Sensorimotor Adjustments After Unilateral Spinal Cord Injury in Adult Rats

Submitted to the College of Graduate Studies and Research of the University of Saskatchewan for partial completion of the Doctor of Philosophy degree in the Department of Veterinary Biomedical Sciences at the University of Saskatchewan and pertaining to Comparative Neuroscience

by

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ABSTRACT

A variety of behavioural tests were used to examine both sensory and motor function of freely behaving unilaterally spinal cord-injured and uninjured rats. The first experiment was designed to determine whether sensory and motor differences existed between uninjured Fischer, Lewis, Long-Evans, Sprague-Dawley and Wistar rats using endpoint, quantitative kinematic, and kinetic measurements. The second experiment examined differences in sensorimotor responses to cervical spinal cord hemisection in Lewis, Long-Evans and Wistar rats. For the third experiment, reflex and locomotor abilities of unilateral cervical or thoracic spinal cord hemisected Long-Evans rats were determined using endpoint, semi-quantitative kinematic, and kinetic measurements. The fourth experiment was designed to investigate the importance of the rubrospinal tract and ascending dorsal column pathways to overground locomotion. This experiment was conducted to help explain the behavioural observations made following cervical spinal cord hemisection. Furthermore, this experiment examined the effects of combined unilateral rubrospinal and dorsal column injury on overground locomotion using endpoint and kinetic measurements. Finally, the fifth experiment set out to investigate the contribution of tracts running in the ventrolateral spinal cord on overground locomotion in freely behaving Long-Evans rats. These animals were assessed using endpoint and kinetic measurements.

The results of these studies revealed that motor and sensory functions are not similar for all uninjured strains of rats. Specifically, Fischer rats tend to have considerable differences in their morphological features and sensorimotor abilities compared to the other strains examined. Results from the other experiments indicate that adult freely behaving female rats develop a characteristic gait when pathways important for locomotion are injured unilaterally, regardless of strain. The rubrospinal tract and ascending dorsal column pathways appear to be important for both skilled and flat-ground locomotion as well as forelimb use while rearing. Pathways traveling within the ventrolateral pathway, however, are not necessary or sufficient for locomotion or limb useage while rearing when injured by themselves. Animals with ventrolateral spinal funiculus injuries regain normal forelimb use and skilled locomotor abilities. Injury to the ventrolateral spinal funiculus, however, results in mild (compared to rubrospinal and dorsal column injured animals) yet long-lasting locomotor changes based on ground reaction force determination. These findings are in agreement with the current opinion that there is a substantial amount of functional redundancy of pathways traveling in the ventral and ventrolateral funiculi.

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DEDICATION

I dedicate this work to my wife, Cheryl. The completion of this thesis was made possible only through her unconditional love, support, and encouragement.

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List of Abbreviations

BBB	Basso, Beattie, Bresnahan
BW	Body weight
CBS	Combined behavioural score
CH	Cervical hemisected
CNS	Central Nervous System
CPG	Central pattern generator
CS	Coeruleospinal tract
DC	Dorsal column
DCML	Dorsal column medial lemniscal pathway
DSCT	Dorsal spinocerebellar tract
$E_{1,2,3}$	Extension phase 1, 2 or 3
Ek	Kinetic energy
Ep	Potential energy
EWMN	Eshkol-Wachmann Movement Notation
F	Flexion phase
FC	Fasciculus cuneatus
FG	Fasciculus gracilis
g	Acceleration due to gravity
GABA	Gamma aminobutyric acid
h	Height of body center of mass
kVp	Kilovolts peak
LatCST	Lateral corticospinal tract
LatSTT	Lateral spinothalamic tract
LED	Light emitting diode
m	Mass
mA	Milliamperes
Main CST	Main corticospinal tract
mAs	Milliampere seconds
MedSTT	Medial spinothalamic tract
MLR	mesencephalic locomotor region
PSDCP	Post-synaptic dorsal-column pathway
ReticST	Reticulospinal tract
RF	Reticular formation
RSCT	Rostral spinocerebellar tract
RST	Rubrospinal tract
SHT	Spinohypothalamic tract
SMT	Spinomesencephalic tract
TH	Thoracic hemisected
v	Velocity
VCST	Ventral corticospinal tract
ULVL	Unilateral, ventrolateral
VSCT	Ventral spinocerebellar tract
VST	Vestibulospinal tract

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Chapter 1. LITERATURE REVIEW

1.1 Introduction

The bulk of the work within this dissertation pertains to the behavioural modifications that laboratory rats make following unilateral spinal cord injury. In particular, pathways traveling in the dorsal, dorsolateral and ventrolateral pathways have been examined. These include: 1) the ascending pathways traveling within the fasciculus gracilis and cuneatus (namely the post-synaptic dorsal column system and the dorsal column medial lemniscus pathway), 2) the rubrospinal tract, 3) descending and ascending pathways traveling within the ventrolateral funiculus. The rationale for examining each of these pathways is not arbitrary, but rather is because of the importance of these pathways for either sensory or motor performance in rats. Specific background information highlighting the rationale for examining the effects of injury to these pathways is provided in the following review of spinal cord pathways (Section 1.2) and of course the appropriate experimental chapters. Some of the experimental work within this dissertation also addresses the effects of strain on the behavioural abilities of uninjured rats.

The purpose of Chapter 1 is to provide an introduction to this dissertation by reviewing 1) the neuroanatomy of the rat spinal cord, 2) important ideas pertaining to the neural control of locomotion, 3) important concepts concerning the biomechanics of locomotion, 4) methods used for assessing sensorimotor abilities of rats, and 5) injury paradigms used for experimental spinal cord injury studies in rats. Understanding these topics is important for understanding the purposes and methodologies of the work presented herein.

1.2 Neuroanatomy of the rat spinal cord: a comparative approach

1.2.1 Introduction

Prior to embarking upon the determination of the relative importance of particular pathways within the spinal cord of the rat, it is important to understand the neuroanatomy of the rat's spinal cord. The basic terminology for identifying particular layers within the spinal cord is presented in Fig.1.1. Briefly, the white matter of the spinal cord contains the axons of descending, ascending and propriospinal neurons. Spinal white matter is referred to in quadrupedal species (from most dorsal to ventral) as the dorsal funiculus, dorsolateral funiculus, ventrolateral funiculus, and ventral funiculus. The spinal gray matter houses the neuron cell bodies for ascending pathways, for interneurons, and for motor neurons.

In 1952, Rexed (Rexed 1952) developed a scheme whereby he divided the spinal cord gray matter into laminae based upon cytoarchitecture (Fig.1.2).

This laminar classification scheme is still used today. Rexed divided the gray matter into 10 layers. The laminae are numbered chronologically from dorsal to ventral. Lamina 10, however, is located circumferentially around the central canal. This system was developed in cats, however, and may not apply to all species.

Often times, neuroanatomy is extrapolated from one species and considered to be identical in another. In part this has stemmed from the lack of neuroanatomical research into particular species. For example, the direct pathway from the cerebral cortex to the spinal cord (corticospinal tract) is thought to be a relatively phylogenetically new tract found in higher species of animals. This tract is not found in birds, is poorly developed in horses, is located in the dorsal column of rats and oppossums, while being found in the dorsolateral funiculus of the spinal cord in cats, dogs, and people (Verhaart 1962; Brown 1971; Webster et al. 1990). From this simple example, it is evident that neuroanatomical differences exist between different species.

The purpose of this chapter is to highlight the neuroanatomy of the rat spinal cord and briefly compare and contrast the rat's spinal cord anatomy to that of other species. In addition, the rubrospinal tract and the associated red nucleus are described in more detail because of the vast amount of knowledge regarding this system and because of the background information required to

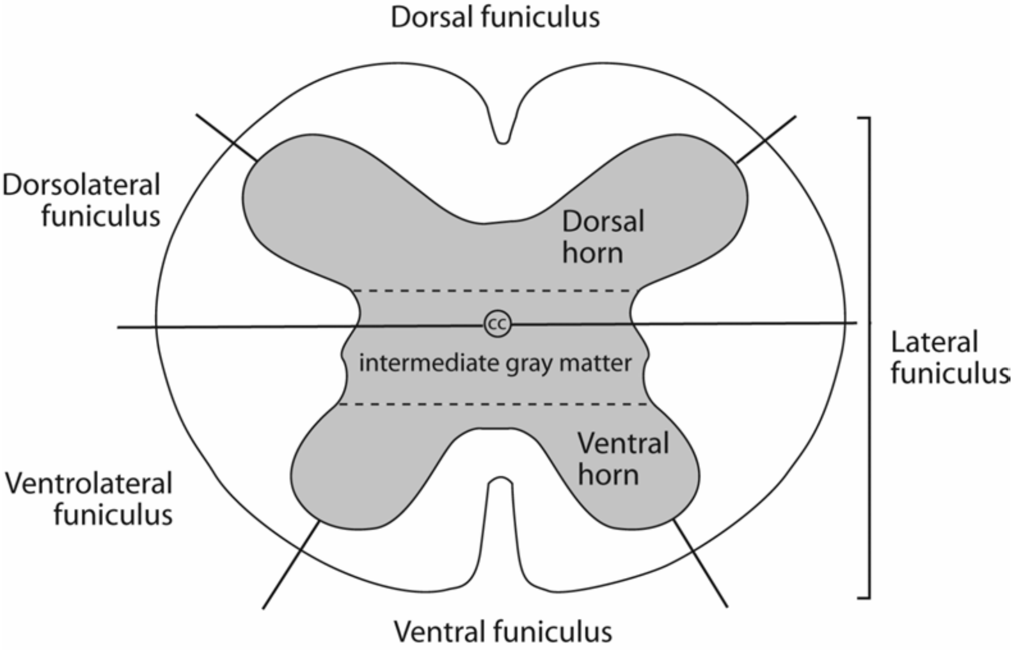


Fig. 1. 1 Location of the dorsal, ventral, lateral, dorsolateral and ventrolateral funiculi of the spinal cord white matter. The gray matter is divided into the dorsal and ventral horns and the intermediate gray matter.

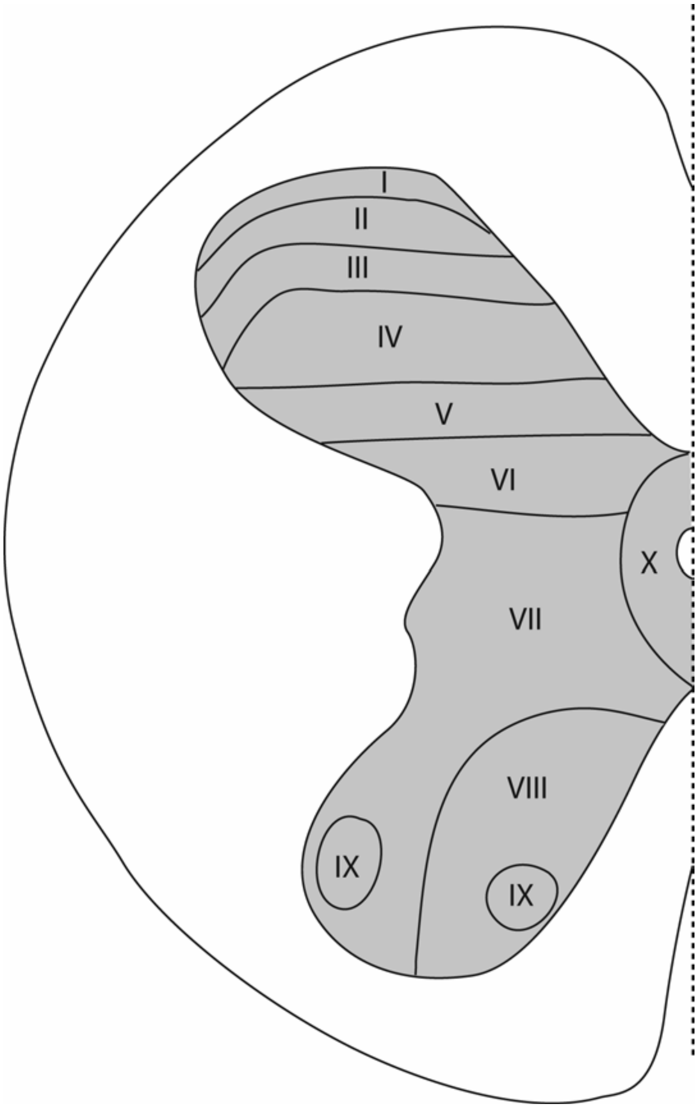


Fig. 1. 2 Rexed's laminae within the cervical spinal cord of the cat. The spinal gray matter is divided into 10 laminae based upon neural cytoarchitecture. Laminae I – IV and VII and IX are known as the 1) marginal zone (I); 2) substantia gelatinosa (II); nucleus proprius (III, IV); 3) nucleus dorsalis (VII); and 4) medial and lateral motor nuclei (IX).

fully understand one of the experiments in this thesis (Chapter 8). The reader is referred to Fig.1.3 for diagrammatic representation of spinal cord pathways.

1.2.2 The Dorsal Column

The dorsal columns are found in the dorsal funiculus of the spinal cord. The dorsal column pathways comprise approximately 1-5 % of the total cross-sectional area in teleosts (bony fishes) and elasmobranches (cartilaginous fishes), 16% for rats and cats, and 27% for humans (Wall 1970). In the rat, the dorsal columns contain both ascending pathways (dorsal column-medial lemniscal pathway and ascending post-synaptic dorsal column pathway) and descending pathways (corticospinal tract) to and from the brain.

