbs simultaneously.

Our results indicate that locomotor function in iSCI rats improves over time but is significantly different from that of intact rats. BBB locomotor scores improved until 6 weeks post iSCI and attained a plateau thereafter. However, 2D and 3D kinematic results showed continued improvement in locomotor function beyond 6 weeks. Based on the BBB score at day 7, animals were divided into two groups. Rats whose BBB

scores were greater than or equal to 14 were grouped as mild injured and others as moderate injured. In early stages after injury, often, the degree of injury can be masked by spinal shock as discussed by Kaegi et al [16]. The classification of groups based on BBB at day 7 reduces the uncertainty in the degree of injury. Though, BBB reflects the extent of injury, it often cannot detect subtle changes of recovery process. Lankhorst et al [44] derived a 7-point BBB subscore to evaluate fine motor control function in the animals. The BBB score reflects all locomotor behavior by one score value and the value increases as motor functions are recovered. Most often, the animals tend to recover motor tasks such as toe clearance, paw position earlier that tasks such as consistent forelimb-hindlimb coordination. On these occasions, the BBB subscore provides essential information on factual locomotor recovery. Moreover, the BBB score is reported as an average of the right and left scores, which suggests that the contusion injury is symmetrical but often contusion injuries may not be symmetrical. Though, BBB locomotor rating score is fairly easy to perform and time efficient, our current results demonstrates that 2D/3D kinematic analysis is imperative to bring out intricate details during recovery process.

Spinal cord injury causes alterations in balance control and reduction of base of support as indicated by increased stance width (Fig. 3.1), increased hindfoot rotation angle and widening of the HL limb segments (Fig. 3.2 & 3.3). Several investigators are using footprint [9, 31, 45, 46], catwalk (automated footprint) [12], Thoraco Lumbar Height(TLH) [44, 46] analyses for assessing base of support. Though there have been several modifications to foot print analysis, the method still remains time consuming. Cheng et al. [36] introduced a novel method to record and analyze the footprints using a video system. Although this method improved the quality of the data and able to extract temporal aspects of locomotion and gait patterns, it is still time consuming. Hamers et al. automated the analysis further to reduce the processing and analyzing time. We adopted the same idea but used infrared markers on the ball and heel instead of recording the paw print area. The heel often does not make contact with the walking platform while eliciting weight-bearing locomotion and hence cannot pickup the position of heel. Most of the injured animals tend to walk with their paws angled and our method reliably calculates the rotational angle since our method allows identifying the heel

position even when the heel is not touching the platform. On the other hand, Thoracolumbar height(TLH: indicator of weight bearing capacity could be deceivable for two reasons; 1) the adults rats are still growing and therefore there will be an intrinsic change in TLH; 2) after injury, the gait of the rat is altered to support it's weight, such as angled walking and wide stance this means height may increase but the impairment still exists.

Few-reported hindfoot rotation values [9] differ from our rotation angle values. This difference can be attributed to variation in the method of calculation. Our results show that after injury, the stance width (base of support) is increased and Stride length is decreased. These results are similar to the values reported earlier [9, 12, 47]. After injury, the velocity is also decreased. Since the stride length is dependent of velocity, stride length was normalized with velocity (SRv). This index did not show significant difference.

3D kinematics allows detection of intricate and minute deficits in gait, especially the higher motor deficits and also provides indirect information on sensory deficits. Though, the ideal way for multi-segmental joint angular measurements is provided by using x ray technique, we and several other investigators [8, 31, 32, 48, 49] used passive markers for angular assessment. Our results from chapter 2 are comparable with the results obtained using x ray technique [50], proving the viability of this technique. After injury, major abnormalities were seen in knee and ankle angles. Metz et al [9] reported that the hip, knee and ankle angles were increased. Our results indicate that there is no considerable change in the hip angle. The knee angle is significantly decreased and the ankle angle is increased. The detailed angular assessment information such as variations in extension, flexion angles, is lacking in the literature. Our study provides such data. After injury, the knee flexion as well as range of motion are severely reduced. The ankle is oveflexed and overextended and its excursion is increased. In addition, the biphasic pattern seen in pre-injury ankle angle trajectory is lost. After injury, impaired knee and ankle angles remain unchanged until the end of the study. Decreased touch-down angle values in the ankle angle indicates that the stance phase is initiated in advance while the hindlimb is still flexing. On the other hand, increased lift-off angle value indicate the delayed onset of swing phase

while the hindlimb is still extending. Thus, in all rats, after injury, the percentage stance duration is increased and percentage swing duration decreased [9]. Interestingly, the FL kinematics did not alter significantly though their usage during uncoordinated locomotion increases.

After injury, intralimb and interlimb coordination is also altered. The maximum extension of hip and knee occurs in phase. Even though, the FL-HL coordination is impaired, the overall left-right segmental coordination is preserved. This finding corroborates the presence of commissural interneurons(CIN) in the spinal cord CPG [51]. The axons of CIN traverse the midline to form synapses onto motor neurons and/or other interneurons (including CINs) situated in the contralateral hemicord thus automatically activating the other side when one side is activated. Variations in the overall segmental left-right coordination are high and could be explained by comparing the one-one intra segmental left-right coordination. The coordination contours for all the joints are preserved except for the ankle. The butterfly pattern (Fig. 3.10) observed pre-injury is lost. After injury, the knee coordination contour pattern is preserved (Fig. 3.10; after zooming) but the range is severely reduced.

Preliminary neural measures for ankle joint indicate that increased GM activity and presence of co-contraction are observed after injury. In conclusion, our detailed 2D and 3D kinematic techniques reveal the intricate and minute deficits in assessment of locomotor function in neurodegenerative diseased subjects,

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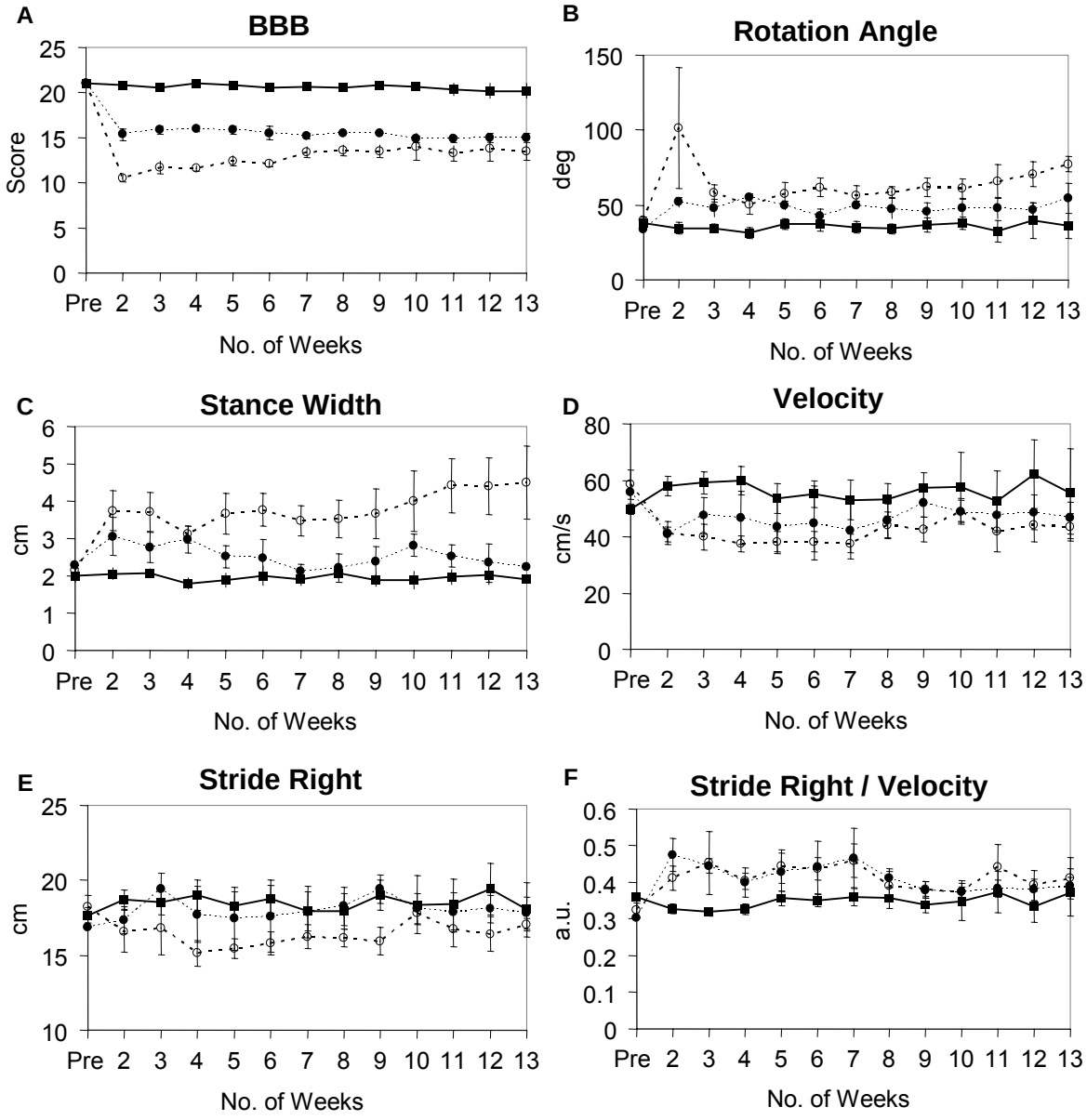