1.2.2.1 Corticospinal tracts

The corticospinal tract arises from neurons in lamina V (Betz cells) of the sensory-motor cortex and is found only in mammals (see (Terashima 1995) for review; see also (Webster et al. 1990). These cells send their axons through the internal capsule and cerebral peduncles where they course ventrally in the brainstem and decussate incompletely at the level of the medulla ((Terashima 1995) for review; see also (Brosamle and Schwab 1997) (Brosamle and Schwab 2000)). The corticospinal tract is divided into uncrossed and crossed components. The main crossed corticospinal tract descends in the ventral portion of the dorsal funiculus (Fig.1.3) in oppossums,

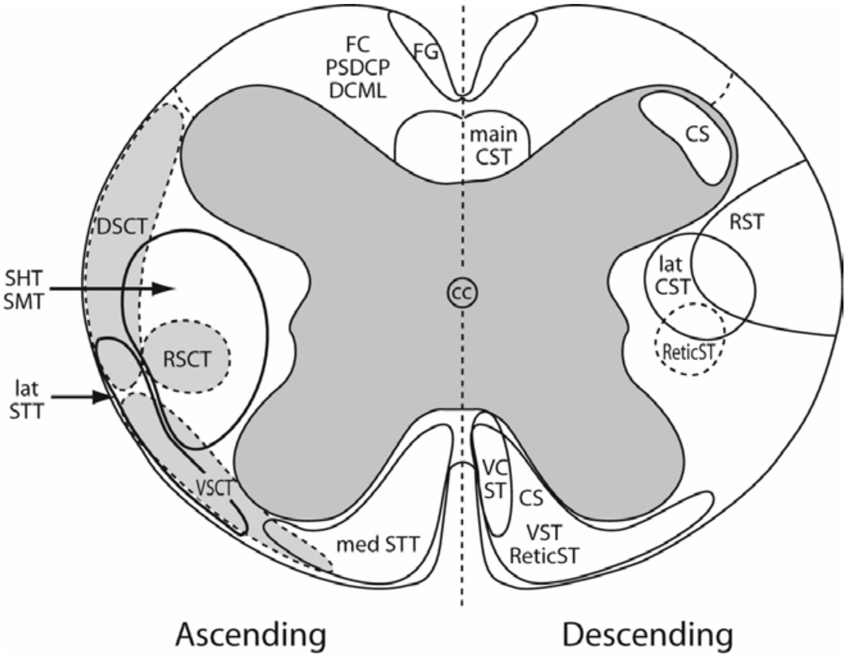


Fig. 1. 3 Approximate areas containing ascending and descending pathways within the rat spinal cord. Ascending pathways: DSCT**=dorsal spinal cerebellar tract (Yamada et al. 1991);** VSCT**=ventral spinocerebellar tract (Yamada et al. 1991; Xu and Grant 1994; Terman et al. 1998);** RSCT**=rostral spinocerebellar tract (Xu and Grant 1994; Terman et al. 1998);** SHT**=spinohypothalamic tract (Katter et al. 1996a; Kostarczyk et al. 1997);** SMT**=spinomesencephalic tract (Zemlan et al. 1978);** LatSTT**=lateral spinothalmic tract (Giesler, Jr. et al. 1981; Dado et al. 1994c);** MedSTT**=medial spinothalamic tract (Giesler, Jr. et al. 1981; Dado et al. 1994c);** FC**=fasciculus cuneatus;** FG**=fasciculus gracilis;** PSDCP**=post-synaptic dorsal column pathway (Giesler, Jr. et al. 1984);** DCML**=dorsal column medial lemniscal pathway (Willis and Coggeshall 1978). Descending pathways: Main** CST**=main crossed corticospinal tract (Brown, Jr. 1971; Terashima 1995; Brosamle and Schwab 1997);** LatCST**=lateral crossed corticospinal tract;** VCST**=ventral uncrossed corticospinal tract (Terashima 1995; Brosamle and Schwab 1997);** CS**=coeruleospinal tract (Clark and Proudfit 1992);** VST**=vestibulospinal tract (Houle and Jin 2001; Matesz et al. 2002);** ReticST**=reticulospinal tract (Fox 1970; Houle and Jin 2001);** RST**=rubrospinal tract (Brown 1974; Antal et al. 1992).**

rodents, and in some prosimian primates (Brown, Jr. 1971; Martin and Fisher 1968; Campbell et al. 1966). This main corticospinal tract is, however, found in the dorsolateral funiculus of the spinal cord in other animals including lagomorphs, carnivores, and primates (Brown, Jr. 1971). A smaller number of uncrossed fibers are found traveling in the ventromedial funiculus of the spinal cord and are known as the ventral corticospinal tracts (Buxton and Goodman 1967; Terashima 1995; Vahlsing and Feringa 1980; Brosamle and Schwab 1997; Brosamle and Schwab 2000) and a small crossed portion of fibers in the dorsolateral portion of the spinal cord (dorsolateral corticospinal tract) in the rat (Vahlsing and Feringa 1980; Brosamle and Schwab 1997; Brosamle and Schwab 2000). The ventral uncrossed component is derived from approximately 1-3% of the total number of corticospinal neurons (Terashima 1995). In some species such as primates, dogs and cats, the corticospinal tract is well developed. The corticospinal tract in domestic farm animals such as sheep and horses, however, is inconspicuous. Instead, these species have a prominent corticotegmental tract (originating from the same neurons as the corticospinal tract) known as Bagley's bundle. The corticospinal tract, however, extends only to the upper cervical portions of species such as armadillos, goats, sheep and horses (Verhaart 1962), while extending to midthoracic levels in oppossum and terminating in dorsal horn (Martin and Fisher 1968). In the rat, however, the corticospinal tract descends along the spinal cord to the lumbosacral levels and projects to the dorsal horn and the intermediate zone of the gray matter (Brown, Jr. 1971;

Terashima 1995). Corticospinal fibers have been shown to send collaterals to many different brainstem nuclei including red nucleus, pontine nuclei, inferior olivary nuclei, and dorsal column nuclei (O'Leary and Terashima 1988; O'Leary et al. 1990). The main corticospinal tract has been found to innervate neurons located in laminae IV to VI, and to motor neurons located in the ventral horn (Antal 1984; Casale et al. 1988; Rouiller et al. 1991). The uncrossed corticospinal tract has been found to synapse on neurons located predominantly in the intermediate laminae of the spinal cord (Brosamle and Schwab 2000).

The role of the corticospinal tract for performing motor functions has been intensively investigated for reaching and grasping tasks. Lesions of this tract, via pyramidotomy, results in impairments in grasping and digit use in many mammalian species including opossums, hamsters, rats, raccoons, cats and primates (Buxton and Goodman 1967; Lawrence and Kuypers 1968a; Lawrence and Kuypers 1968b; Castro 1972; Gorska and Sybirska 1980; Hore et al. 1973; Kalil and Schneider 1975; Lawrence and Hopkins 1976). Brosamle and Schwab proposed that the ventral corticospinal tract is responsible for similar motor control as the main corticospinal tract (Brosamle and Schwab 2000). Recently it has been shown that rats were less successful at retrieving pellets, as a result of proximal forelimb dysfunction, when all corticospinal tracts had been transected compared to when just the main dorsal corticospinal tract was injured (Weidner et al. 2001). It was also demonstrated that any functional recovery regained by rats with only dorsal corticospinal tract injury was lost immediately upon injuring the ventral uncrossed pathway (Weidner et al. 2001). Rats having either dorsal corticospinal tract or ventral corticospinal tract transections had impaired ability to retrieve food pellets soon after surgery; however, these animals regained pellet retrieval ability four weeks following surgery. From this study the ventral component of the corticospinal tract, it seems, can be used to compensate for behaviour previously mediated by the dorsal corticospinal tract and is equally as important as the dorsal corticospinal tract for skilled forelimb use.

In addition to reaching and grasping behaviours, it appears as though the corticospinal tract is important in performing tactile placing. Tactile placing reactions occur when an animal places its paw onto a solid surface when the surface is brought toward and touches the paw. It has been shown that tactile placing responses are absent initially following pyramidotomy or motor cortex ablation in dogs and cats (Hukuda et al. 1973). The development of tactile placing reactions has been shown to develop with the descent of the corticospinal tract in rats. Donatelle (Donatelle 1977) has shown that placing reactions are first seen between 4-7 days postnatally for the forelimbs, and between postnatal days 9 and 13 for the hind limbs. Interestingly, corticospinal axons are present in the cervical spinal grey matter at day 5 postnatally and have extended to the lower lumbar and sacral spinal grey matter by day 9 postnatally.

1.2.2.2 Dorsal column medial lemniscal and post-synaptic dorsal column pathways

Ascending fibers terminating on dorsal column nuclei have been identified in a variety of animal classes including *Agnatha* (Jawless fishes), *Chondrichthyes* (Cartilagenous fishes), *Osteichthyes* (bony fishes), *Amphibia*, *Reptilia*, *Aves*, and *Mammalia* (for review see (Munoz et al. 1997)). Fibers ascending from the dorsal column-medial lemniscus pathway are made of fibers from primary afferents originating from the dorsal roots. The ascending post-synaptic dorsal column pathway (PSDC), however, arises from neurons located in the dorsal horns immediately adjacent to the substantia gelatinosa (lamina II) throughout the entire length of the spinal cord in rats (Giesler et al. 1984). Fibers from the dorsal column-medial lemniscal pathway and the PSDC ascend ipsilaterally to the brainstem in a somatotopical organization. Axons ascending from the lumbar spinal cord are found within the more medially located fasciculus gracilis (Fig.1.3), while those ascending from the cervical spinal cord travel within the laterally located fasciculus cuneatus (Fig.1.3) (Giesler et al. 1984). Fibers running in the fasciculus cuneatus and gracilus end on neurons within the cuneate and gracile nuclei within the medulla, respectively. These nuclei then relay information to the contralateral nuclei in the ventrobasal thalamus and the somatosensory cortex. The dorsal column-medial lemniscus system is thought to relay information pertaining to discriminative touch and kinesthesia. The postsynaptic dorsal column pathway, however, is thought to be important for signaling visceral pain (Willis et al. 1999), mechanosensation (Angaut-Petit 1975), and both mechano- and thermal nociception (Angaut-Petit 1975).

1.2.3 Dorsolateral Funiculus

1.2.3.1 The red nucleus and rubrospinal tract

The rubrospinal tract was first described in 1883 (Kennedy 1990). The cell bodies of the rubrospinal tract are located in the midbrain at the level of the rostral colliculus, ventrolateral to the oculomotor nucleus, and dorsal to the substantia nigra (Boseila et al. 1975). These cell bodies are organized as paired nuclei known as the red nuclei (Boseila et al. 1975). The nuclei receive their name based on their appearance in sectioned fresh brain. The nucleus has a reddish appearance in freshly sectioned human brain. It is not known, however, what causes the nucleus to appear red (Massion 1988). It has been postulated that the red colouration is the result of increased blood supply to the red nucleus compared to surrounding tissues; however, studies examining vascular patterns of this nucleus do not support this hypothesis (Massion 1988).

A red nucleus has been defined by neuroanatomists as a nucleus that is found in the mesencephalic tegmentum with contralaterally projecting fibers extending to the spinal cord or the brainstem as well as having afferent input from the contralateral cerebellum. Consequently, red nuclei have been described in many classes of animals including amphibia (anurans), reptilia (quadrupedal reptiles), aves, and mammalia (ten Donkelaar 1988). The nucleus is only vestigial in anurans. Teleosts (bony fish) and elasmobranchs (cartilagenous fish such as sharks and dogfish) lack a red nucleus and rubrospinal tract, although, a red nucleus has been described in rays (*Raja clavata* and *Dasyatis sabina*), which use their pectoral fins for locomotion.

The red nucleus is variable in size depending upon the species being examined. For example, the red nucleus of dromedary camels is 8.35 mm long (rostro-caudal) X 5 mm wide (mediolateral) X 3.7 mm high (dorsoventral) (Adogwa and Lakshminarasimhan 1982), 5.88 mm X 2.86 mm X 2.99 mm in the buffalo (Paily, 1935), 6.09 mm X 3.66 mm X 2.37 mm in the pig (Otabe and Horowitz 1970), 5.72-4.3 mm X 2.5 mm X 2.3 mm in *Macaca sp.* (Jerath 1964), 3.06 mm long in the cat (Adogwa and Lakshminarasimhan 1982), and 0.332 mm in diameter in the mouse (Jerath 1964). The size of the red nucleus may be a reflection of the length of the limbs used during locomotion. That is, the longer the limbs, the larger the red nucleus and hence the larger the rubrospinal tract (ten Donkelaar 1988). Of course, red nucleus size may be related to body size not limb size. Afterall, it is known that, generally speaking, appendage length increases with body size.

This may be important for coordination and use for motor control of flexor muscles (discussed below).

The red nucleus reaches maximum size soon after birth (by 3 months in rats). It’s volume begins to regress, however, beginning six months postnatally in this species (Boseila et al. 1975). The red nucleus has both magnocellular and parvicellular components (Jerath 1964). In some species the magnocellular portion is lost (very small in primates) (Jerath 1964; Massion 1967). In other species, such as the cat, there is no distinct magnocellular or parvicellular portion (Adogwa and Lakshminarasimhan 1982). The magnocellular component is well developed in animals such as the oppussum, whereas, the parvicellular portion is better developed in mammals such as primates. Interestingly, the inferior olivary nucleus which receives afferents from the parvicellular portion of the red nucleus is better developed in mammals having primarily a parvicellular red nucleus. The inferior olivary nucleus sends afferents to the dentate nucleus of the cerebellum which is also better developed in animals having a red nucleus predominantly composed of parvicellular neurons.

The magnocellular portion receives input from the cerebral cortex and interposed nucleus of the cerebellum, and sends efferents to the spinal cord (Oka and Jinnai 1978; Flumerfelt 1978). The parvicellular portion of RN receives afferent information from the parietal association cortex and the dentate nucleus of the cerebellum (in cats) and sends efferents to the ipsilateral inferior olive in rats, cats and monkeys (Flumerfelt 1978; Oka and Jinnai 1978; Kennedy et al. 1986) . Therefore, it has been suggested that the parvicellular portion is important in the control of highly coordinated movement or posture.

Multiple neurotransmitters have been identified in the red nucleus using immunohistochemistry. Red nucleus neurons appear to be responsive to all of them (see (Keifer and Houk 1994) for review). Salient features of the neurotransmitters found in the red nucleus are taken from Keifer and Houk and are presented herein (Keifer and Houk 1994). Glutamate, glycine, GABA, serotonin, dopamine, norepinephrine, and Ach immunopositivity have been found in mammalian red nuclei, while glutamate, glycine, GABA and serotonin immunopositivity are found in reptilian red nuclei. Glutamatergic synapses onto the red nucleus are from the corticorubral and cerebellorubral pathways (Nieoullon et al. 1988), glycinergic synapses from cerebellorubral pathways (Ottersen et al. 1987), and serotonergic synapses from the dorsal raphe nucleus (Smeets and Steinbusch 1988; Bernays et al. 1988). Intrinsic rubral interneurons are gabaergic (Keifer et al. 1992; Vuillon-Cacciuttolo et al. 1984) . Rubrospinal axons appear to be excitatory as demonstrated by the difficulty to co-localize rubrospinal synaptic boutons with GABA or glycine immuno-positive neurons in the spinal cord (Antal et al. 1992).

The rubrospinal tract originates from both small (parvicellular) and large neurons (magnocellular) in the caudal 2/3 of the red nuclei (Shieh et al. 1983) in the rat, whereas in other species (primates) the rubrospinal tract arises exclusively from the magnocellular portion of the red nuclei (Kennedy 1990). Red nucleus axons decussate at the level of the mesencephalic tegmentum to extend predominantly contralaterally in the dorsolateral funiculus of the spinal cord (Fig.1.3) (Brown 1974; ten Donkelaar 1988). It has been estimated, however, that up to 7% of the rubrospinal tract arises from the ipsilateral red nucleus in monkeys (Massion 1967). Meanwhile, others have shown that the rubrospinal tract completely decussates (Zemlan et al. 1979). The red nucleus is arranged somatotopically. Neurons projecting to the cervical spinal cord are found in the dorsal and dorsal medial regions, those projecting to lumbosacral segments of the spinal cord are located ventrally and ventrolaterally (Shieh et al. 1983).

It has been shown that the rubrospinal tract extends throughout the entire length of the spinal cord in the opossum, yet only a few fibers extend to the uppermost cervical levels in humans (Martin and Dom 1970; Nathan and Smith 1982). In the rat, rubrospinal axons can extend as far as the lumbar spinal cord (Antal et al. 1992). Ipsilateral rubrospinal axon terminals may constitute 10-28% of synaptic boutons within the spinal cord (Antal et al. 1992). Rubrospinal projections make connections to neurons in Rexed's laminae V, VI, and VII throughout the length of the spinal cord in many species (Martin and Dom 1970). These projections are present at birth (Shieh et al. 1983). Axons arising from the dorsomedial portion of the red nucleus project to forelimb motor neurons whereas ventrolaterally located red nucleus neurons project to hind limb motor neurons (Huisman et al. 1981). It has recently been shown that the rubrospinal tract makes connections with distal and intermediate muscle motor neurons but not proximal muscle motor neurons in adult rats (Kuchler et al. 2002).