Figure 3.1: Changes in BBB measures and 2D kinematic measures. A) BBB, (B) Rotation Angle, (C) Stance Width, (D) Velocity, (E) Stride Right, and (F) Stride Right/Velocity at pre-injury (pre) and 2-13 weeks post-injury. ■--- sham (Group 1, n=6), ●… Group 2a iSCI (n=3), ○… Group 2b iSCI (n=7). For 2D kinematics, only data from iSCI rats capable of plantar foot placement is presented (see text for details).

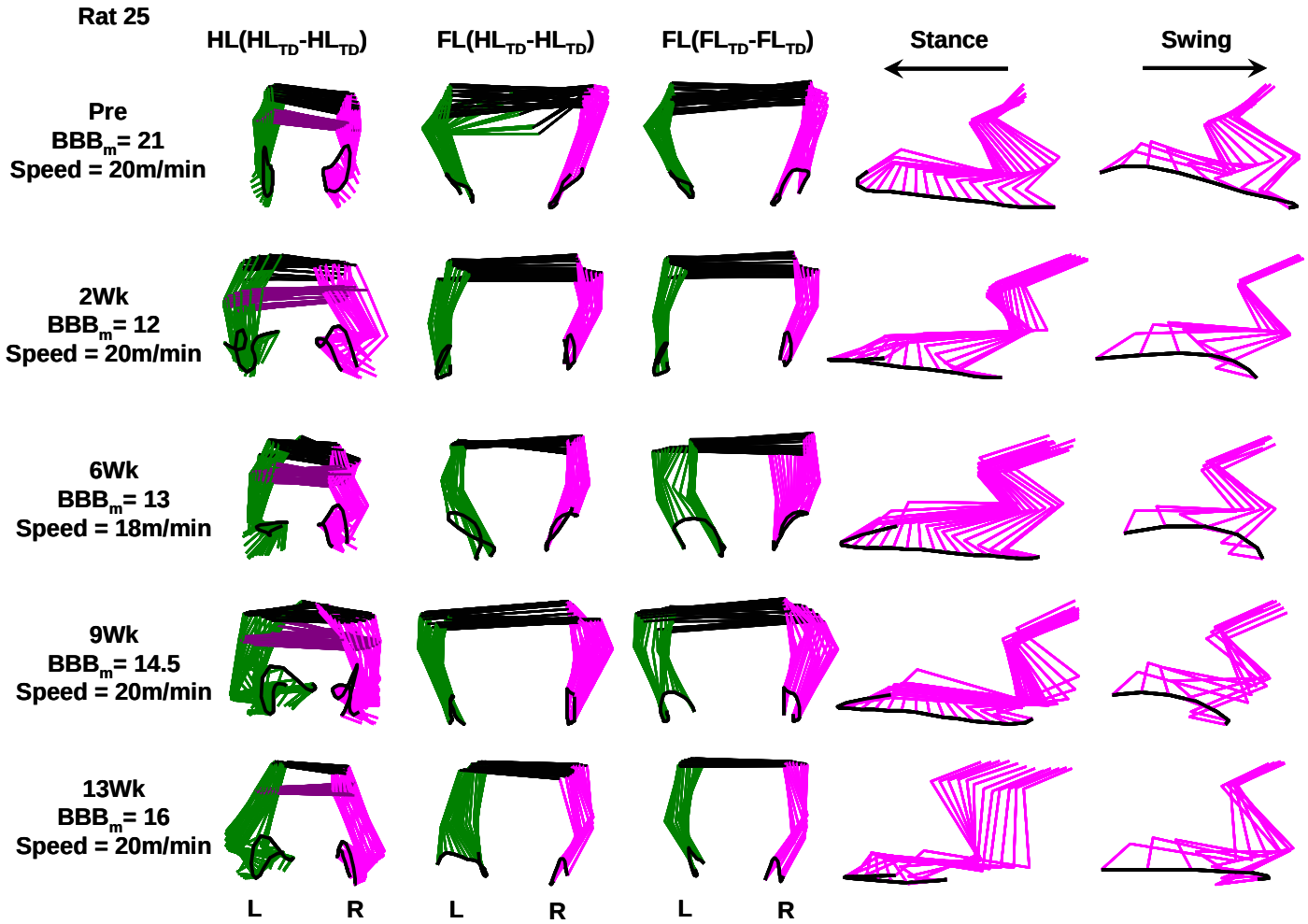

Figure 3.2: Stick figure representation of limb segments in iSCI Rat 25. Rear view of hindlimb (HL) (column 1) and forelimb (FL) (column 2) segments from HL_{TD}- HL_{TD}. Rear view of FL (column 3) from FL_{TD}-FL_{TD}. Sagittal view of HL stance phase (column 4) and swing phase (column 5). Left - right hip, pelvis and shoulder markers are connected (see text for the limb segment description). In rear view HL, ankle is traced and in FL wrist is traced while in sagittal view toe is traced. Left limb segments are indicted in green and right limb segments are indicted in pink. TD: Touch Down.

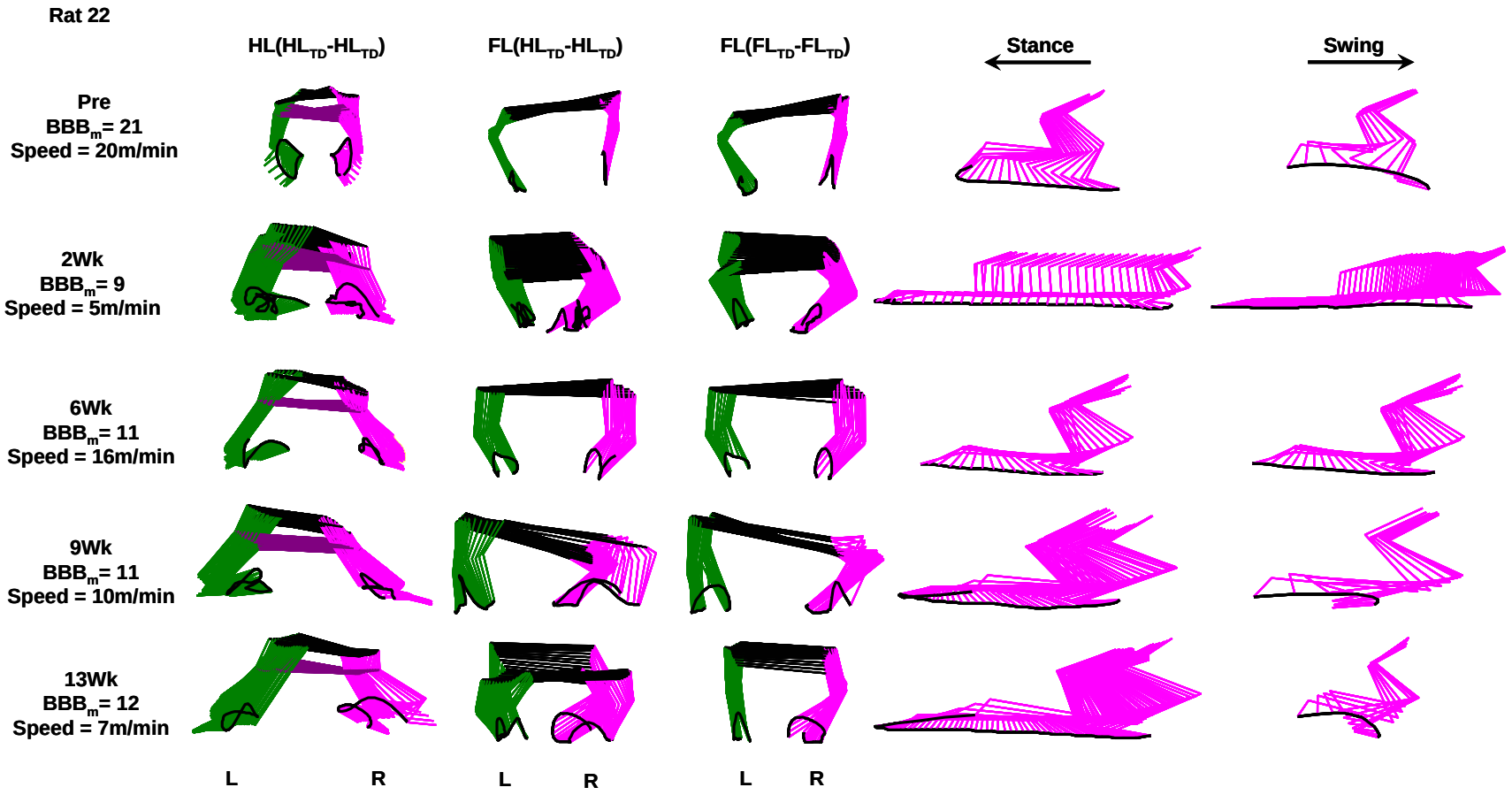

Figure 3.3: Stick figure representation of limb segments in iSCI Rat 22. Rear view of hindlimb (HL) (column 1) and forelimb (FL) (column 2) segments from HL_{TD}- HL_{TD}. Rear view of FL (column 3) from FL_{TD}-FL_{TD}. Sagittal view of HL stance phase (column 4) and swing phase (column 5). Left - right hip, pelvis and shoulder markers are connected (see text for the limb segment description). In rear view HL, ankle is traced and in FL wrist is traced while in sagittal view toe is traced. Left limb segments are indicted in green and right limb segments are indicted in pink. TD: Touch Down.

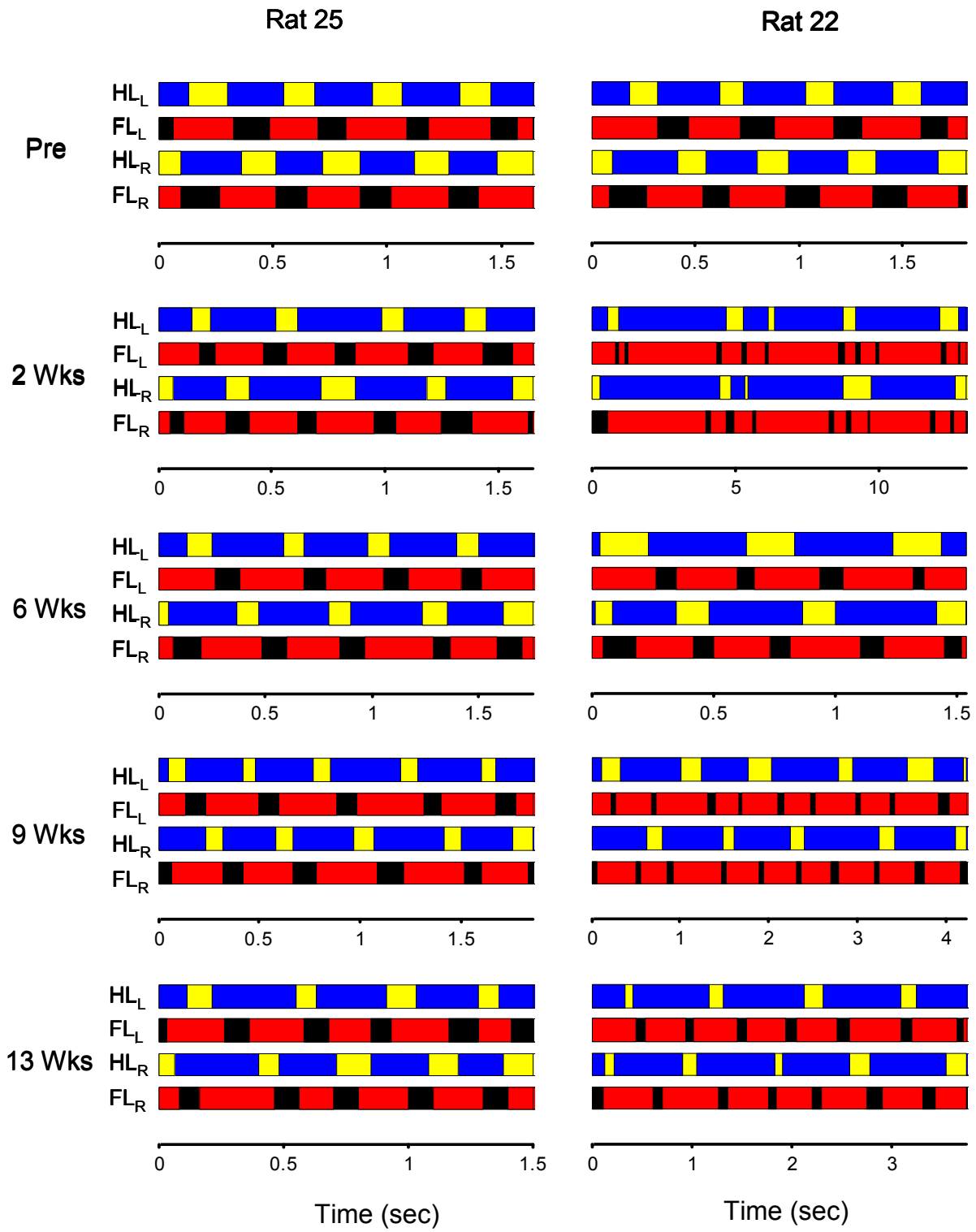

Figure 3.4: Footfall patterns: Comparison of relative interlimb swing and stance phase durations and stepping gait pattern in Rats 25 and 22 over time. In HL (hindlimb), blue and yellow indicate stance and swing phases and in FL (forelimb), red and black indicate stance and swing phases. L: Left and R: Right.