The rubrospinal tract has mainly polysynaptic excitatory effects on flexor motor neurons and inhibitory effects on extensors (Hongo et al. 1969a; Orlovsky 1972a). However, electrical stimulation of the red nucleus can also elicit inhibition of flexors and excitation of extensor motor neurons (eg. excitation of toe extensors) (Hongo et al. 1969a). Electrical stimulation of the red nucleus causes flexion of contralateral limb flexors (Gassel et al. 1965). Obliteration of the red nuclei does not cause marked disturbances in locomotion in adult cats (Shik and Orlovsky 1976). Thalamic cats (do not have corticorubral system) were studied with intact cerebellum or were decerebellate and rubrospinal neurons were recorded during treadmill locomotion (Orlovsky 1972a). This study found that decerebellation abolished cyclic modulation of rubrospinal neurons during locomotion and decreased mean frequency of discharge of RN neurons. Rubrospinal neurons were found to be most active during swing and end of stance phases of locomotion (ie. when flexors most active). It has been shown that the major source of rhythmic locomotor input (ie. from the central pattern generators) to the red nucleus is through the ventral spinocerebellar tract (Arshavsky et al. 1988) and the spinorubral pathway (Vinay et al. 1993).

Several hypotheses concerning the role of the rubrospinal system in controlling movement exist (for review see (Massion 1988)). One hypothesis is that the red nucleus is important during movement execution via cerebello-rubrospinal circuits (see (Massion 1988)) and not during movement initiation or preparation. In another hypothesis the red nucleus is thought to be important during the braking phase and postural fixation of movement through interactions with the cerebral cortex (corticorubral projections) (Tsukahara et al. 1968). Other evidence, showing that red nucleus neurons are active before the onset of movement, points toward the red nucleus having a role in movement initiation (see (Massion 1988)). It has even been suggested that a lesion of the rubrospinal tract (RST) would result in activation of the rubro-olivary projection and subsequent switching of motor activity from the red nucleus via the cerebellum to the corticospinal fibers and ventrolateral complex of the thalamus leading to compensation of deficits induced by lesioning of the RST (Kennedy 1990; Fanardjian et al. 1999). This proposal suggests that the rubrospinal tract is redundant and not necessary or sufficient for motor skills. Recently, it has been shown that destruction of the red nucleus, unilaterally, produces alterations in the ground reaction force pattern of freely locomoting rats. These alterations were a manifestation of altered interlimb coordination (Muir and Whishaw 2000). One could argue that because switching of rubrospinal tract to corticospinal tract requires an intact red nucleus that these locomotor differences resulted because switching had not occurred. One of the aims of this thesis is to determine whether unilateral rubrospinal tract lesions, do in fact result in long lasting locomotor deficits thereby providing evidence not supporting the hypothesis that the rubrospinal tract is redundant and can be compensated for by the corticospinal system.

1.2.3.2 Spinocerebellar tract

The spinocerebellar tract has been described in numerous classes of animals including *Agnatha* (Jawless fishes) (Munoz et al. 1997), *Chondrichthyes* (Cartilagenous fishes) (Munoz et al. 1997), *Osteichthyes* (bony fishes) (Munoz et al. 1997; Finger 2000), *Amphibia* (Gonzalez et al. 1984), *Reptilia* (Kunzle 1983; Bangma and ten Donkelaar 1982), *Aves* (Necker 1989; Yamamoto et al. 2001), and a wide variety of species of *Mammalia* (Tracey et al. 1988; Terman et al. 1998; Grottel 1975; Matsushita and Hosoya 1979; Snyder et al. 1978; Petras and Cummings 1977; Cummings and Petras 1977) (for more detailed comparative review see (Munoz et al. 1997)).

The spinocerebellar tracts are traditionally divided into 3 tracts, namely the 1) dorsal, 2) ventral and 3) rostral spinocerebellar tracts (Fig.1.3). Sensory information from the caudal dorsal roots project to the cells of origin of the ventral spinocerebellar tract (Border cells and caudal Clarke's nucleus), while information from the rostral dorsal roots of the thoracic spinal cord project to the cells of origin of the dorsal spinocerebellar tract (rostral Clarke's nucleus), and cervical afferents project to the cell bodies of origin of the rostral spinocerebellar tract (nucleus centrobasalis within the cervical enlargement (Stilling's cervical nucleus), and possibly the central cervical nucleus (in the upper cervical segments)) (Snyder et al. 1978; Terman et al. 1998).

The ventral spinocerebellar tract predominantly decussates to the contralateral spinal cord and appears to decussate a second time at the level of the rostral cerebellar peduncle to terminate in the cerebellum ipsilateral to the cells of origin of the ventral spinocerebellar tract (VSCT) (Bloedel and Courville 1981; Tracey et al. 1988). Neurons comprising the dorsal spinocerebellar and rostral spinocerebellar tracts ascend predominantly to the ipsilateral cerebellum (Snyder et al. 1978; Tracey et al. 1988; Xu and Grant 1994), while axons arising from the central cervical nucleus project to the contralateral cerebellum (Xu and Grant 1994).

Other spinocerebellar neurons have recently been described for the sacral and coccygeal regions of the spinal cord. Contralaterally projecting axons from these neurons, however, are not traditionally considered part of the dorsal spinocerebellar tract, but do travel to the cerebellum via the dorsolateral funiculus. The cell bodies for the sacral and coccygeal spinocerebellar fibers are found in Stilling's nucleus and the ventrolateral nucleus (Terman et al. 1998).

Traditionally, it has been accepted that fibers from the dorsal spinocerebellar tract enter the cerebellum via the caudal cerebellar peduncle and fibers from the ventral spinocerebellar tract enter the cerebellum via the rostral cerebellar peduncle (Yamada et al. 1991). Recently, however, it has been shown that a significant number of ventral spinocerebellar fibers enter the cerebellum via the caudal cerebellar peduncle in rats (Yamada et al. 1991). Spinocerebellar fibers entering the cerebellum are known as mossy fibers and terminate on granule cells within the granule cell layer of the cerebellar cortex and on the interposed and fastigial cerebellar nuclei (Bloedel and Courville 1981). As can be predicted from the anatomical location of the cells of origin from the three spinocerebellar tracts, the rostral spinocerebellar tract projects information from the forelimbs, the dorsal spinocerebellar tract transmits information from the back and a portion of the hind limbs and tail, while the ventral spinocerebellar tract transmits information from the hindlimbs (Bloedel and Courville 1981). The spinocerebellar system relays both proprioceptive and exteroceptive information to the cerebellum (for review see (Bloedel and Courville 1981)).

Little information exists as to the importance of the spinocerebellar system in freely locomoting animals. One study, however, found that damage to the spinocerebellar system does not result in changes in the freely behaving cat's gait. In this instance, bilateral lesioning of the dorsal spinocerebellar tract within the thoracic spinal cord in adult cats did not result in abnormal temporal gait patterns (English 1985).

1.2.4 Ventrolateral and Ventral Spinal Tracts

1.2.4.1 Vestibulospinal tracts

Vestibulospinal tracts are recognized in a variety of animals including agnathans (Ronan 1989), cartilaginous (Chondrichthyes) and bony (Osteichthes) fish (Oka et al. 1986; Prasada Rao et al. 1987), amphibians (Sanchez-Camacho et al. 2001), reptiles (ten Donkelaar et al. 1980), birds (Webster and Steeves 1988), and mammals. (Nudo and Masterton 1988) (for review see (Sanchez-Camacho et al. 2001)). Vestibulospinal tracts are derived, principally, from three nuclei of the vestibular system in the rat. The mammalian vestibulospinal system is divided into lateral, medial, and caudal components (Peterson et al. 1978). The lateral vestibulospinal tract originates from the lateral vestibular nucleus (Deiter's nucleus) while the medial vestibular tract arises from the medial vestibular nucleus, and the caudal vestibulospinal tract which arises from the caudal portion of the medial vestibular nucleus and the descending vestibular nucleus (Bankoul and Neuhuber 1992). The vestibulospinal tracts descend mainly ipsilateral in the ventral and ventrolateral funiculus in the rat (Fig.1.3) (Houle and Jin 2001; Matesz et al. 2002). Pictorial descriptions of the course of vestibulospinal axons in the rat spinal cord are rare. However, in the cat, the lateral and medial vestibulospinal tracts remain ipsilateral, while the caudal vestibulospinal tract descends bilaterally within the ventral and dorsolateral funiculi (Peterson et al. 1978). Vestibulospinal fibers have been found to terminate in small numbers in laminae II-VII but mainly in laminae VIII and IX (Matesz et al. 2002).

Medially projecting spinal cord fibers, such as the vestibulospinal tract, are important in maintaining posture by exerting their effects on axial motor neurons. Moreover, tracts running within the ventral funiculus are thought to be essential for maintaining normal locomotion, as destruction of these pathways have induced severe locomotor deficits in cats and rats (Brustein and Rossignol 1998; Loy et al. 2002a). Vestibulospinal fibers have been found, however, to exert excitatory effects on extensor motorneurons and inhibitory effects on flexor motor neurons of the proximal hind limbs of cats (Grillner et al. 1970). Recently it has been suggested that the lateral vestibular nucleus has an important role in maintaining and increasing extensor tone bilaterally in locomoting cats (Matsuyama and Drew 2000b; Matsuyama and Drew 2000a).

1.2.4.2 Reticulospinal tracts

The reticulospinal fibers originate from neurons located in the midbrain, pons and medulla (Satoh 1979). This spinal pathway has been observed in agnathans (Ronan 1989), cartilaginous (Chondrichthyes) (Smeets and Timerick 1981) and bony (Osteichthyes) fish (Oka et al. 1986; Prasada Rao et al. 1987) , amphibians (Sanchez-Camacho et al. 2001), reptiles (ten Donkelaar et al. 1980), birds (Webster and Steeves 1988), and a whole host of mammals (Nudo and Masterton 1988). There are approximately 13 medullary reticular nuclei and approximately 13 pontine and mesencephalic reticular nuclei identified in the rat (Newman 1985). Many of these nuclei project to both the cerebral cortex and the spinal cord (Newman and Liu 1987). Those fibers originating from the midbrain descend in the ventromedial funiculus and extend only to the level of the midthoracic spinal cord in rats (Waldron and Gwyn 1969). Fibers originating from the medullary reticular formation extend to the lower lumbar segments traversing in the ventral portion of the spinal cord in rats (Fox 1970; Shapovalov and Gurevitch 1970). The reticulospinal system provides serotonergic input to the spinal cord (Satoh 1979) which has been shown to be important for locomotion (Rossignol et al. 1998).

Reticulospinal tracts have been implicated in controlling a wide variety of behaviours of rats and cats including the lordosis (Zemlan et al. 1983) and startle reflexes (Yeomans et al. 2002), and locomotion (Noga et al. 1991). The reticulospinal tract has been found to be important in transmitting information necessary for initiating and maintaining normal locomotion in many species including lamprey (McClellan 1988), avian species

(Sholomenko and Steeves 1987), rats (Loy et al. 2002a), cats (Steeves and Jordan 1980; Eidelberg et al. 1981), and primates (Lawrence and Kuypers 1968b; Eidelberg et al. 1981).

Medullary reticular nuclei are important in producing locomotion (Noga et al. 1991). As eluded to already, the neurons making these nuclei are serotonergic (Dahlstrom and Fuxe 1964; Anden et al. 1965; Huisman et al. 1981). These nuclei project axons to the spinal cord in the ventrolateral, and ventromedial funiculi of the spinal cord (Fig.1.3). It is thought that the fibers running in the ventral and ventrolateral funiculi are important for locomotion as demyelination of these fibers results in severe locomotor deficits (Loy et al. 2002a).

1.2.4.3 Coeruleospinal tract

The coeruleospinal tract has been identified as an important source of noradrenergic input to the spinal cord since the late 1970s (Commissiong et al. 1978). The cell bodies of this spinal pathway arise from the locus coeruleus within the pons (Commissiong et al. 1978). The coeruleospinal tract descends bilaterally (decussating within the spinal cord) (Commissiong 1981). The exact intraspinal course and termination pattern of coeruleospinal axons appears to be, in part, dependent upon the source of the particular rat strain (Fig.1.3) (West et al. 1993). For example, Sprague-Dawley rats obtained from one vendor demonstrate dorsal horn termination patterns with axons traveling within lamina I-II, and the dorsolateral funiculus, while Sprague-Dawley rats obtained from another source demonstrate ventral horn termination patterns with axons traveling in the ventral funiculus (Clark and Proudfit 1992). Furthermore, an antinociceptive property in the animals having dorsal terminations is not present in animals having ventral horn terminations (West et al. 1993). In cats, the locus coeruleus has been shown to synapse on lumbar motorneurons (Fung et al. 1991). Consequently, the coeruleospinal tract probably plays a role in nociception and/or motor control depending on the species and/or the particular strain of animal.

1.2.4.4 Spinoreticular tracts

The medial spinoreticular tract arises from neurons located in the lateral part of the neck of the dorsal horn and from laminae VII and VIII (Chaouch et al. 1983). The spinoreticular tract is found throughout the length of the spinal cord (Fig.1.3) (Chaouch et al. 1983). Electrophysiological evidence shows that neurons of the medial spinoreticular tract are important for sending information to the bulbar and pontine reticular formation about painful and non-painful cutaneous stimuli (Menetrey et al. 1980). This tract is most likely important in relaying sensory impulses from other structures including joints, muscles, and tendons (Menetrey et al. 1982).

1.2.4.5 Spinomesencephalic tract

The spinomesencephalic tract runs in both the dorsolateral and ventrolateral funiculi of the spinal cord (Fig.1.3) and projects bilaterally to the mesencephalon (Zemlan et al. 1978). The tract ascends to the midbrain to terminate in the intercollicular nucleus, deep layers of the rostral colliculus, the external nucleus of the inferior colliculus, lateral portions of the central grey, and the cuneiform nucleus (Menetrey et al. 1982). The origin of the spinomesencephalic tract has been identified as neuron clusters surrounding the dorsal horn. Cells are located in the marginal zone, the dorsolateral funiculus nucleus, the lateral part of the neck of the dorsal horn and the dorsal grey commissure throughout the length of the spinal cord. Projections arising from the marginal zone and neck of the dorsal horn are predominantly contralateral, and those fibers arising from the nucleus of the dorsolateral funciculus project bilaterally (Menetrey et al. 1982). The mesencephalic tegmentum has been shown to be a major target for somatosensory input arising from the spinal cord, dorsal column nuclei and the lateral cervical nucleus (Menetrey et al. 1982). The spinomesencephalic tract is thought to be important in transmitting information regarding pain (Menetrey et al. 1982).

1.2.4.6 Spinothalamic tract

The spinothalamic tract can be divided into lateral and ventral components. Cell bodies of the lateral spinothalmic tract are located deep within the gray matter (medial lamina VII) and within the dorsal 2/3 of the dorsal horn (laminae I, III-V), while cell bodies of the ventral tract are found within the ventral most areas of the dorsal horn (laminae VI-VII) and within the intermediate gray matter (Giesler, Jr. et al. 1981). Depending upon the location of the cell bodies, some of the axons of these neurons remain ipsilateral or decussate within the spinal cord and ascend to the contralateral thalamus (Dado et al. 1994a; Kobayashi 1998). Recent studies have even classified spinothalamic tract neurons, based on cytoarchitecture, as remaining ipsilateral or decussating and whether they travel to the medial or lateral portions of the thalamus (Kobayashi 1998). Generally speaking, however, axons traveling within the lateral spinothalamic tract ascend to synapse in the lateral thalamus while those ascending in the ventral funiculus ascend to the medial thalamus (Giesler, Jr. et al. 1981). Recently, electrophysiological studies have shown that spinothalamic tract axons course more dorsally within the dorsolateral funiculus within the upper cervical spinal segments (Fig.1.3) (Dado et al. 1994c). It has been shown, physiologically, that spinothalamic tract neurons are highly responsive to noxious stimuli (Dado et al. 1994b). Consequently, the spinothalamic tract is thought to be important for pain processing.