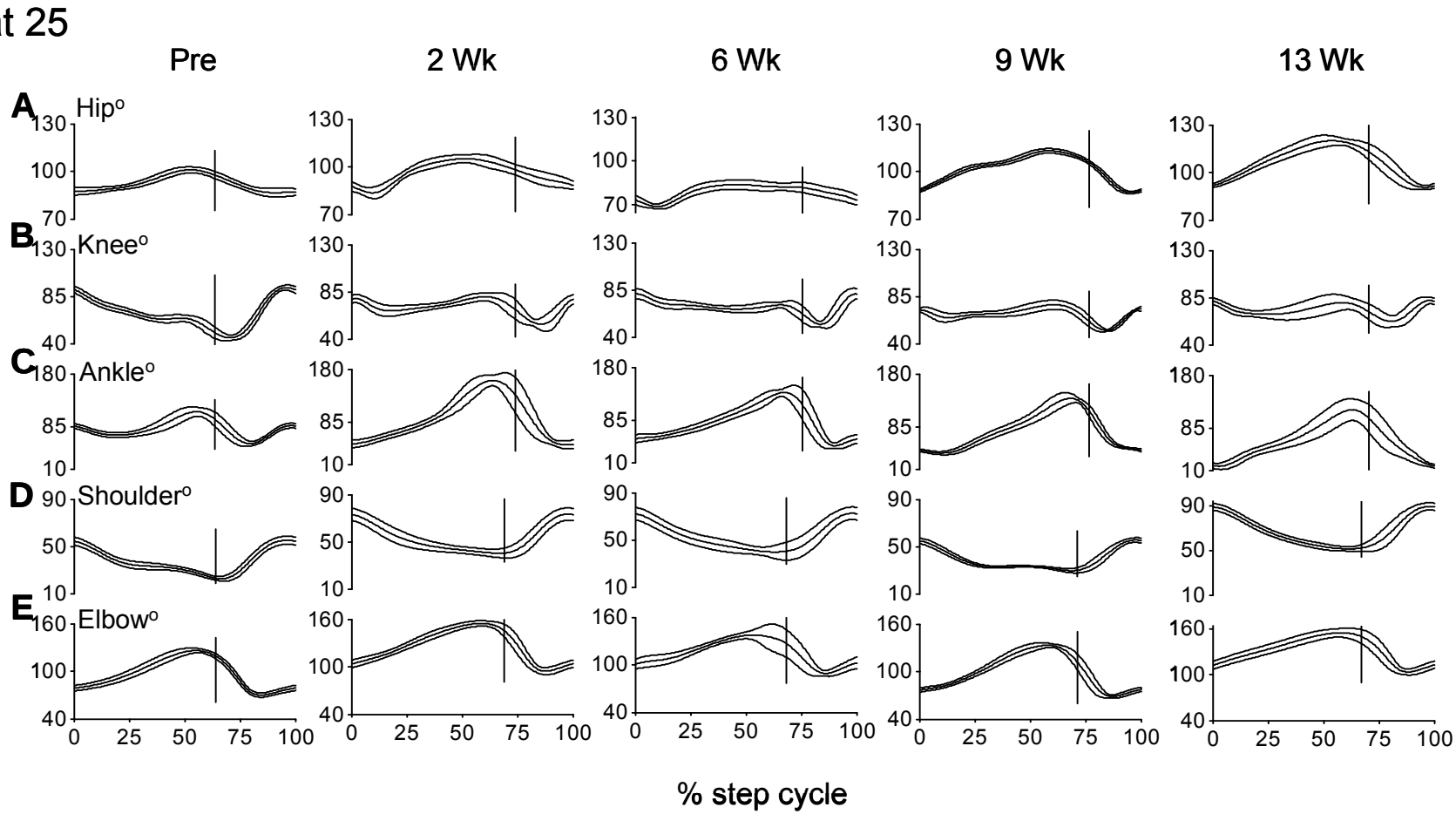

Figure 3.5: Average angle trajectories of iSCI Rat 25 (fast locomotor recovery) over time. Column 1 illustrates average angle trajectories at pre-injury and columns 2, 3, 4, and 5 represent the angle trajectories at 2, 6, 9, and 13 weeks post-injury respectively. Each column illustrates the average +/- 1SD (dashed line) angle trajectories of (A) Hip, (B) Knee, (C) Ankle (D) Shoulder, and (E) Elbow. Vertical dotted line: Lift-off event marker

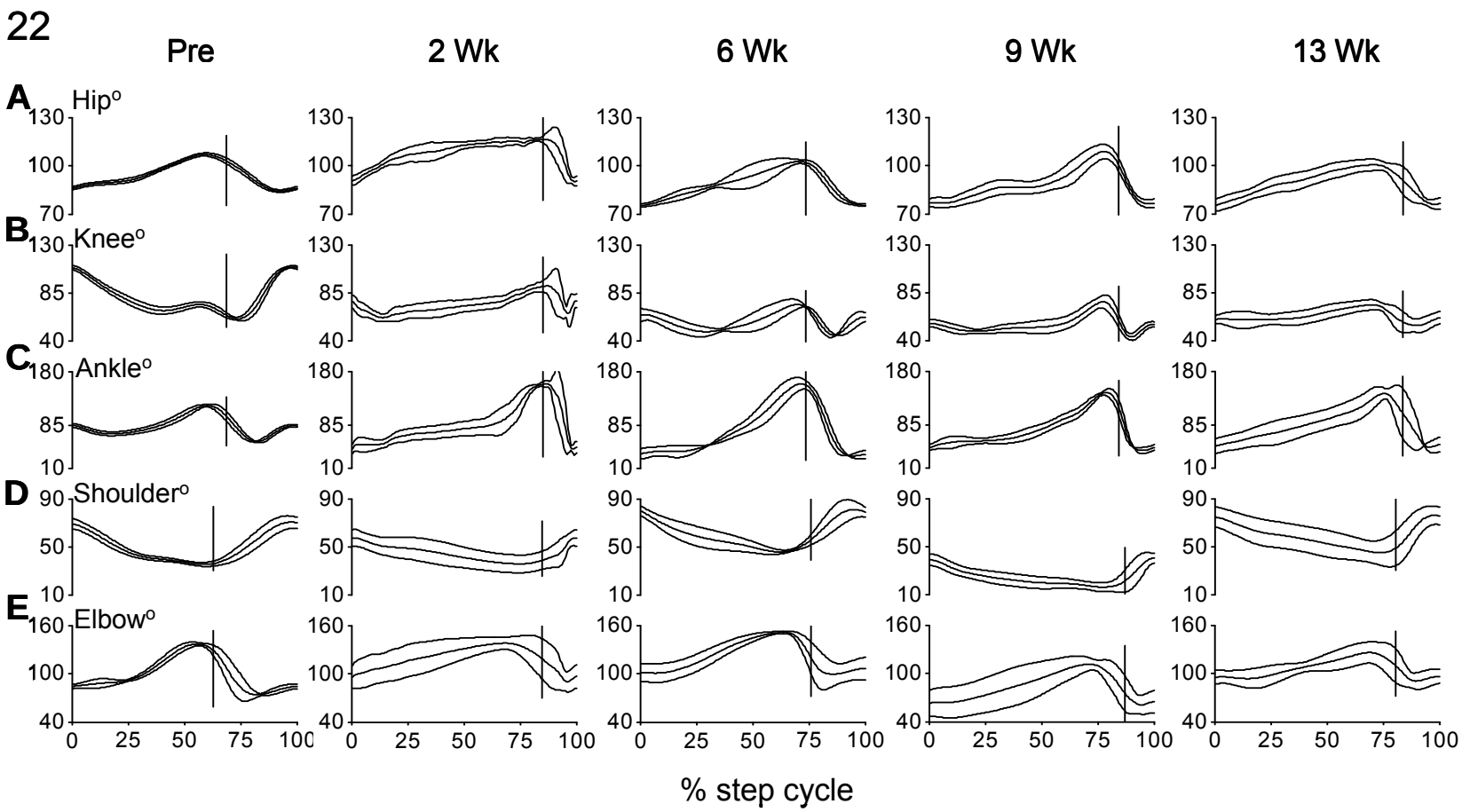

Figure 3.6: Average angle trajectories of iSCI Rat 22 (slow locomotor recovery) over time. Column 1 illustrates average angle trajectories at pre-injury and columns 2, 3, 4, and 5 represents the angle trajectories at 2, 6, 9, and 13 weeks post-injury respectively. Each column illustrates the average +/- 1SD (dashed line) angle trajectories of (A) Hip, (B) Knee, (C) Ankle (D) Shoulder, and (E) Elbow. Vertical dotted line: Lift-off event marker.