1.2.4.7 Spinohypothalamic tract

Direct connections from the spinal cord to the hypothalamus were first discovered in monkeys in 1949 and 1951 (Giesler, Jr. et al. 1994). Recently, the presence of a spinohypothalmic tract has been described in cats (Katter et al. 1991) and in rats using anatomical and physiological tracing methods

(Burstein et al. 1987; Dado et al. 1994c). Neuron cell bodies of the spinothalamic tract are found within lamina I, (lateral reticulated area), and X (Giesler, Jr. et al. 1994). These cells bodies send their axons to the contralateral spinal cord and ascend in the dorsal portion of the lateral spinothalamic tract and deep within the dorsolateral funiculus (Fig.1.3) (Katter et al. 1996a; Kostarczyk et al. 1997). Spinohypothalamic tract axons traverse the brainstem and thalamus to enter the hypothalamus ipsilateral to their cell bodies (Giesler, Jr. et al. 1994). Electrophysiologic studies confirm that spinohypothalamic tract neurons respond to noxious stimuli (Burstein et al. 1991; Dado et al. 1994b; Katter et al. 1996b) . Recently, it has been shown that the spinohypothalamic tract is important in transmitting visceral pain (Zhang et al. 2002). Spinohypothalamic neurons also have substance-P like immunoreactivity, providing further evidence of their likely role in transmission of pain to the hypothalamus (Li et al. 1997). It is suspected that the spinohypothalamic tract, through its direct stimulation of the hypothalamus, participates in mediating autonomic, endocrine and affective responses to nociceptive stimuli (Burstein et al. 1996). Such responses to painful stimuli include elevations in blood pressure, increased blood flow to the heart and skeletal muscles and decreased blood flow to the viscera and skin, elevations in cortisol, and changes in an animal's demeanor (Giesler, Jr. et al. 1994).

1.3 Neural control of locomotion

Locomotion involves any form of purposeful movement that results in an organism moving from point A to point B. As is readily obvious organisms may swim, crawl, fly ,walk, gallop or hop. Each of these forms of locomotion has been adopted by particular species based on the environment in which they live. This section of the thesis pertains to the neural control of terrestrial locomotion in quadrupedal species of animals.

1.3.1 Introduction

Work by Charles Sherrington revolutionized the way that we think about locomotion and reflexes today. In the early 1900s, Sherrington described a locomotor-like (stepping) response that could be elicited by cutaneously stimulating a spinal animal's paw (Sherrington 1910). This early work led to the idea that locomotion was a manifestation of an animal's limbs moving because of a chain of proprioceptive reflexes resulting from the primary cutaneous stimulation of the animal's paw (this became known as the reflex chain theory of locomotion). The reflex chain hypothesis has been summarized as follows: "Contraction of one muscle group results in a signal for contraction of another group and so on until the entire step cycle is completed." (Shik and Orlovsky 1976). In 1911 Graham Brown described the central pattern generation of locomotion, where the neural circuitry responsible for the alternating pattern of limb movements exists entirely within the spinal cord (Brown 1911). Brown showed using deafferented acute spinal preparations that, unlike the hypothesis proposed by Sherrington, this alternating pattern of movement is not due to a chaining of simple reflexes; rather, the entire network for locomotion is found within the spinal cord and will function independent of any sensory inputs. Brown proposed that generation of alternating activity might be due to two centers (one for flexors, another for extensors) within the spinal cord with mutual reciprocal inhibition (known today as the half-center hypothesis). It is Brown's half-center hypothesis which forms the foundation for our understanding of locomotion, particularly stepping, today. The purpose of this section is to provide an introduction to the neural mechanisms of stepping, initiation of locomotion and interlimb coordination.

1.3.2 Neural mechanisms of stepping

The location of the neural circuitry controlling stepping has been known since 1911 (Brown 1911). This particular region within the spinal cord is commonly known as the central pattern generator (CPG). Individual limb extension/flexion patterns seen during stepping and scratching arise from these CPGs. Central pattern generators are not simply made up of one or two neurons, but are made up of many neurons. Central pattern generators are not discretely localized to one spinal segment but, in the case of the pelvic limb central pattern generator of the neonatal rat, extend from the last thoracic spinal segment through the caudal lumbar spinal segments and are found in the ventral third of the spinal cord (Kjaerulff and Kiehn 1996; Kremer and Lev-Tov 1997). Specifically, lumbar CPGs are found medially within the spinal cord and are localized at the level of the ventral commissure (Kjaerulff and Kiehn 1996). The forelimb central pattern generating network of neurons are located within the last two cervical and first thoracic spinal cord segments in the neonatal rat (Ballion et al. 2001).

Each limb has its own central pattern generator (Forssberg et al. 1980; Viala and Vidal 1978) and is thought to be divided into two half centers as proposed by Brown (Brown 1911). The half center hypothesis divides the central pattern generator into circuits that control flexor muscles and those controlling extensor muscles. Forelimb centers are connected with one another (Ballion et al. 2001) as are hindlimb centers (Kjaerulff and Kiehn 1996; Stokke et al. 2002). No supraspinal or afferent stimuli are required for the CPGs to perform the typical alternating pattern involved with stepping. Supraspinal and afferent stimuli are, however, necessary to adjust and fine-tune stepping (Pearson 1995; Bouyer and Rossignol 1998).

1.3.3 Supraspinal initiation of locomotion

Neural control of locomotion can be either voluntary (via supraspinal mechanisms) or involuntary (spinal mechanisms). Descending inputs from the cerebral cortex are involved with the voluntary initiation of locomotion.

Voluntary initiation of locomotion is thought, however, to occur from indirect connections from the cerebral cortex to the spinal cord because cortical stimulation results in locomotion even if the corticospinal tracts are injured (Grillner 1981) . The cortex could potentially initiate locomotion by its interactions with the basal ganglia, the medial or lateral hypothalamus, the mesencephalic locomotor region, or the medullary reticular formation (Jordan 1998).

The mesencephalic locomotor region (MLR) is a region within the mesencephalon (midbrain) that when stimulated in a decerebrate animal will result in quadrupedal locomotion. Increasing the strength of the stimulus has been shown to change an animal's gait from a walk to a trot and even to a gallop (Shik et al. 1969).

Recently, the MLR has been described as all nuclei within the mesencephalon (midbrain) that become activated during locomotion (Jordan 1998) . Until recently, it has been thought that the neural mechanism involved in locomotor initiation is common to all forms of locomotor behaviour. A classification scheme of locomotor initiation systems was originally proposed by Sinnamon (Sinnamon 1993) and later reviewed by Jordan (Jordan 1998). This classification scheme divides neural control of locomotion into 3 distinct categories: 1) the exploratory system, 2) the primary appetitive system, and 3) the primary defensive system. It is thought that locomotion results from activation of these different systems because of different needs of the animal. The exploratory system comprises the basal ganglia, the appetitive system includes the perifornical/lateral hypothalamic locomotor region, and the medial hypothalamus and central gray matter are part of the defensive system. Regardless of this particular classification scheme, all of these regions must act through the pontomedullary reticular formation and reticulospinal tracts to activate the central pattern generators of the fore and hind limbs (Steeves and Jordan 1980).

1.3.4 Interlimb coordination during locomotion

As previously mentioned, the mesencephalic locomotor region is required to initiate locomotion in decerebrate animals. Electrical stimulation of this region results in locomotor activity of all four limbs. If the intensity of the stimulus is increased the interlimb coordination of the limbs changes from a walk to a trot, and if the stimulus is increased further galloping develops (Shik et al. 1966). Each of these gaits is determined by the coordination that occurs between ipsilateral fore and hind limbs, and also between contralateral limb pairs.

Interlimb coordination, also known as gait, is the temporal coordination of limb footfalls. Gaits are thought of as being either symmetric (alternating) or asymmetric (nonalternating) (Grillner 1981). Symmetric gaits occur when the left and right fore and hind limbs alternate evenly in time. Asymmetric gaits occur when footfalls of fore and/or hind limbs occur together as couplets and do not alternate evenly in time. The neural mechanisms controlling gaits and gait changes are not fully understood. However, it has been shown that animals change gaits in part because of metabolic efficiency (Hoyt and Taylor 1981) and also because of critical forces (forces where musculoskeletal injury may occur) acting on the limbs (Farley and Taylor 1991). This implies that gait transitions in animals could be in part, cortically mediated. The best understood aspect of gait modifications, however, lies within the intraspinal neural circuitry that controls the alternating patterns (flexion and extension) of limb movement.

Interlimb coordination can occur via coupling between contralateral limbs through short propriospinal neurons connecting two adjacent central pattern generators. Coupling between ipsilateral fore- and hindlimbs also occurs (Ballion et al. 2001); however, there is no evidence for diagonal coupling between, for example, a forelimb and contralateral hind limb center (Duysens et al. 2000). Evidence supporting central pattern generator coupling is seen in cats having undergone midline myelotomy and/or spinal cord hemisections. Cats with midline myelotomy alone were able to locomote with relatively normal interlimb coordination which implies that contralateral limb coupling is not essential for interlimb coordination (Kato 1988). In cats having undergone midline myelotomy with lateral thoracic spinal cord hemisection, the isolated hind limb generated its own rhythmic oscillations and was not coordinated with any other limb (Kato 1989; Kato 1990). Taken together, these results indicate that the rostrocaudal central pattern generator (CPG) coupling is more important than contralateral CPG coupling for interlimb coordination. These studies also confirm the notion of the existence of individual limb central pattern generators which can be stimulated with simple afferent input (Kato 1990; Kato 1989).

1.4 Biomechanics of locomotion

1.4.1 Introduction

The serious examination of locomotion began in the late 1880's by an English artist/photographer born Edward Muggeridge (aka Helios, more commonly known as Eadweard Muybridge). Muybridge was hired by the railroad tycoon Leland Stanford to determine whether or not horses had a period of time during a canter when none of the limbs were on the ground (van Weeren 2001). Through the development of a series of tripping devices that were hooked to a series cameras, Muybridge was able to take successive photographs of the locomoting horse to document that horses have a period during trotting (now known as the flight phase) where no limbs are on the ground (van Weeren 2001). It was Muybridge's photographic descriptions of locomotion that began the study of the biomechanics of locomotion. With the development of Muybridge's new technology the description of many different gait patterns were described. It is this seminal work of Muybridge that began the revolution of biomechanic research in locomoting animals.

This brief review will focus on the biomechanics of locomotion, specifically pertaining to: 1) the definition and description of gaits and how velocity changes particular stride variables, and 2) the two basic mechanisms that terrestrial limbed animals use to minimize energy expenditure while locomoting overground.

1.4.2 Gaits and the influences of velocity

As mentioned above, the term “gait” is used to describe the coordinated temporal footfall pattern that occurs during locomotion. Also mentioned previously, gaits can be either symmetric or asymmetric. Symmetrical gaits occur when left and right fore and hind limb footfall patterns occur evenly spaced in time. Asymmetric gaits are those gaits that have fore and/or hind limbs alternate in couplets and are not evenly spaced in time. Examples of symmetrical gaits include the walk, trot, pace or rack, while asymmetric gaits include the canter, rotary or transverse gallops (see Fig.1.4). Interesting aspects of gaits include the effects of velocity on temporal limb cycle variables. Before the effect of velocity is discussed, however, it is necessary to define several key terms pertaining to gaits. Within each limb cycle there is a period of time when the limb is on the ground, and a period of time when the limb is off the ground. These two different components of the step cycle are referred to as stance and swing periods (Leach 1993). Alternatively, the step cycle can be divided into extension and flexion phases. The extension phase

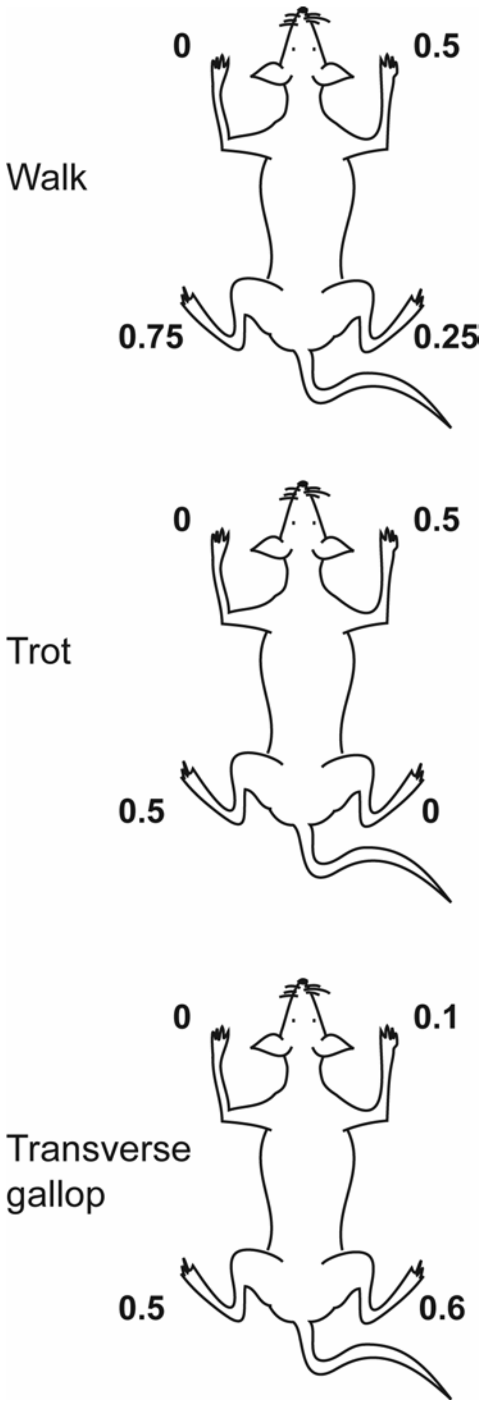


Fig. 1. 4 Drawings of rats indicating the relative phases between the limbs during the walk, trot and transverse gallop. Asymetric gaits have unequal temporal spacing between limbs while symmetrical gaits have equal temporal spacing between limb pairs or individual limbs.

consists of three different components, namely E1 to E3, while there is only one flexion phase. E1 occurs during the onset of limb extension just prior to contacting the ground, E2 occurs during the middle of stance, and E3 occurs at the end of stance (Engberg and Lundberg 1969). The flexion phase occurs during the first half of the swing period. Consequently:

$$\text{stance} = E2 + E3 \tag{1.1}$$

$$\text{swing} = E1 + F \tag{1.2}$$

A stride occurs when there has been a complete cycle of limb motion. For example, during a trot diagonal limb pairs are on the ground at the same time (Fig. 1.4). One complete stride of a trot might be from the onset of stance of the left forelimb/right hindlimb to the next onset of stance of the same diagonal limb pair. Other terms important in describing various gaits pertain to whether the animal is on the ground or not. For example, during a trot, there is typically a period of time where none of the animal's limbs are on the ground referred to as the suspension phase (Leach 1993).