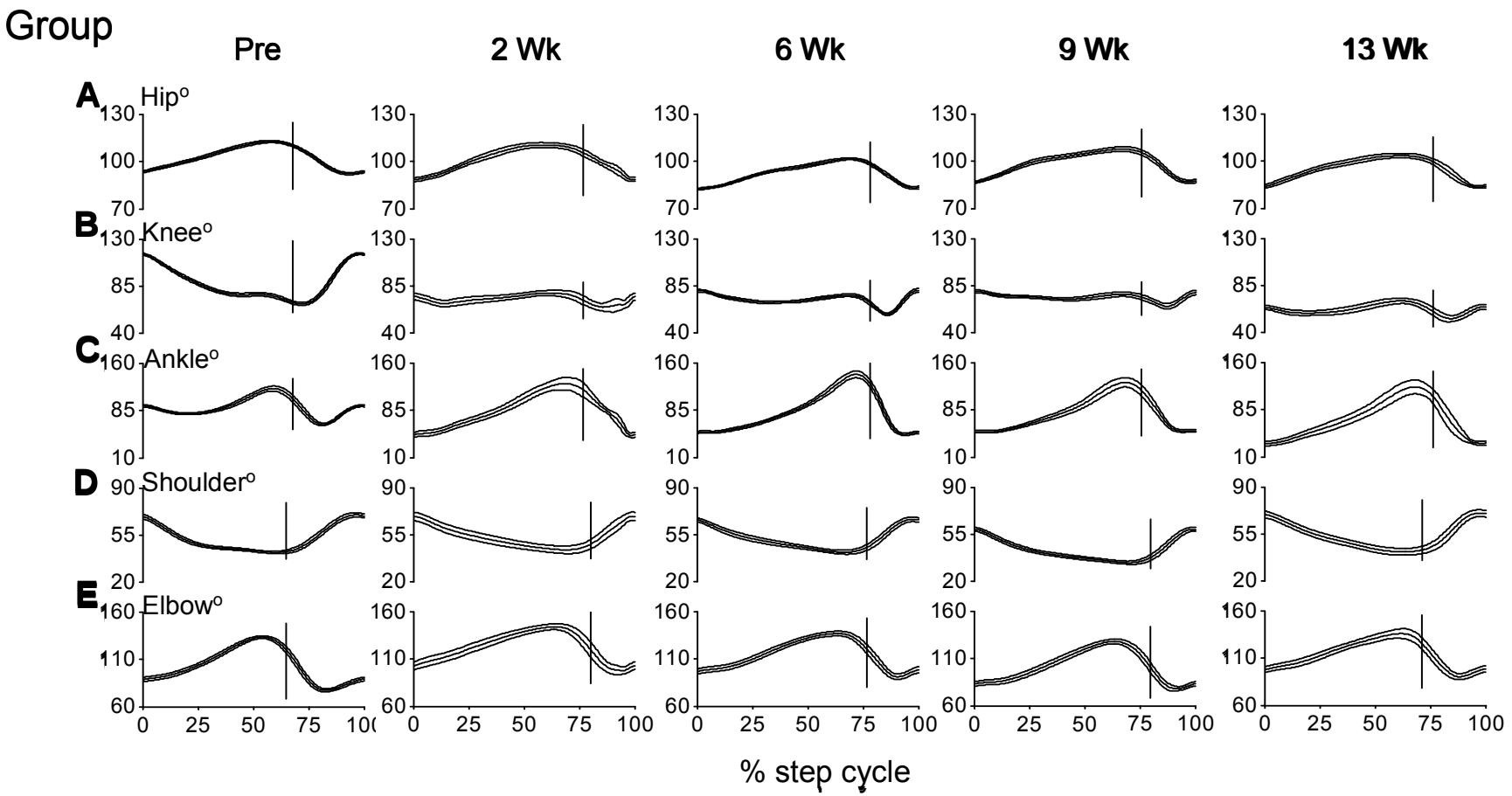

Figure 3.7: Average angle trajectories of iSCI rats over time. Column 1 illustrates average angle trajectories at pre-injury (n=7) and columns 2, 3, 4, and 5 represents at 2 (n=4), 6(n=7), 9(n=7), and 13 (n=4) weeks post-injury respectively. Each column illustrates the average (4-16 cycles per rat; solid line) +/- 1SEM (dashed line) angle trajectories obtained from a group of 4 or 7 rats (See text for further details). (A) Hip, (B) Knee, (C) Ankle (D) Shoulder, and (E) Elbow angle trajectories. Vertical dotted line: Lift-off event marker.

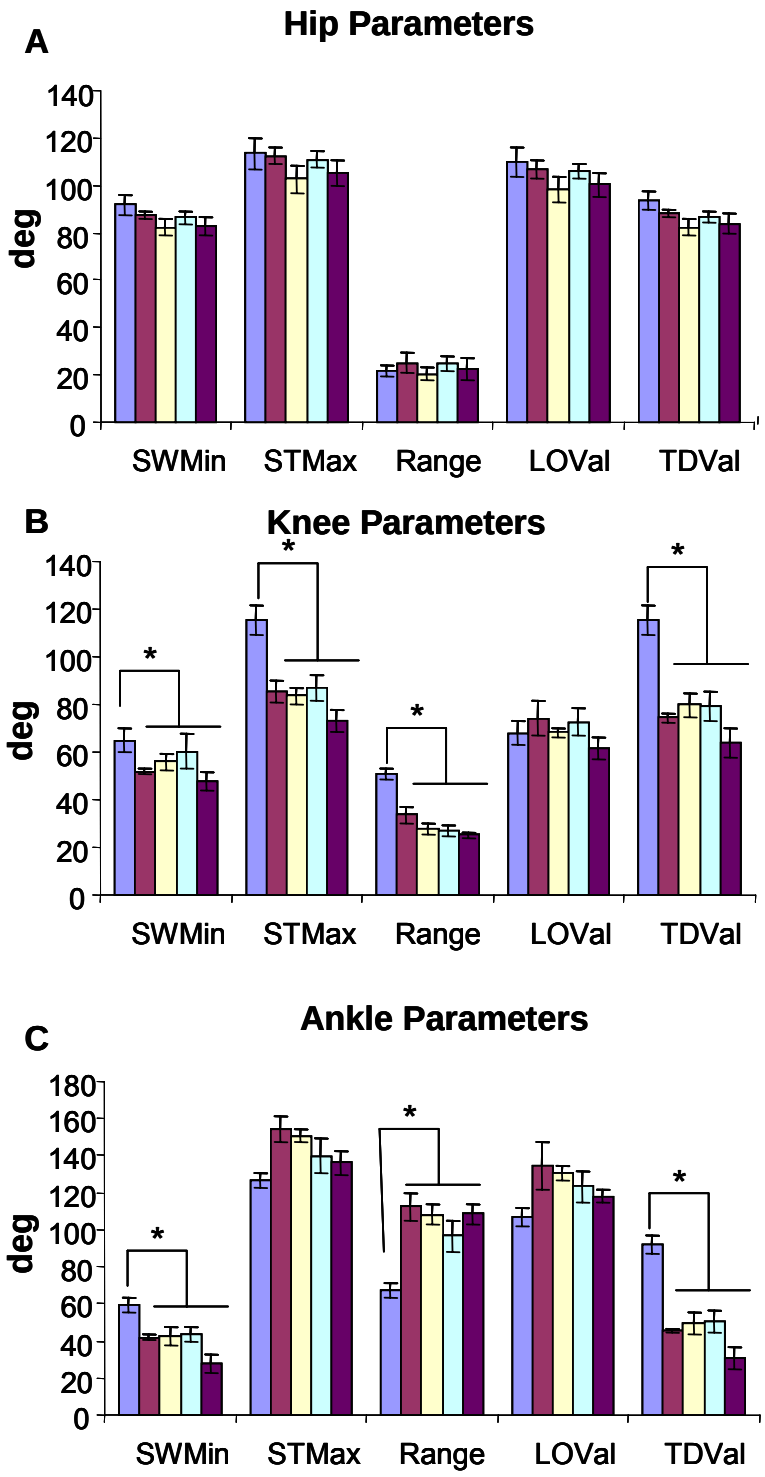

Figure 3.8: Hindlimb mean joint angle values in iSCI rats over time. SWMin- Swing minimum; STMax - Stance Maximum; LOVal – Lift off value, TDVal – Touch down value. Mean +/- SEM for 4 or 7 rats. Hip parameters are illustrated in (A), Knee in (B) and Ankle in (C). The knee and ankle parameters are altered after injury (See text for details). Blue: Pre-injury; mauve: 2 wks; yellow: 6 wks; green: 9 wks; purple:13 wks post iSCI.

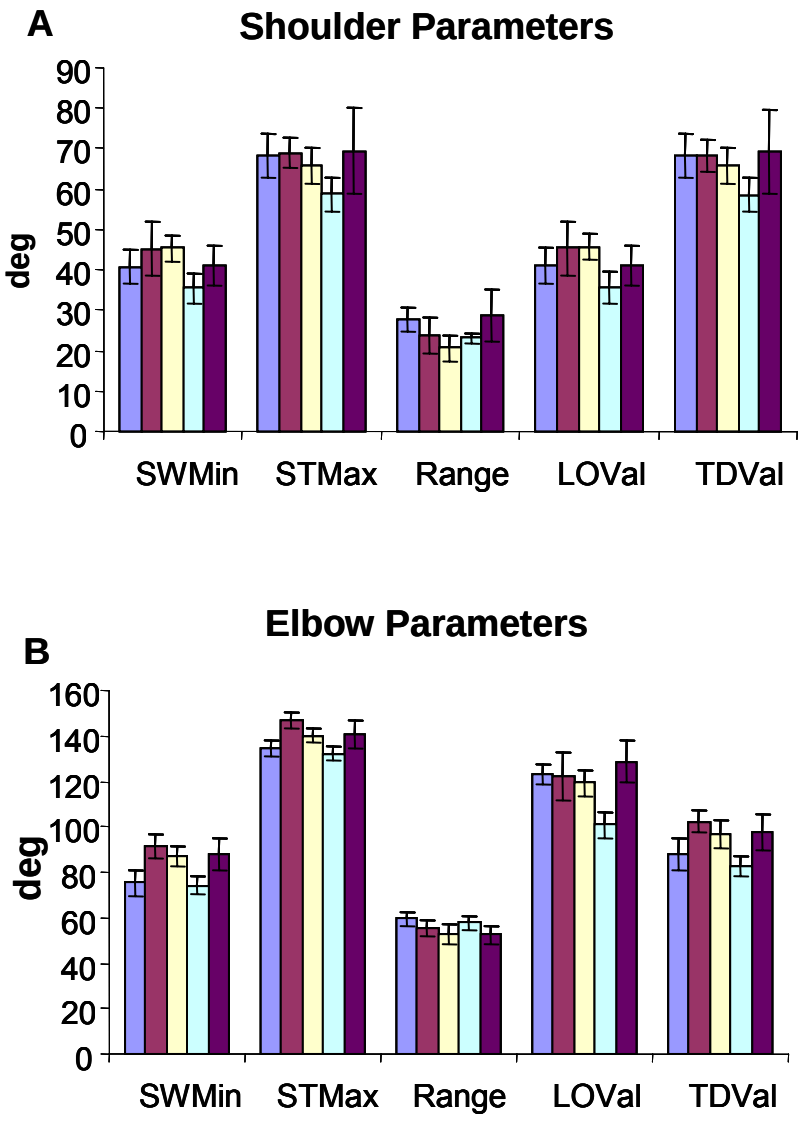

Figure 3.9: Forelimb mean joint angle values in iSCI rats over time. SWMin- Swing minimum; STMax - Stance Maximum; LOVal – Lift off value, TDVal – Touch down value. Mean +/- SEM for 4 or 7 rats. Shoulder parameters are illustrated in (A) and Elbow in (B). Blue: Pre-injury; mauve: 2 wks; yellow: 6 wks; green: 9 wks; purple:13 wks post iSCI.

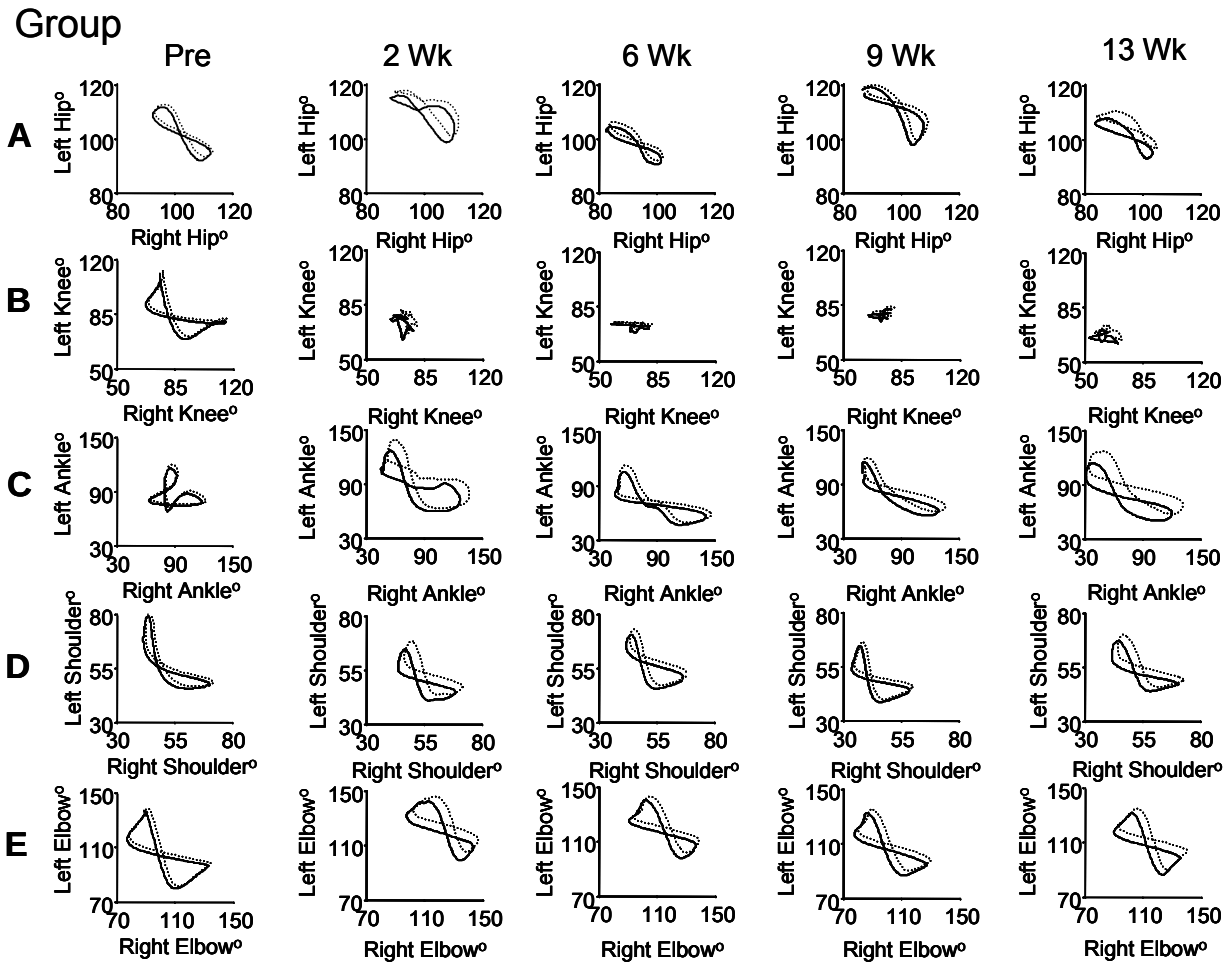

Figure 3.10: Left-right interlimb coordination of iSCI rats over time. Angle-angle plots illustrate the coordination between the joints of contralateral limbs during treadmill walking. Data (average (solid line) +/- 1SEM (dashed line) from 4 or 7 rats (4-16 cycles) per rat shows the maximum, minimum and range of excursion of each joint. After injury, A) the typical figure-eight pattern seen for left-right coordination of Hip (A) is preserved but the range is altered. B) Knee excursion is severely impaired and the pattern is lost. C) The “butterfly” ankle coordination pattern in ankle is replaced by a simple “figure-eight” pattern indicating the loss of double burst in the angle trajectory. D) Coordination of shoulder and E) Elbow are not affected by injury.