Two important aspects of gait and its relationship to velocity pertain to stride length and stride frequency. It has been found that as an animal's velocity increases so does the stride frequency (Fig. 1.5). This relationship, however, is not linear and is depicted in Fig.1.5. As velocity increases stance duration tends to decrease and swing duration remains approximately the same and

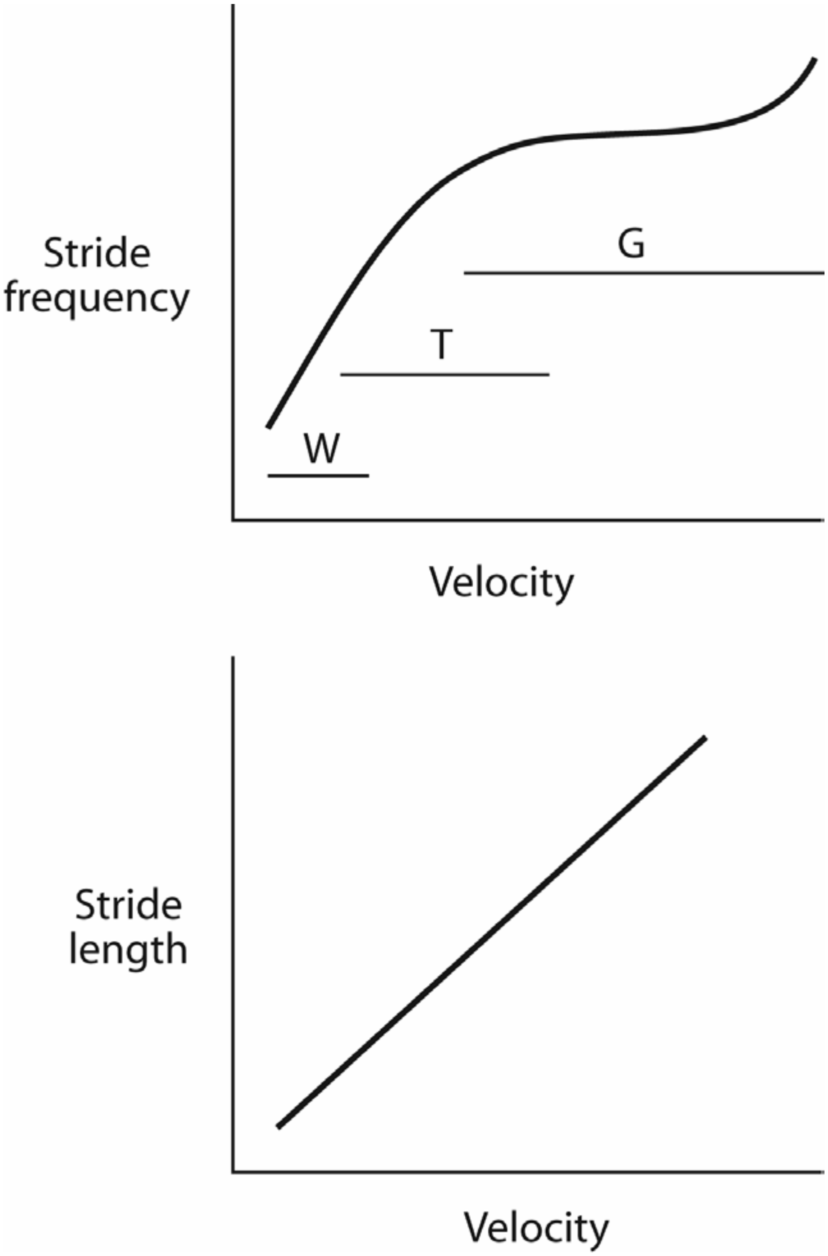


Fig. 1. 5 The relationship between stride frequency and length with velocity during walking (W), trotting (T) and galloping (G). A) Generally, stride frequency increases with velocity during quadrupedal locomotion. B) Stride length increases linearly with overground velocity. Stance duration typically decreases and swing duration remains relatively the same with increasing velocity. Consequently, animals cover more ground at higher velocities thus increasing stride length.

only slightly decreases with increasing speed (Gorska et al. 1998). As velocity increases so does stride length. This relationship is linear and is depicted in Fig.1.5. These relationships are consistent among a variety of different species, and have been shown to be consistent with laboratory rats.

Regardless of the gait used by an animal, all animals appear to have a preferred speed that they perform a particular gait. Animals prefer to perform particular gaits within a narrow range of speed to help conserve energy. The term – economy of locomotion – is used to describe the net energy cost, and is described in terms of milliliters of oxygen consumption per kilogram of body weight per meter of horizontal distance traveled (Hoyt and Taylor 1981).Gaits performed at very low or very high speeds result in increased oxygen consumption per meter of distance moved (Hoyt and Taylor 1981). However, it has been shown that freely behaving animals will travel at the speed that is most energy efficient within a particular gait (Hoyt and Taylor 1981).

1.4.3 Two basic mechanisms for minimizing mechanical energy during locomotion

Two basic mechanisms for minimizing energy expenditure have been described. One of these mechanisms is seen during walking, the other during trotting. Consequently, gaits can be categorized according to their mechanism of conserving energy as opposed to being described with respect to the temporal pattern of footfalls. Therefore, temporal gaits can be described energetically as belonging to one of three different groups: 1) walking, 2) trotting (running in bipeds), or 3) a combination of walking or trotting (eg. skipping in bipeds, galloping in quadrupeds) (Cavagna et al. 1977; Minetti 1998).

During walking, energy is conserved by converting one form of energy into another. In this instance, walking has been described as being similar to an inverted pendulum or an egg rolling end-over-end. That is, kinetic and potential energies are approximately 180 degrees out of phase with one another. During walking, gravitational potential energy is converted to forward kinetic energy, and kinetic energy is converted back to gravitational potential energy. As an animal walks overground its body center of mass rises and by definition potential energy increases.

$$Ep = mgh \tag{1.3}$$

where: Ep = potential energy

m = body mass; g= acceleration due to gravity

h= height of the body center of mass

As the center of mass rises, however, the animal is slowing down and forward kinetic energy is decreasing.

$$Ek = 0.5\ mv^2 \tag{1.4}$$

where: Ek = kinetic energy

m= mass

v= horizontal velocity

At midstance, potential energy is at its peak, and as the body's center of mass moves forward the animal speeds up (increasing forward kinetic energy) in the forward direction and the body center of mass descends (reduction of potential energy due to gravity). As is readily apparent, animals are slowing down as they rise up and are speeding up as they fall forward and downward. This form of energy conservation is seen in both quadrupedal and bipedal species from a variety of animal classes including Reptilia, Aves, and Mammalia (Cavagna et al. 1977; Farley and Ko 1997) .

The other basic mechanism that quadrupedal and bipedal species use to minimize energy expenditure is seen during trotting (running in bipeds). In this instance, forward kinetic energy and gravitational potential energy are not out of phase with each other. Instead, in the case of trotting, elastic energy is stored and released during locomotion and the animal basically springs from diagonal limb pair to diagonal limb pair. During trotting, diagonal limb pairs contact the ground at approximately the same time. Upon impact with the ground, the body center of mass is decreasing (potential energy is decreasing) and the animal is slowing down concurrently (forward kinetic energy is decreasing). At the same time energy is being stored in the muscles, tendons, and other connective tissue as elastic energy. Following midstance, this elastic energy is released in the forms of gravitational potential energy and forward kinetic energy. That is the animal springs up and forward and is speeding up. Unlike walking, trotting results in the animal speeding up as it moves forward and upward and slowing down as it moves forward and downward. This form of mechanical energy conservation is also seen in a various reptiles, birds and mammals (Farley and Ko 1997; Cavagna et al. 1977).

Finally the third mechanism of reducing energy expenditure is galloping in quadrupeds or skipping in bipeds. Galloping can be imitated by two people walking approximately 75% out of phase while trotting can be mimicked by two people running 50% out-of-phase (Minetti 1998). During slow quadrupedal galloping, as an animal places its hind limbs on the ground (immediately after the flight phase) Ep decreases and forward kinetic energy increases (Cavagna 1977). After falling forward the forelimbs hit the ground as a couplet (Cavagna 1977). After the second forelimb hits the ground the animal's Ep and Ek simultaneously decrease and rises and it's Ek decreases similar to what is seen during walking. As the animal begins another flight phase, however, Ep and Ek are in-phase with one another, a consequence of the animal pushing up and forward with the limb about to leave the ground (Cavagna 1977). Variations of these combined uses of pendulum and spring models for conserving energy are observed depending on the exact gait (eg. canter versus galloping).

1.5 Methods used for assessing sensorimotor abilities of rats

1.5.1 Introduction

Behaviour results from the integration and coordination of sensory and motor information within the central nervous system. The outward appearance of this information is seen in locomotion, skilled movements, or other forms of motor behaviour. Behaviour is studied for a variety of reasons. It may be studied to determine a specific function of an area within the central nervous system or it may be used to assess the efficacy of a particular treatment. Criteria for assessing functional recovery following spinal cord injury have been made (Goldberger et al. 1990). It has been recommended that a behavioural test should: a) be sensitive and quantitative, b) be conditioned (trained) behaviours (to negate motivational factors), and c) the behaviour studied is appropriate for the species being used in experiments. Suggested behaviours should test for sensorimotor postural reflexes, coordinated movements of limbs (ie. locomotion), and sensory tests. Moreover, methods of assessing these behaviours can be lumped into broad categories including

1) endpoint measures, 2) qualitative kinematics, 3) quantitative kinematics, and 4) kinetics.

Spinal cord injury, whether caused by transection or compression or an ischemic event, results in an impairment, or change of motor function. These changes may be reflected as lower motor neuron versus upper motor neuron signs. Lower motor neuron signs are due to damage of local reflex arcs. Signs include flaccid paralysis and decreased or absent local reflexes. Upper motor neuron signs are due to damage to upper motor neurons (ascending or descending). The signs seen include hypertonia of limb muscles, conscious proprioceptive losses, paresis or paralysis, loss of deep pain, and normal to increased local reflexes. Because these changes can be graded following injury, it is important that a method of assessing functional recovery is sensitive enough to detect changes in both sensory and motor function. The method should also reject any bias due to subjectivity of the behavioural examiner. If the method of assessing a given behaviour can detect changes in sensory or motor function it should also be able to allow elucidation of the mechanism causing the behavioural change. This chapter attempts to introduce different methods of assessing functional recovery (behaviour) following spinal cord injury in an experimental setting. Methods of assessing behavioural recovery, more specifically motor behavioural recovery, will be grouped into one of the aforementioned four categories. Benefits and limitations of each of the behavioural groups will be addressed. A statement of when a given method might be used will also be provided.

1.5.2 Methods used to assess functional recovery

Several methods of assessing behaviour following spinal cord injury have been developed. These methods can be grouped into one of four types. As already mentioned, the four methods of assessing behaviour include endpoint measures, qualitative or quantitative kinematics, or kinetics (Muir and Webb 2000). It is important to understand that a chosen method of assessing an animal's behaviour may provide little information regarding how that behaviour reflects the functional recovery of the spinal cord. Of paramount importance is the fact that certain methods of assessing behaviour in a given species may not be suitable for that species being studied. Issues such as these will be discussed in the following section.

1.5.2.1 Endpoint measures

In general, endpoint measurements are results of given tasks (See Table 1.1 for examples). For example, can a rat grab a pellet of food and eat it without dropping it or can an animal walk across a meshed grid or ladder without falling through? The endpoint measure method of assessing functional recovery has several benefits and drawbacks. Benefits of endpoint measurements include the ease at which they can be performed, the relative inexpensiveness of equipment needed (depending on the test performed), the time to learn the methods are quick, and the time taken to complete the analyses is relatively short.

This technique does not, unfortunately, reveal what exactly is going on with the animal system. It is impossible to determine whether the animal has compensated in some way. For example, there are many ways that a pellet might be obtained and moved to a rat's mouth. It has been shown that rats will modify their behaviour to be able to eat the pellet (the endpoint) (Whishaw et al. 1991; McKenna and Whishaw 1999). As a result, simply counting the number of successful attempts to grab a pellet and eat it is probably not assessing the functional recovery of the spinal cord effectively. It definitely does not lend any information about compensatory changes employed.

Quantitative data retrieved from endpoint measure analysis includes determining how many times a rat successfully completes the desired endpoint, for example - the number of times a rat's foot fell through a grid. Several researchers have compiled complex grading systems by assigning values to responses of a variety of tasks. These values are then converted to a

Table 1. 1 Behaviours analyzed using the endpoint measurement technique (table not all inclusive)

BEHAVIOUR	**ENDPOINT MEASURED**	**REFERENCES**
food pellet reaching	•time taken to obtain x# pellets •success rate - # of pellets successfully obtained	*Z'Graggen et al., 1998 (Z'Graggen et al. 1998)* *Kartje-Tillotson and Castro, 1980 (Kartje-Tillotson and Castro 1980)* *Schrimsher and Reier, 1992, 1993 (Schrimsher and Reier 1992; Schrimsher and Reier 1993)*
rope climbing	•# foot slips/total # steps	*Carlini et al., 1967 (Carlini et al. 1967)* *Z'Graggen et al., 1998 (Z'Graggen et al. 1998)*
grid walking	•# of foot falls (through grid) / # of steps	*Kunkel-Bagden et al., 1993 (Kunkel-Bagden et al. 1993)* *Z'Graggen et al., 1998 (Z'Graggen et al. 1998)* *Prakriya et al., 1993 (Prakriya et al. 1993)*
sticker removal	•# times sticker removed/ # attempts to remove sticker	*Diener and Bregman, 1998 (Diener and Bregman 1998)*
Toe spread response	•how far the toes spread apart after suddenly lowering the animal	*Blight, 1994 (Blight 1994)*
withdrawal reflex	•the degree to which an animal withdrawals its limb following cutaneous stimulation of the distal end of the extremity	*Blight, 1991 (Blight et al. 1991)* *Gale et al., 1985 (Gale et al. 1985)*
placing reflexes	•does the animal place its foot on a surface that it has made contact with	*Gale et al., 1985(Gale et al. 1985)* *Schrimsher and Reier, 1992 (Schrimsher and Reier 1992; Schrimsher and Reier 1993)*

tail flick	•does the animal flick its tail away from a focused source of heat	*Gale et al., 1985 (Gale et al. 1985)*
inclined plane	•determining the degree at which an animal can no longer maintain its position on an inclined plane	*Rivlin and Tator, 1977 (Rivlin and Tator 1977)*
walking on beams	•length of time, and number of steps necessary to cross a beam •number of errors (foot slips) crossing the beam	*Kunkel-Bagden and Bregman, 1993 (Bregman et al. 1993)*
righting behaviour	•length of time to move from supine to prone positions	*Diener and Bregman, 1998 (Diener and Bregman 1998)* *Kunkel-Bagden et al., 1992 (Kunkel-Bagden et al. 1992)* *Kerasidis et al., 1987 (Kerasidis et al. 1987)*
staircase test	•# of food pellets retrieved	*Montoya et al., 1991 (Montoya et al. 1991)*

score, as in the combined behavioural score (CBS) method (Gale et al. 1985; Kerasidis et al. 1987). Regardless of the scoring method, the scores received by an animal do not lend any information about how an animal is able to complete a task. For example, a rat may score 88 on the CBS, or score 1 for the placing response. These numbers simply tell us that the animal does not ambulate well, or that it does not place its foot as readily. One can see that these numbers simply identify if a task, or tasks, can be performed or not.

In summary, the endpoint measures method of assessing functional recovery following spinal cord injury has been used extensively in behavioural neuroscience. Its usefulness is limited. Endpoint measures simply indicate whether tasks can be performed or not, although attempts to quantify the behaviour have been made (Gale et al. 1985). It does not provide any information about why, or how a task may or may not be performed. As well, if a task is performed it may not be performed in a usual manner. In this case endpoint measures are not sensitive enough to detect such a response.

1.5.2.2 Qualitative kinematics

Kinematics is that phase of mechanics which deals with the possible motions of a material body (Dorland 1994). Kinematical analysis follows the position of limbs and body as an animal moves past an imaging device. Kinematic analysis can be either qualitative or quantitative. Qualitative kinematic analysis simply describes the movement of limbs in space, it does not measure things such as translational or angular velocities or accelerations. Examples of qualitative kinematic analysis used in assessing motor function include the Eshkol-Wachmann Movement Notation (EWMN) (Eshkol and Wachmann, 1957 as cited in (Whishaw et al. 1991), Tarlov grading system (Tarlov et al. 1953), or open field locomotor scores (Basso et al. 1995).

In the example of EWMN, the body is treated as multiple segments. EWMN simply demonstrates changes of one body segment with respect to another. By doing this one is able to determine exactly how an animal is moving. This method has been used to demonstrate changes in the manner in which a rat grasps a pellet following motor cortex lesions (Whishaw et al. 1991). This method demonstrates that an animal may still be able to accomplish a task, yet is able to accomplish the task in a different way. This method of assessing functional recovery provides more precise information about how a task might be completed, as opposed to an endpoint measure method of analysis which simply tells the observer if a task can be performed or not.

Other qualitative kinematic analyses include the open field locomotor scoring systems which include the BBB-scale, and the Tarlov system. The premise behind these systems is to give animals scores which indicate how well they have recovered following spinal cord injury. These scoring systems are complex and the numbers assigned to given behaviours are arbitrary. It has been shown that the consistent and accurate application of these scoring systems requires considerable experience, yet results of such systems can be compared between institutions (Basso et al. 1996b).

Qualitative kinematic analysis provides more information about how behaviours occur when compared to the simple endpoint measure methods. Results of the qualitative kinematic analysis methods still contain some subjectivity, and do not provide solid factual evidence of what or how an animals' behaviour is occurring. This method does screen individuals for motor impairment. That is, this method will demonstrate if an animal has altered its behaviour to complete a given task.

1.5.2.3 Quantitative kinematic analysis

Quantitative kinematic analysis is that branch of kinematics which attempts to quantify how the body moves in space. It attempts to quantify the timing, distance, and angular variables of a movement (Clayton 1996). As opposed to qualitative kinematic analysis, quantitative analysis attempts to dispose of any subjectivity. For example, qualitative analysis describes that a rat supinates their paw at exactly the same time as it withdraws the paw after grabbing a food pellet (Whishaw et al. 1991). Quantitative analysis might, however, include measuring the angle of limb trajectory while grasping a pellet of food.

Quantitative kinematic analysis of an animal's gait requires that an animal's behaviour is recorded by either videotaping or some other method of recording. Identification of constant points on an animal's body (either by using skin markers or metal implants), computer software and hardware for analysis, and some sort of timing device are also required. The equipment used for quantitative kinematic analysis is relatively expensive when compared to the methods already discussed, especially compared to the endpoint measure method. Data that can be collected from this type of analysis includes the stride period, the stride length, average velocities during the stride, and joint angles throughout the stride. Quantitative kinematic analysis has been used to study recovery of locomotion following spinal cord injury (Brustein and Rossignol 1998; Muir 1999).

Quantitative kinematic analysis provides an effective way of determining how locomotion during the swing phase changes following spinal cord injury. However, this method requires substantial formal training of an observer when analyzing data. This method really only provides information about what a limb is doing when in motion. Therefore less information is obtained using this form of analysis during the stance phase. As well, a large amount of error can be incurred when 1) the subject is being filmed from too great a distance (cannot see body markers), 2) body markers are placed over areas where there is ample skin (skin will move thereby moving the marker from place to place) (Audigie et al. 1998), and 3) if the motion being analyzed is too small (eg.- a digit of a rat).

Steps have been made to eliminate some of the error. X-ray kinematography has been used for quantitative kinematic analysis (Boczek-Funcke et al. 1994; Kuhtz-Buschbeck et al. 1994; Boczek-Funcke et al. 1996; Kuhtz-Buschbeck et al. 1996). X-ray kinematography utilizes the radiodense properties of metal objects. Metal objects are implanted into the site being used as a reference point. This method has the advantage over conventional skin markers because it eliminates error of marker movement from skin movement over a body part. However, this particular technique does require invasive measures for placement of the metallic marker.

As already mentioned, the largest disadvantage of kinematic analysis (qualitative or quantitative) for locomotion research is that forces imparted by a limb on the ground are not measured.

1.5.2.4 Kinetic analysis

Kinetics is that branch of physics which studies the effect of forces in the production or modification of motion (Funk and Wagnall's 1989). Kinetic locomotion analysis provides information regarding the forces generated (in the case of ground reaction forces) on the ground as an animal locomotes. Kinetic analysis has been used extensively in animals to determine the effects of such things as surgical correction of cranial cruciate ligament rupture (Budsberg et al. 1988), experimentally induced synovitis (Rumph et al. 1993), the ontogeny of bipedal locomotion in chickens (Muir et al. 1996), voluntary gait modification in cats (Lavoie et al. 1995), and most recently changes occurring from lesions within the central nervous system including corticospinal tract (Muir and Whishaw 1999a), red nucleus lesions (Muir and Whishaw 2000), and spinal cord hemisection (Webb and Muir 2002a).

Kinetic analysis requires the following equipment and facilities: gait path, force transducers (force plates), signal-conditioning electronics, computer software and hardware, and velocity monitoring electronics. It has been suggested that multiple force plates are required for accurate determination of simultaneous force measurement recordings (Bertram et al. 1997). Ground reaction forces are obtained during locomotion in vertical, fore-aft, and medio-lateral directions. These forces can then be analyzed to reveal velocity, acceleration, kinetic energy, and potential energy of the body center of mass during locomotion (Cavagna et al. 1977; Webb and Muir 2002a). By determining these values one is then able to assess how a limb is being used, whether it is used as a strut, or as a spring (Cavagna et al. 1977). This method can also inform the observer about how the other limbs are being used. These changes provide quantitative information about what an animal is doing during locomotion. Force plates can be designed for very small measurements (eg. forces from mice (Clarke and Still 1999)) or for large measurements obtained from heavy animals like horses (Clayton et al. 2000).

Force plate analysis provides quantitative information about an animal's locomotion. Ground reaction forces obtained are not restricted to one gait, that is, analysis can be performed in an animal at the walk, trot or the gallop (Cavagna et al. 1977). Ground reaction force determination is precise and can be used to measure a wide range of forces. The analysis of these forces reveals how limbs are being used during locomotion. The information obtained from this method can be used to determine specific contributions of a variety of injuries. Unfortunately, equipment used for this analysis is expensive and elaborate, and specific formal training of the behaviourist is required to perform this method of analysis. However, this method of analysis could be easily standardized between institutions, and laboratories. Standardization of a behavioural analysis method would prove beneficial because data from one lab could be compared directly with data from another lab.

1.6 Injury paradigms for experimental spinal cord injury studies in rats

1.6.1 Introduction

Since the time of Sherrington and Brown, spinal cord injury models other than complete transection have been developed to facilitate research in the areas of neural regeneration, locomotion, and a whole host of other disciplines. These models can be categorized as complete or incomplete injuries. Incomplete injuries can be categorized as unilateral or bilateral. The method used to invoke a particular spinal cord injury can be contusion, concussion, laceration or ischemic insult to the spinal cord. Spinal cord injury models may also occur at different levels along the spinal cord. For example, injury may occur above or at the level of the cervical or thoracolumbar enlargements. The ideal model for any spinal cord injury experiment depends upon the intended goal of the study. This section highlights different models used by researchers in the field of spinal cord injury and briefly identifies the particular usefulness of each model.

1.6.2 Complete spinal cord injury models

Complete spinal cord injury models are created by sharp transection of the spinal cord within the lower thoracic spinal segments. Consequently, this model involves laceration of the meninges. This model of spinal cord injury has been used for a variety of purposes including studies evaluating 1) the central pattern generator of the lumbar spinal cord (Barbeau et al. 1987; Barbeau and Rossignol 1991), and 2) regeneration within the spinal cord (Ramon-Cueto et al. 2000). The benefits of using complete transection models result from the fact that all descending supraspinal input to the spinal cord below the level of transection has been removed. This permits evaluation of the central pattern generator following various pharmacologic

(Barbeau et al. 1987; Barbeau and Rossignol 1991) and/or sensory stimuli (Barbeau and Rossignol 1987) without the confounding influences of the supraspinal circuitry, and to evaluate the influence of supraspinal structures on the central pattern generator by comparing intact animals with transected animals (Hiebert et al. 1994). In the instance of regeneration studies, however, this model of spinal cord injury has the benefit of being able to identify axons that have descended within the spinal cord below the level of injury. For example, injecting a retrograde tracer of one colour into the spinal cord immediately after surgery would demonstrate completeness of injury by the absence of that tracer within the brain. If another tracer of a different colour is injected, after a particular therapy and period of time, results in retrograde labeling of neurons within the brain then one can be confident that regeneration had occurred. In incomplete spinal cord injury models, this is difficult (if not impossible) to determine as some of the axons are left intact at the injury site. Furthermore, complete transection models allow one to determine whether supraspinal influences contribute to behavioural recovery following therapeutic intervention when combined with re-transection of the spinal cord (Kwon et al. 2002). That is, if behavioural recovery occurs following a particular treatment and the degree of recovery is lost following transecting the spinal cord a second time then the behavioural recovery observed initially was most likely due to the effect that treatment has on regenerating fibers.

1.6.3 Incomplete spinal cord injury models

Incomplete spinal cord injury models constitute the vast majority of models used in spinal cord injury studies today. These models are used in studies evaluating various treatments for spinal cord injury (Bethea et al. 1999; Ramon-Cueto et al. 2000; Kamencic et al. 2001), for evaluating the importance of particular pathways in locomotion (Webb and Muir 2002b), to study autonomic dysreflexia (Weaver et al. 2001), and in the assessment of chronic central pain (Hulsebosch et al. 2000). Incomplete spinal cord injuries can be unilateral or bilateral and can be induced by compressing, contusing, lacerating injuries or by inducing ischemia. Advantages to using partial spinal cord injury models include: 1) very specific injury to particular pathways or regions of the spinal cord can be made; 2) spinal cord injured animals require significantly less post-operative care; and 3) some of these models mimic naturally occurring spinal cord injury in humans. Disadvantages of these models include: 1) the inability to confidently identify whether regeneration has occurred following a specific therapy; and 2) the inability to discern loss of function from behavioural compensation.

1.6.3.1 Compression

Compression models are thought to be useful for imitating what occurs during naturally produced spinal cord injuries in humans. Spinal cord compression models involve compressing the spinal cord with or without incising the dura mater. This can be accomplished using modified aneurismal clips (Rivlin and Tator 1978; Khan and Griebel 1983), inflating balloons intraspinally and intra- or extradurally with air or liquid (Tarlov et al. 1953; Khan and Griebel 1983; Vanicky et al. 2001), and by placing a weight onto the spinal cord epidurally for a period of time (Black et al. 1986; Farooque 2000). Compression models have been useful for illustrating the effect of duration of compression with both behavioural and neuropathological outcomes (Rivlin and Tator 1978). In the instance of the balloon compression model it has been demonstrated that the balloon volume dictates the degree of spinal cord injury.

1.6.3.2 Contusion

Contusion models have been described as early as 1911 (Kwon et al. 2002). Contusion models have been shown to simulate the majority of spinal cord injuries occurring in humans (Metz et al. 2000a). Contusion models, like naturally occurring human spinal cord injuries, result in hemorrhagic necrosis at the injury epicenter, with subsequent development of cystic cavitation surrounded by glial scarring. Contusion injury to the spinal cord is simulated in 1 of 2 different ways. Contusion can be simulated by either 1) dropping a weight from a predetermined height onto the centre of the spinal cord or 2) electromechanically displacing the spinal cord for a specified amount of time (for specific technical review see (Kwon et al. 2002)).

With the weight drop method, the amount of damage to the spinal cord depends upon energy transmitted to the spinal cord. This energy is dependent upon the mass of the weight and the velocity that the weight achieves upon impact of the spinal cord (equation 1.4) and the velocity that the weight returns from the spinal cord after impact (Kwon et al. 2002). For example, if the weight is dropped from a height, the energy transmitted to that spinal cord would be zero if the weight bounced off of the spinal cord at exactly the same impact velocity. The electromechanical devices used to deliver a particular amount of energy to the spinal cord do not bounce from the spinal cord. Instead, the impactor remains in place for a period of time. The most commonly used weight drop model is created using the New York University (NYU) impactor, while the most common electromechanical device is the Ohio State University (OSU) impactor (Kwon et al. 2002). Both of these devices can however, result in reliable, graded forms of spinal cord injury providing that conditions such as animal strain, age, and anesthetic agent are controlled (Kwon et al. 2002).

1.6.3.3 Laceration

The vast majority of naturally occurring spinal cord injuries in humans result from contusion and compression to the spinal cord resulting from vertebral fracture (Kakulas 1999). In some instances, however, spinal cord injury can result from laceration by knives, or other forms of penetrating injuries (National Spinal Cord Injury Statistical Center 2000). Partial spinal cord injury models involving sharp transection are useful for modeling these forms of naturally occurring injury. Lacerating partial spinal cord injuries are created by cutting the meninges and spinal cord with a sharp instrument such as a scalpel blade (Ballermann et al. 2001), a modified tapered needle (Webb and Muir 2002b), or with scissors (Webb and Muir 2002a). This technique of spinal cord injury results in either unilateral or bilateral damage. Damage is characterized pathologically by hemorrhage, transection of spinal axons, necrotic death of neurons within the spinal gray matter, and variable amounts of microcystic cavitation. In the case of unilateral spinal cord injuries, asymmetric sensorimotor behaviours develop (Webb and Muir 2002a, b). These asymmetries facilitate investigations studying the importance of particular pathways that might otherwise go undetected following bilateral spinal cord injury.

1.6.3.4 Ischemia

In all cases of spinal cord injury, some degree of ischemia results. In some cases, ischemia is the primary cause of spinal cord injury. For example, surgery performed on the abdominal aorta might require transient occlusion of the aorta with subsequent ischemia to the spinal cord (Cambria et al. 2002).

Fibrocartilagenous embolism of the spinal vasculature may also result in ischemic myelopathy (McLean et al. 1995; Cauzinille 2000) . Regardless, an ischemic spinal cord injury model has been developed to mimic these clinical phenomena using rats. The model consists of inserting a Fogerty catheter through the femoral artery to the level of the subclavian arteries. The Fogerty catheter balloon tip is then filled with saline to permit occlusion of the thoracic aorta and the duration of ischemia has been anywhere from 6 to 40 minutes (Taira and Marsala 1996). This model also involves the concurrent control of proximal arterial pressure. The effects of proximal arterial pressures examined included 40-120 mm Hg (Taira and Marsala 1996). This spinal cord ischemia model results in apoptosis and necrosis within the gray and white matter (Follis et al. 1993; Kato et al. 1997). However, as one might expect, increasing length of time of ischemia results in more dramatic spinal gray matter damage, and reduced proximal arterial pressure results in decreased collateral circulation to the spinal cord and worsening spinal gray matter histopathology (Taira and Marsala 1996).

Overall, the development of spinal cord injury models has resulted in the advancement of knowledge in the area of spinal cord injury research. Each model has its own innate limitations and benefits. Some models require sophisticated apparatuses in order to produce reliable and reproducible results, while other models rely upon relatively simple techniques requiring high degrees of surgical skill by the surgeon. Choosing the spinal cord injury model for a particular research project is dependent upon the desired outcome of the study and the scientific hypotheses to be answered.

1.7 Summary

Over the past century several advances have been made in our understanding of the neuroanatomy of the rat spinal cord and locomotion (both it's neural control and biomechanics), and the development of methods used to evaluate behavioural recovery following experimental spinal cord injury and models to mimic spinal cord injury in humans. This review has provided a general overview of these topics. This foundation knowledge is essential for understanding the premise and methodologies used in the following chapters.

Chapter 2. GENERAL OBJECTIVES

The main objectives of the studies presented herein were to 1) determine whether different strains of rats have similar sensorimotor abilities and to 2) evaluate sensorimotor abilities of spinal cord injured rats using a battery of behavioural tests. Many of the experiments were designed to provide evidence supporting the importance of particular spinal cord pathways (those thought to be important) for performing various sensorimotor tasks, especially locomotion.