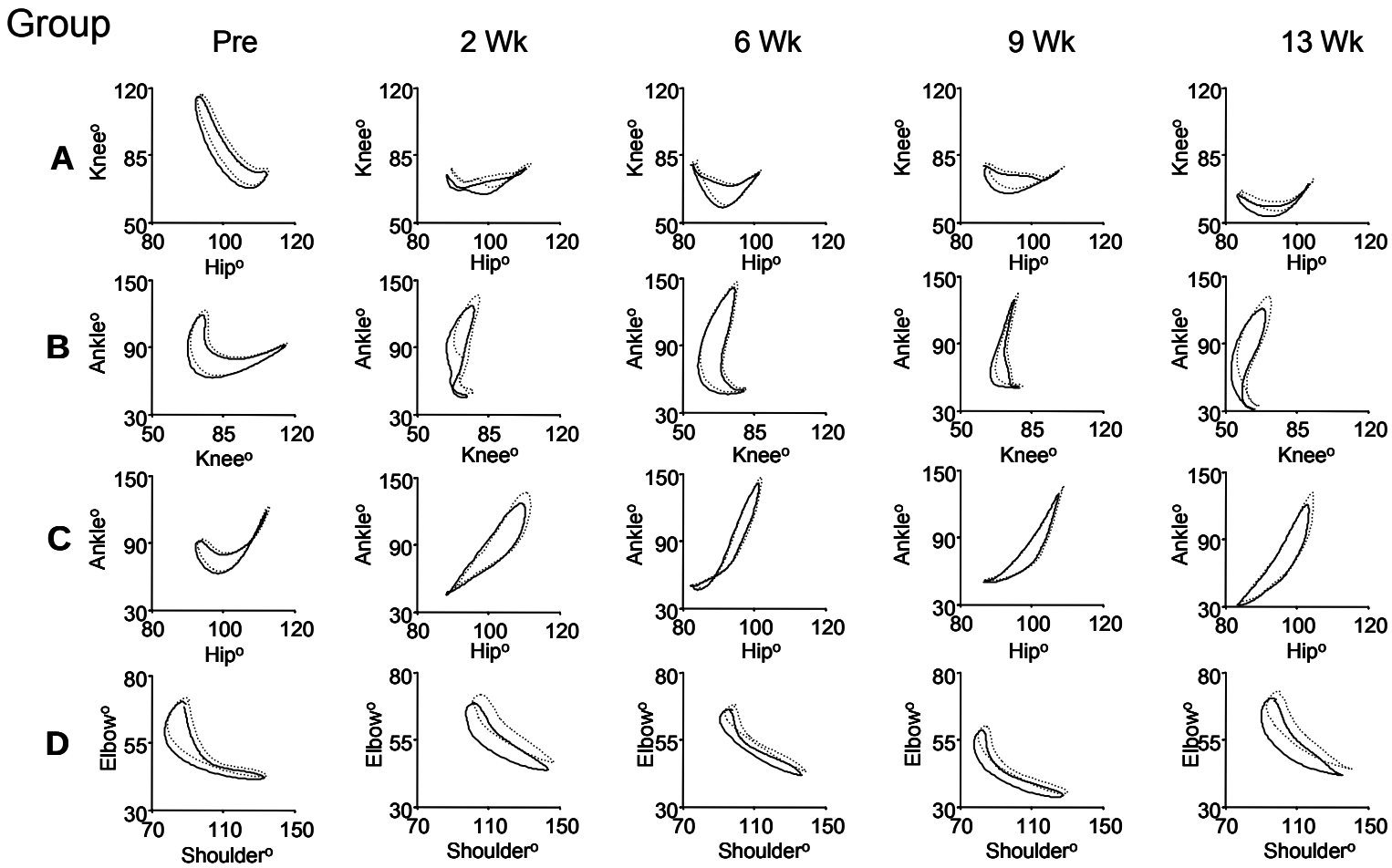

Figure 3.11: Intralimb coordination of iSCI rats over time. Angle-angle plots illustrate the coordination between the joints of the same limb during treadmill walking. Data (average (solid line) +/- 1SEM (dashed line) from 4 or 7 rats (4-16 cycles) per rat shows the maximum, minimum and range of excursion of each joint. After injury, in the hindlimb the typical coordination patterns of Knee vs. Hip (A), Ankle vs. Knee (B) and Ankle vs. Hip (C) were altered. Injury did not affect the pattern of Elbow vs. shoulder (D) forelimb intralimb coordination.

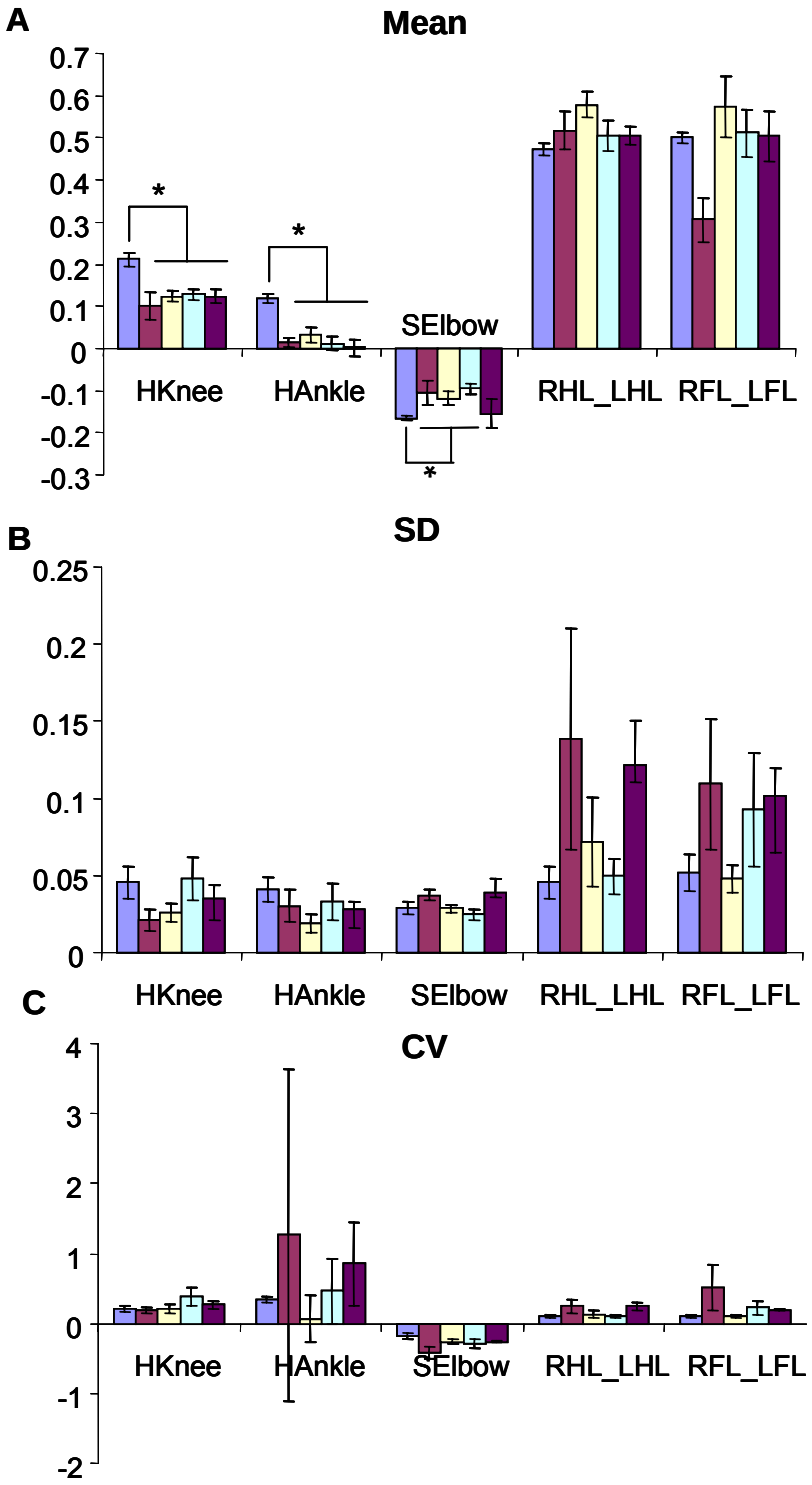

Figure 3.12: Phase measures of iSCI rats over time. (A) In hindlimb, group mean ± SEM phase values of Hip to Knee (Hknee) and Hip to Ankle (HAnkle), Shoulder to Elbow (SElbow). (B) Standard deviation of the above measures (C) Coefficient of variation of the above measures. Blue: Pre-injury; mauve: 2 wks; yellow: 6 wks; green: 9 wks; purple:13 wks post iSCI.

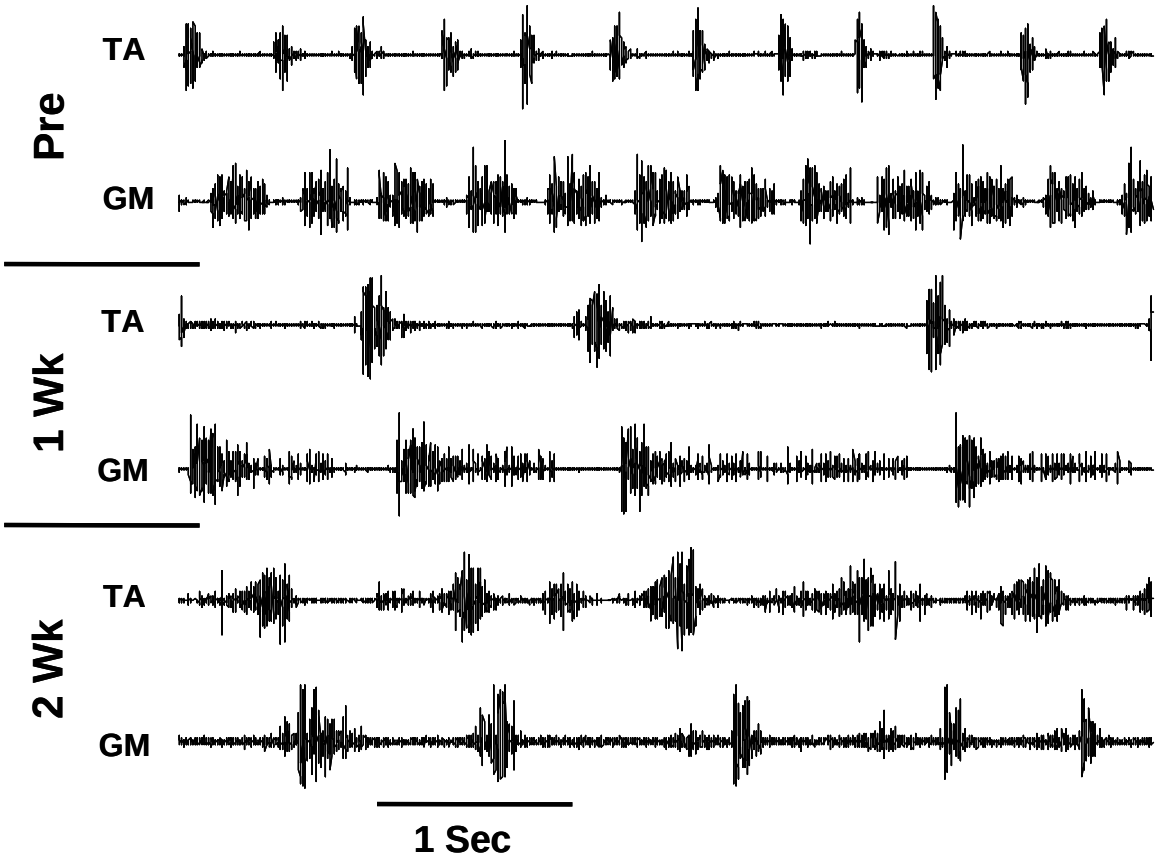

Figure 3.13: Comparison of flexor and extensor EMG activity of the ankle joint during treadmill walking in iSCI rat. TA: ankle flexor and GM: ankle extensor, of iSCI at pre-injury, 1 and 2 weeks post-injury. At 1-week post-iSCI, the burst duration of GM increased significantly when compared to the pre-injury GM activity. At 2-week post-iSCI, the burst duration of GM decreased.