Rats are one of the most common species used for spinal cord injury research. Spinal cord injury research involves the use of a variety of different strains of rats. More than one particular strain might be used within a particular laboratory and/or between laboratories. It has been assumed, in the spinal cord injury field, that all rats behave similarly regardless of strain. Discrepancies in results obtained from different laboratories using similar therapies exist. These discrepancies may result from different strains of animals being used between laboratories. The objectives of chapters 5 and 6 were to determine if the strain of a particular animal affects the animal's sensorimotor abilities both before and following spinal cord injury.

Understanding the importance of various spinal pathways for performing sensorimotor tasks in rats may help guide researchers in developing therapies for spinal cord injury. For example, particular pathways may be more important for overground locomotion than others. In this instance it may be important to aim therapies for spinal cord injury at specific spinal cord pathways, or regions, rather than targeting the entire spinal cord. The objectives of the remaining experimental chapters (chapters 7-9) were to identify specific spinal pathways or spinal cord regions as being important (in freely behaving rats) for various sensorimotor tasks, especially locomotion.

Chapter 3. HYPOTHESES

1. From observing various rats it is clear that mature rats of different strains share morphological similarities and differences in physical stature. It is hypothesized that differences in long bone length exist between strains of rats.

2. Numerous behavioural (both spontaneous and chemically-induced) differences have been reported between various strains of rats. Strain-dependent differences in locomotor ability have not, however, been described. It is hypothesized that sensorimotor, especially locomotor, abilities are different between strains of rats and these strain dependent differences affect the behavioural abilities of rats following unilateral spinal cord injury.

3. Unilateral injury to the central nervous system (excluding the spinal cord) has been shown to produce characteristic assymetrical changes in a rat's locomotor abilities. It is hypothesized that unilateral spinal cord injury will result in a characteristic change in locomotion, but will depend upon the cranio-caudal level of the injury.

4. The rubrospinal tract appears to be important for excitation of appendicular flexor muscles in the decerebrated cat preparation. Unilateral injury to the red nucleus has been shown to result in long-lasting locomotor changes. It it is hypothesized that unilateral injury to the rubrospinal tract will result in long-lasting changes in sensorimotor and locomotor abilities of adult freely behaving rats. These changes will show similarities to rats with unilateral cervical spinal cord hemisection.

5. Both sensory and motor alterations are affected following dorsal column injury in monkeys, cats and rats. Traditionally it is thought that the dorsal columns serve a role in tactile discrimination and relay information pertaining to other exteroceptive stimuli. These proposed or known functions may be important for locomotion. It is hypothesized that unilateral injury to the ascending pathways traveling within the fasiculus cuneatus and gracilis will result in long-lasting changes in sensorimotor and locomotor abilities of adult freely behaving rats. These changes will show similarities to rats with unilateral cervical spinal cord hemisection.

6. The vestibulospinal and reticulospinal pathways are two major paths providing descending input to the spinal cord from the brain. The vestibulospinal tract has been shown to be important for extensor

muscle excitation in the decerebrate preparation. The reticulospinal tract is important for locomotor initiation in the decerebrate and fictive preparations. Recently, it has been shown that these pathways demonstrate a considerable amount of functional redundancy with respect to freely behaving locomotion in rats. Locomotion has been, until recently, evaluated grossly in rats. It is hypothesized that unilateral injury to the ventrolateral spinal funiculus will result in mild but long-lasting changes in sensorimotor and locomotor abilities of adult freely behaving rats. These changes will show similarities with cervical spinal cord hemisected rats.

Chapter 4. GENERAL METHODS AND PROCEDURES

4.1 Training to locomote for food reward

All animals were trained to travel along the length of a 182 X 20 cm runway for a food reward. Animals were also trained to run the length of the runway with a horizontal ladder placed equidistant between its ends. Training was successful when the animal was able to complete these tasks consistently.

4.2 Anesthesia and analgesia

Rats undergoing experimental spinal cord injury or retrograde tracing were premedicated 30 minutes prior to general anesthesia with 0.05 mg/kg buprenorphine hydrochloride (Buprenex, Reckitt & Colman Pharmaceuticals, VA, USA) and 0.05 mg/kg atropine (MTC Pharmaceuticals, ON, Canada) administered subcutaneously. Pre-emptive analgesia allowed a surgical plane of anesthesia to be induced using 35-40 mg/kg sodium pentobarbital (Somnotol, MTC Pharmaceuticals, ON, Canada) administered intraperitoneally (Roughan et al., 1999). Following induction of anesthesia, rats were kept warm on a recirculating warm water blanket. Animals were administered 100% oxygen nasally throughout the duration of the procedure. Respiratory rates, heart rates, and pedal withdrawal responses were assessed periodically throughout the surgical procedure to ensure that a surgical plane of anesthesia was maintained. All rats were administered an additional dosage of buprenorphine (0.05 mg/kg) subcutaneously 12 hours after the initial dose thereby providing 24 hours of analgesia.

4.3 Surgical Procedures

All rats were administered 30 mg/kg methylprednisolone sodium succinate (MPSS) (Solu-Medrol, Pharmacia & Upjohn Inc., ON, Canada) intraperitoneally upon induction of general anesthesia. MPSS was administered to prevent exacerbation of the mechanical injury from secondary injury mechanisms (Oudega et al. 1999; Webb and Muir 2000). Rats were routinely surgically prepared and draped. Rats underwent either cervical or thoracic dorsal laminectomies at either vertebra C3 or T9/T10 respectively using a Zeiss Stemi 2000C surgical microscope for magnification. Briefly, skin and superficial muscle were incised, and epaxial musculature was bluntly dissected from the appropriate dorsal spinous process and the lamina of the vertebrae. The dorsal spinous process was removed using a pair of microrongeurs. Laminectomy of the appropriate vertebra was performed using microrongeurs. A durotomy was made using microscissors and the spinal cord was injured using a modified 22 gauge needle (for dorsal column, rubrospinal and ventrolateral lesions) or with microscissors (for cervical or thoracic spinal cord hemisections). An autologous fat graft, obtained subcutaneously near the surgery site, was placed over the laminectomy site to prevent fibrous adhesions to the spinal cord and dura (Pospiech et al. 1995).

Overlying muscles were closed using 3-0 braided polyglycolic acid (Dexon II, Davis & Geck, NJ, USA) in a simple continuous pattern. Skin was closed using skin staples (Appose ULC, Davis & Geck, NJ, USA). All rats had skin staples removed 10 days following surgery. Sham-operated animals were treated similarly except that no damage was done to the spinal cord.

4.4 Behavioural Assessments

4.4.1 Ladder Crossing

The number of times a rat's paw or limb falls between the rungs of a horizontally placed ladder during locomotion has been used to evaluate motor abilities of brain (Soblosky et al. 1997) and spinal cord injured rats (Soblosky et al. 2001). Briefly, a ladder was constructed such that 1/4" doweling separated 1" apart (center of one doweling to center of next doweling) were used as rungs. The ladder was placed equidistant from the ends of a clear plexiglass runway. Animals were videotaped (Super VHS video recorder; Panasonic AG-456U S-VHS, Secaucus, NJ) at 30 frames/second while crossing either 25 or 10 rungs of the ladder. Ten runs were collected for each rat. A “run” was considered successful if the rat moved across the ladder without hesitation. Video tape was analyzed field by field (60 fields/s) and the total number of footfalls was recorded and expressed as percentage footfall for each rat ((total number of footfalls/total number of ladder rungs)X 100).

4.4.2 Forelimb support while rearing

Rats naturally use their forelimbs for balance and stabilization while rearing against a wall. This naturally occurring behaviour has been exploited to describe the behavioural ability of rats with unilateral, experimentally induced stroke (Schallert et al. 2000), brain injury (Schallert et al. 2000), dopamine depletion (Schallert et al. 2000), and spinal cord injuries (Liu et al. 1999; Ballermann et al. 2001). Each rat was placed in a clear plexiglass cylinder (46 cm high X 40 cm diameter), set on a clear piece of glass, and videotaped from an angled mirror placed below the glass table top for 5 minutes (Fig. 4.1). Videotapes were retrospectively reviewed field by field (60 fields/sec) and the total number of times the left, right or both limbs were used for support while rearing was recorded. The criteria used to determine whether right, left or both limbs were being used was similar to that used in other studies (Liu et al. 1999). Briefly, individual limbs (right or left) were scored when the rat would place the individual limb on the wall of the cylinder. Animals were scored as using both forelimbs if 1) both limbs simultaneously hit the wall of the cylinder or 2) for each time the animal walked along the side of the cylinder using alternating left and right limbs. For example, if a rat reared and placed it's right forelimb on the cylinder it would be recorded as a right placement. If the rat did not lift it's right forelimb from the cylinder wall before placing it's left forelimb on the wall a "both forelimb" would be recorded. For every subsequent step the rat made while having a forelimb in contact with the wall "both forelimb" would be recorded. A "right or left forelimb" could not be scored until the rat removed all forepaws from the plexiglass wall.

4.4.3 von Frey Filament Testing

Withdrawal latencies to mechanical stimuli are commonly used in experiments involving the assessment of pain (Mills et al. 2001) or sensory function. I chose to evaluate sensory abilities using withdrawal thresholds using von Frey filaments.

Briefly, rats were placed in a clear plexiglass box placed over an opaque plexiglass perforated surface (30 cm X 30 cm platform, 1.5 mm diameter perforations 5 mm apart) mounted on a frame such that there was easy access to the bottom of the opaque plexiglass. It has been shown that a decrease in variation occurs when using a hard, opaque surface for rats to stand on compared the more traditional use of wire mesh (Pitcher et al. 1999). Animals were placed in the box, with the top covered, until exploratory behaviour subsided. Calibrated von Frey filaments (Semmes-Weinstein Monofilaments, Stoelting Co., Wood Dale, IL) were used as previously described (Pitcher et al. 1999) to evaluate the force required to elicit hind limb withdrawal (mechanical threshold). Threshold was recorded as that force required to elicit a withdrawal 3 to 4 out of 4 times. Von Frey filaments were calibrated to the nearest 100th of a gram of force, using a Mettler balance,

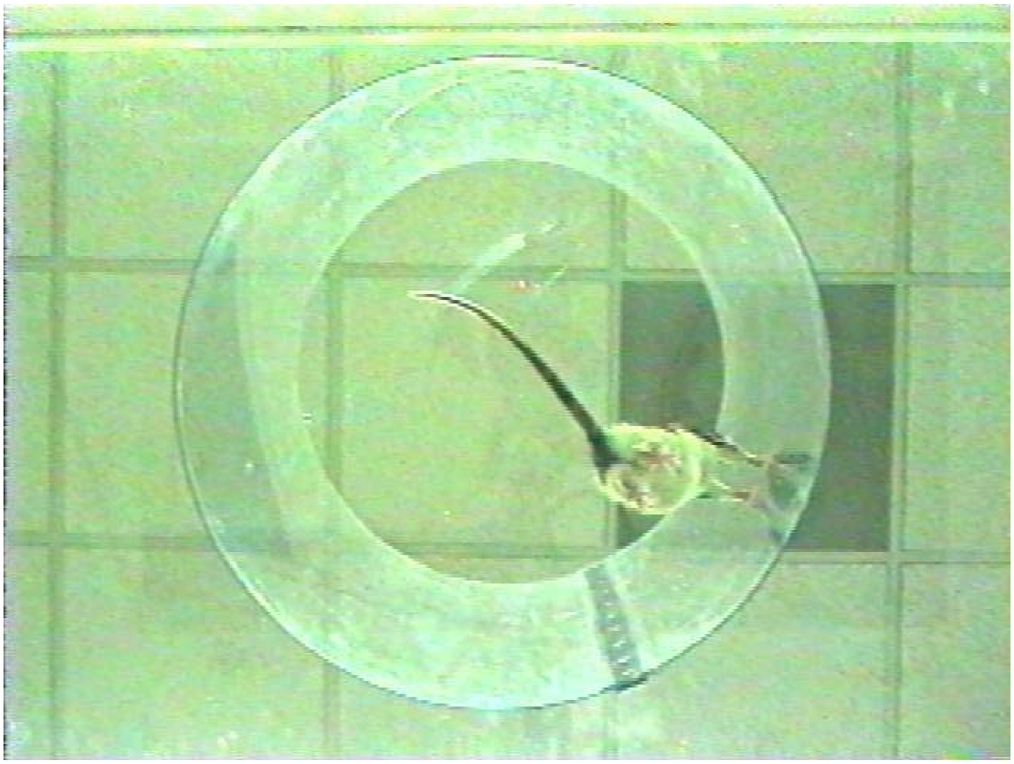


Fig. 4. 1 Captured video field of a rat during the forelimb preference task.

prior to use as force elicited for each filament can change with environmental and physical factors (eg. amount of use, temperature, and humidity).

4.4.4 Kinematic Measurements

Each rat was marked with a black, non-toxic marker along the skin covering the length of their fifth metatarsal bone to the point of the calcaneous (point of the hock) for each hind limb. Animals were video taped from a caudal position as they trotted along a runway for a food reward (Fig. 4.2). Animals had to successfully complete at least 10 runs. A run was deemed successful if the animal moved without hesitation and was trotting. No galloping or bounding gaits were measured kinematically. Video tapes of each animal were reviewed at 60 fields/s and two different fields/limb/run at midswing were captured and digitized using Northern Eclipse 6.0 software (Northern Eclipse 6.0, Empix Imaging Inc., Mississauga, ON). Angle measurements from the edge of the 5th metatarsal to a line drawn from the point of the calcaneous perpendicular to the ground were determined electronically (Northern Eclipse 6.0, Empix Imaging Inc., Mississauga, ON) for each captured field for a given rat (Fig. 4.2). These angles were averaged to yield an individual hock angle for a given limb for a particular animal. These individual hock angles were kept separate for left and right limbs for each animal of a given strain. These individual hock angles were used to determine the mean group hock angles for each experimental group.

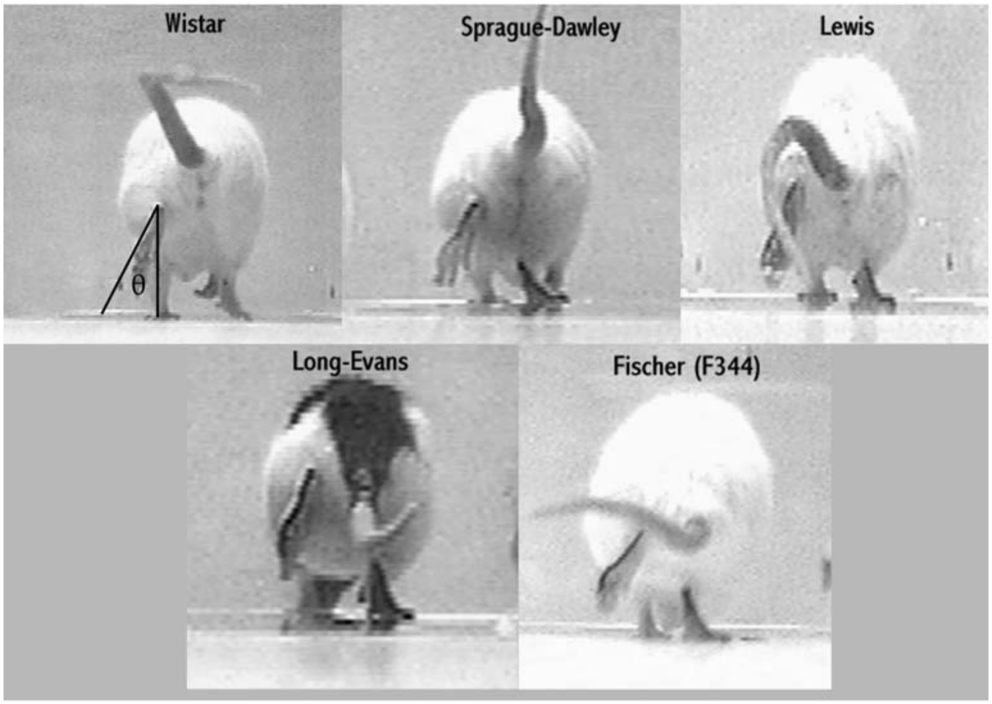


Fig. 4. 2 Captured video fields illustrating distal hind limb abduction angles. Distal hind limb abduction angles were determined by measuring the angle made between a line made perpendicular to the ground to the point of the calcaneous and a line parallel with the 5th metatarsal.