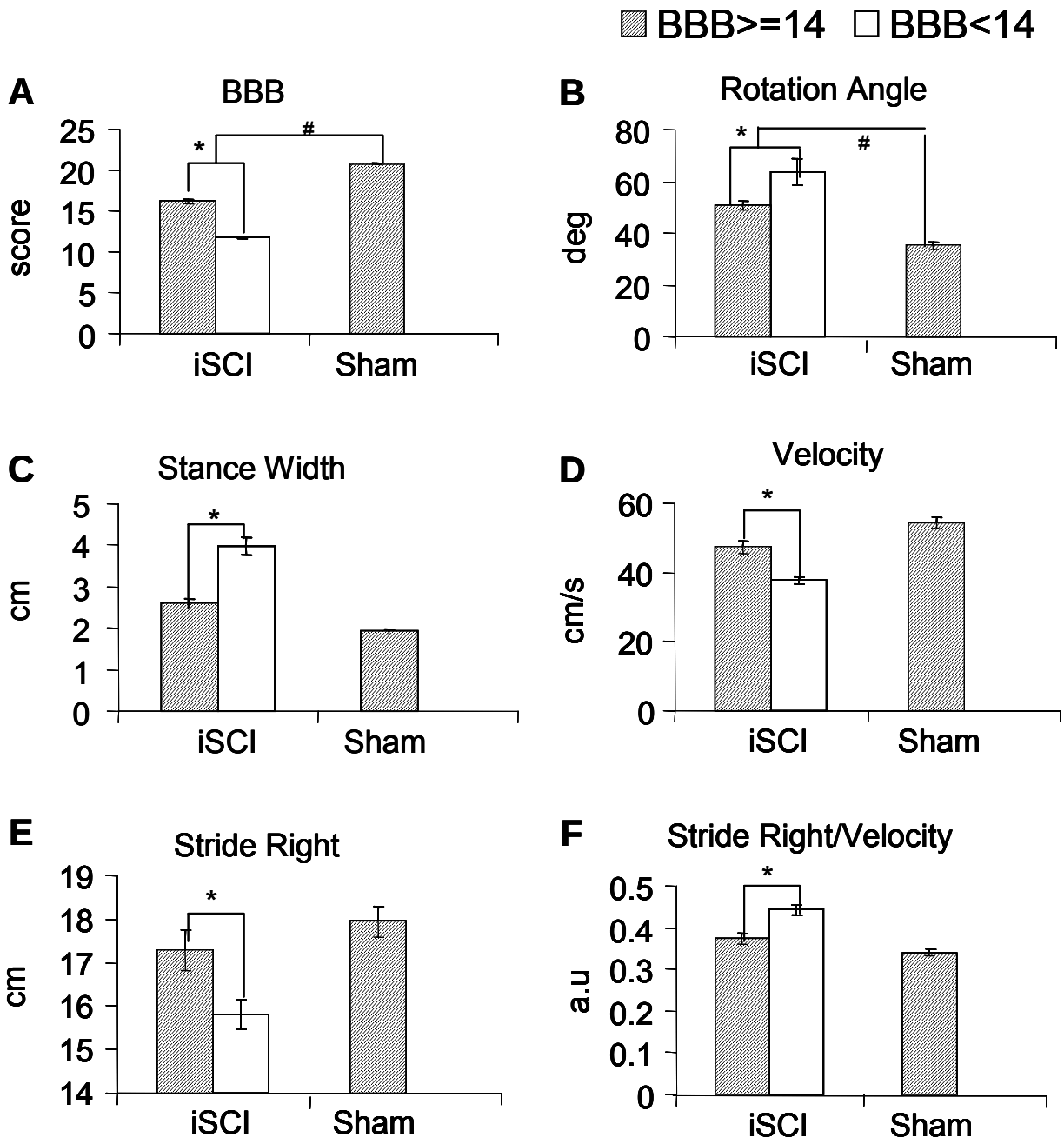

Figure 3.14: Effects of injury: BBB vs. 2D kinematics. 2D measures in iSCI rats were grouped by the level of injury (criteria BBB<14 and BBB $\geq$ 14). The values of (A) BBB, (B) Eversion Angle, (C) Stance Width, (D) Stride Right, (E) Velocity, and (E) Stride Right/Velocity were compared between two groups.

Chapter 4: Future work

The long-term goal of this research is to develop and implement strategies to enhance recovery of locomotor function in human subjects with incomplete Spinal Cord Contusion Injury (iSCI). In order to achieve this goal, three step by step specific issues need to be addressed:

1. To develop a tool to understand and characterize the underlying mechanisms of the normal locomotor function
2. To characterize or quantify the locomotor function in incomplete spinal cord injury subjects and
3. Implement strategies to enhance the recovery of locomotor function

This thesis report addressed the first two issues. Before proceeding further, the problems experienced with EMG recordings need to be rectified. We found that the novel EMG electrodes used in our study were not stable over time. Signal to noise ratio was very high and was seen as early as two weeks post iSCI. The possible solutions for reducing the noise would be:

- Redesigning a better interconnecting system between electrode wires and the head connector leads.
- Avoiding unnecessary interconnections between the amplifier modules and the EMG electrodes.

Future work:

EMG data from the injured animals needs to be analyzed to account for the changes after injury such as fatigue, co-contraction, spasticity etc. The outcome from these analyses would help either to modify the current strategies or to develop new strategies to enhance locomotor function.

Histological analyses need to be performed to understand the mechanism of recovery of the locomotion after injury. Outcome measures such as lesion volume, white matter sparing are expected to provide essential information on the recovery process of locomotor function. Further, classification of histological sections according to the location of white matter sparing should provide the valuable information on various partially or fully intact pathways. Comparison of the detail deficits with the intact

pathways should lead to the understanding of the possible mechanisms of specific motor task or deficit.

Finally, phase interrelationships of EMG activity with the corresponding kinematics information should provide the baseline information to design open loop and close loop functional electrical stimulation therapy in spinal cord injured individuals.

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Vita

Date of Birth : 13 September 1976
Place of Birth : Karimnagar, Andhra Pradesh, India

EDUCATION:

Bachelors of Engineering in Biomedical Engineering, **Osmania University,** Hyderabad, India. May'94 - Jun'98

PROFESSIONAL POSITIONS:

Visiting Scholar May' 03 to Feb' 04
Harvard-MIT Division of Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA.

Research Assistant Aug' 00 – May'03
Centre for Biomedical Engineering, University of Kentucky, Experimental and Computational Neuroscience Lab, Lexington, KY.

Biomedical Engineer In-charge
Sep'99 – Jul'00
Swarn Jayanthi Samudaik Hospital, Raunchi Bangar, Mathura, Uttar Pradesh, India.

Instructor
Jan'99 – Sep'99 Univ. College of Engineering, Dept. of Biomedical Engineering, Osmania University, Hyderabad, India.

Research Assistant
Jun'98 – Jan'99
Navigational Electronics Research and Training Unit, Osmania University, Hyderabad, India.

HONORS:

- Best second paper award at National Rocky Mountain Bioengineering Symposium '01, Colorado.
- Oral presentation at Biomedical Engineering Society Conference at Duke University, Durham, NC, Oct '01.
- Research Assistantship at Biomedical Engineering Department at University Of Kentucky August'00

PUBLICATIONS, ABSTRACTS AND PRESENTATIONS:

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R.Jung, S.Carlson, E.Knapp, **A.Thota**, B.Thompson, N.Ravi. Kinematics Of Rodent Gait After Incomplete Spinal Cord Injury, *Society of Neuroscience, Orlando, FL, 853.4, Nov 2 - Nov 7, 2002*

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