4.4.5 Kinetic Measurements

As previously mentioned, rats were trained to cross the runway for food reward. Three force platforms used to describe the locomotor abilities of spinal cord injured rats (Webb and Muir 2002a) were used to determine ground reaction forces. These force plates are capable of measuring vertical, fore-aft and mediolateral forces. Each of the three force platforms was 10.5 cm X 11 cm. The platforms were arranged in series and embedded in the center of the runway. Each animal's weight was recorded prior to collecting ground reaction force data. Rats triggered the data acquisition system (RC electronics, San Raphael, CA) and an LED timer by breaking an infrared beam of light placed across the runway and located behind the force platforms. Data was amplified and analogue-to-digital converted (RC Electronics, San Raphael, CA) at 1000 Hz. The LED timer was stopped when the rats broke a second infrared beam of light placed behind the last force platform. The distance between each beam of light was measured prior to collecting data and was used for calculating average velocity. Data was only collected from animals that were trotting. Trotting involves the alternating pattern of diagonal limbs being placed on the ground simultaneously. Animals were video taped at 30 frames/s using a Super VHS video recorder (Panasonic AG-456U S-VHS, Secaucus, NJ) from a lateral position. This permitted retrospective evaluation of the limb pairs that "hit" each plate as well as permitting the determination of the time elapsed between breaking the first and second infrared beams of light (Fig. 4.3).

Fig. 4. 3 Video field of a rat trotting across force platforms. Animals broke an infrared beam of light thereby triggering the data acquisition system and LED timer. The animal then crossed the force plates and broke a second infrared beam of light, thereby turning off the LED timer.

Ground reaction force data was imported as ASCII data to a personal computer. Data was divided into individual runs for each rat, then filtered in the forward and reverse direction using a modified low pass recursive filter (SPSS, Chicago, IL). Force data was expressed in proportion of body weight and normalized over time as a proportion of stride using custom written software (SPSS, Chicago, IL) for each rat. Limb pairs were kept separate and averaged together for each trial for each rat (minimum of 5 runs/trial). Only runs where the animal was traveling between 50-100 cm/s were used to determine the mean ground reaction forces for an individual. This averaged ground reaction force data served as representative individual ground reaction force data for each animal. All individual ground reaction force data was averaged together for a particular strain to provide the mean strain ground reaction force data. Standard error of each strain's group data was calculated using custom written software (Microsoft Visual Basic 6.3, Microsoft Corp.). Animals had to appear to be traveling at a relatively constant velocity both at data collection time and upon inspection of video analysis.

4.4.5.1 Variables of the ground reaction force data examined

1) peak vertical, propulsive and braking forces for fore and hind limbs bilaterally

2) Ipsilateral Individual Limb Peak Vertical Force Symmetry. Calculated bilaterally as:

$$\left(\frac{\text{Peak Vertical Forelimb Force}}{\text{Peak Vertical Hindlimb Force}}\right) \tag{4.1}$$

For Individual Limb Peak Vertical Force Symmetry ratios a value greater than 1 indicates higher peak vertical force on forelimbs compared to hind limbs and values less than 1 indicate higher peak vertical force in the hind limbs compared to the forelimbs.

3) Individual Limb Peak Vertical Force Left to Right Symmetry for the fore and hind limbs. Calculated as:

$$\left(\frac{\text{Peak Vertical Left Forelimb Force}}{\text{Peak Vertical Right Forelimb Force}}\right) \tag{4.2}$$

This was calculated similarly for hindlimbs. For Individual Limb Peak Vertical Force Left to Right Symmetry ratios a value greater than 1 indicates higher peak vertical force on left limb compared to right limb and values less than 1 indicate higher peak vertical force on the right limb compared to the left limb.

4) Individual Limb Peak Braking to Peak Propulsive Forces for fore and hind limbs bilaterally. Calculated as:

$$\left(\frac{|\ \text{Peak Braking Force}\ |}{|\ \text{Peak Propulsive Force}\ |}\right) \tag{4.3}$$

Values greater than 1 for braking to propulsive peak force ratios indicate higher braking forces, values less than 1 indicate higher propulsive forces are being generated.

5) Summed Peak Vertical Force Symmetry. Calculated as:

$$\left(\frac{\text{LFore - RHind Peak Vertical Force}}{\text{RFore - LHind Peak Vertical Force}}\right) \qquad (4.4)$$

For symmetry of peak vertical forces for diagonal limb pairs values greater than 1 indicate higher peak forces generated on left fore-right hind limb pair, values less than 1 indicate higher peak forces generated on right fore-left hind limb pair, values equal to 1 indicate perfect symmetry between diagonal limb pairs.

6) Net Peak Summed Fore-aft Force. Calculated as:

(peak braking + peak propulsive force for left fore-right hind) + (peak braking + peak propulsive force for right fore-left hind) (4.5)

Net peak fore-aft force for one complete stride taken from summed ground reaction forces, values above zero indicate that the animals were accelerating, and less than zero indicate that animals were decelerating. Ideally, the amount of force generated in the propulsive direction should equal the amount of force generated in the braking direction if constant velocity is maintained while traveling over plates.

All variables were calculated or determined using custom written software (Microsoft Visual Basic 6.3, Microsoft Corp.).

4.5 Histological evaluation of lesion site

After all behavioural tests were completed, rats were deeply anesthetized with 70 mg/kg sodium pentobarbital (Somnitol, MTC Pharmaceuticals, ON, Canada) administered intraperitoneally, and were transcardially perfused with 250 mls of heparinized 0.1M PBS followed by an equal volume of 4% paraformaldehyde dissolved in 0.1M PBS. Spinal cords were dissected and post-fixed in 4% paraformaldehyde/0.1M PBS for 24 hours. Spinal cords were routinely processed and embedded in paraffin. Cords were serially and transversely sectioned at 8-10 μm throughout the injury site and mounted on slides coated with poly L-lysine and were stained with either crystal violet or luxol fast blue/crystal violet. All sections were examined by light microscopy to determine the maximum size of the lesion for each rat.

4.6 Retrograde tracing of the red nucleus

One week prior to euthanasia, animals (rubrospinal tract, dorsal column, and ventrolateral funiculus injured animals) underwent a second surgical procedure. The anesthesia and analgesia protocol was as described for the first surgery. A dorsal laminectomy was performed to expose T9/T10 spinal cord segments as described in section 4.3. Following durotomy, 0.2 µl of fluorogold (Fluorochrome Inc., Englewood, CO, USA) (4% (w/v) in sterile saline) was injected into the spinal cord using glass pulled pipettes (150 µm outside diameter) mounted on a blunt-ended needle attached to a 2µl Hamilton syringe using a stereotaxic apparatus for positioning. The fluorogold solution was injected over approximately 2 minutes at each site. Any excess fluorogold was removed from the surface of the spinal cord with cellulose ocular sponges (Ultracell Medical Technologies, North Stonington, CT, USA) upon retracting the glass pipette. Musculature and skin was closed as described for the first surgery. One week following injection of retrograde tracer, animals were deeply anesthetized with 70 mg/kg sodium pentobarbital (Somnitol, MTC Pharmaceuticals) administered intraperitoneally, and were transcardially perfused with approximately 250 mls of heparinized physiologic saline followed by an equal volume of 4% paraformaldehyde dissolved in 0.1 M PBS.

Midbrains of all brains were removed at the same time point as spinal cords were dissected. Midbrains were frozen in embedding media, and kept at -80 degrees centigrade until they could be serially and transversely sectioned at 32 µm on a cryostat. Upon sectioning the midbrain, sections were mounted on positively charged slides. All midbrain sections were examined using a Zeiss Axioskop fluorescent microscope using appropriate filters to illuminate neurons expressing fluorogold. The red nuclei were located and examined throughout their rostrocaudal extent. Only animals having left red nucleus fluorescence were considered to have complete left-sided rubrospinal tract injury.

Chapter 5. FISCHER (F-344) RATS HAVE DIFFERENT MORPHOLOGY, SENSORIMOTOR AND LOCOMOTOR ABILITIES COMPARED TO LEWIS, LONG-EVANS, SPRAGUE-DAWLEY AND WISTAR RATS

5. 1 Abstract

Locomotor and/or sensory behaviour is commonly evaluated in laboratory rats in the field of neuroscience. Many strains of rats, however, have been propagated through intensive breeding programs. With any breeding program, traits are selected for purposefully or inadvertently. The current investigation was conducted to determine whether differences in morphology, sensory or motor behaviours exist using five age-matched strains of laboratory rats. Personal observations of morphological differences between different strains of rats led us to hypothesize that Fischer rats were dissimilar to the other strains in each of the parameters investigated. Evaluation of morphology involved measuring long-bone lengths and body weights of each strain. Motor skills were evaluated by measuring paw preferences while rearing, abduction of the distal portion of hind limbs during locomotion, footfalls through a horizontal ladder during locomotion, and ground reaction forces generated during trotting. Sensory ability was assessed by von Frey testing. Fischer rats had shorter long-bone lengths, weighed less, and had significantly abducted distal portion of their hind limbs during locomotion compared to the other strains. Lewis and Sprague-Dawley rats were less sensitive to

mechanical pedal stimulation compared to Fischer rats. While rearing, all strains of rats tended to use individual forelimbs 25% of the time for each right and left limbs, and both forelimbs together 50% of the time. There were no significant differences in the number of footfalls during the ladder task. Ground reaction force determination revealed that Fischer and Sprague-Dawley rats bore more weight on their hind limbs compared to forelimbs during locomotion, Long-Evans and Lewis rats bore more weight on their forelimbs compared to their hind limbs, while Wistar rats distributed weight evenly between forelimbs and hind limbs during trotting. In conclusion, morphologic, sensory and motor differences exist between the five strains of laboratory rats examined and several of these differences are most pronounced in the Fischer strain.

5.2 Introduction

Rats have been used for experimental purposes since at least the mid 1800's (Lindsey 1979). Since these early experiments, and the domestication of the wild Norway rat (*Rattus norvegicus*), many strains of rats have been developed through intensive breeding programs throughout the world. Breeding and husbandry programs for the laboratory rat in North America were primarily brought about by the Wistar Institute of Philadelphia, USA (Lindsey 1979). Breeding programs have developed based on the needs of various researchers and institutes.

Many strains of rats have been propagated using inbreeding or out-breeding strategies. A particular strain of animal is considered inbred if it was created following more than 20 generations of sibling or parent-offspring matings (inbred animals are considered homozygous but homozygosity may not be truly present until after 40 sibling matings) (Festing 1979; Davisson 2000). Outbred strains, however, are strains that are maintained to have a considerable amount of genetic heterozygosity (Hartl 2001). Confirmation of genetic heterozygosity within outbred strains can be verified using a variety of genetic screening tests (Hartl 2001). Regardless, some of the more popular strains of rats originally developed in the early 1900's are still in use today (for historical highlights of breeding practices see (Lindsey 1979)).

Animal husbandry practices generally select and enhance particular traits of a given species of animal. There is no better example of this than the result of breeding practices of domesticated dogs. Purebred dogs have been bred for selected characteristics for hundreds of years (Young 1985). Consequently, highly breed-specific morphological and sensorimotor characteristics have been developed. For example, dogs can range in size from 1 kg up to 100 kg (The Canadian Kennel Club 1989), and particular breeds appear to be more sensitive to painful stimuli than others (Hansen and Hardie 1993). Although the laboratory rat has been bred for a shorter period of time (Lindsey 1979), these animals can propagate quickly and are intensely bred by many established institutes and animal suppliers. Like many domesticated species of animals, laboratory rats have developed many strain specific characteristics, although they may not be as obvious as in domestic dogs. Because the laboratory rat is a popular animal used for studying sensory and motor behaviours, and particular anatomical characteristics may contribute to these behaviours, the current study was conducted to determine whether differences in morphology and sensory and/or motor behaviours exist between various strains of rats. It was hypothesized that differences exist with regard to specific morphological, sensory, and motor abilities of different strains of rats. Specifically, based on grossly observing morphological differences between different strains of rats, it was hypothesized that the Fischer strain is least similar amongst the strains of rats examined. By using morphologic, endpoint, kinematic and kinetic measurements, the morphologic, sensory, and motor differences that exist between five strains of rats are described. Fischer rats tend to be most dissimilar from the other strains. These differences have implications for investigations involving sensorimotor behavioural manifestations of neurologic disease such as spinal cord injury. The findings are discussed in light of their significance for those studying sensory and motor behaviour in the neurosciences.

5.3 Materials and Methods

5.3.1 Subjects

Eleven female rats of each of 5 different age-matched strains were obtained from Charles River Laboratories, Canada (Quebec, Canada). Animals were approximately 4 months of age and considered to be mature. The strains used included: Fischer (CDF(F-344)/CrlBR); Lewis (LEW/CrlBR); Long-Evans (Crl:(LE)BR); Sprague-Dawley (Crl:CD (SD)IGS BR) and Wistar (Crl:(WI)BR). Fischer and Lewis are inbred albino strains; Long-Evans (pigmented), Sprague-Dawley (albino) and Wistar (albino) rats are outbred strains. The strains, in the present study, were chosen because of their relatively common use in the neurosciences, particularly in spinal cord injury (eg. F-344 (Takami et al. 2002a; Takami et al. 2002b), Sprague-Dawley (Basso et al. 1995; Soblosky et al. 2001), Lewis (commonly used for studying immune-mediated and inflammatory phenomenon in the CNS (Popovich et al. 1997)), Wistar (Bruce et al. 2002; Kamencic et al. 2001), and Long-Evans (Webb and Muir 2002a)). Animals were housed in pairs with 12 h light/12 h dark cycle in the laboratory animal care facility at the Department of Veterinary Biomedical Sciences, Western College of Veterinary Medicine at the University of Saskatchewan. All animals were examined daily and deemed healthy by a licensed veterinarian. All animals were cared for according to the standards set out by the Canadian Council on Animal Care.

5.3.2 Training

All animals were trained according to the description in Chapter 4.

5.3.3 Morphometric analysis

Morphometric evaluation was performed by measuring selected long bone lengths. The long bones measured included, bilaterally, the: 1) femur, 2) tibia, 3) humerus, 4) radius, and 5) ulna. Animals were anesthetized with sodium pentobarbital to permit restraint during radiography. All animals were radiographed in dorsal recumbancy with limbs outstretched using a Picker Transix 800A model radiography unit. Radiographs were taken with a setting of 100 milliamperes (mA), 50 kilovolts peak (kVp), and 1 milliampere-seconds (mAs). All images were obtained using Kodak film and were processed routinely using an automated radiograph developer. Bone lengths were measured from the resultant radiograhic images. Briefly, each bone's length was determined by measuring the straight-line distance between the most proximal and distal points of each bone bilaterally (Figs. 5.1A and 5.1B). All measurements were made to the nearest millimeter. Corresponding right and left long bone lengths were averaged together to yield that particular long-bone length for each animal. In addition to bone length, maximum body weights of each animal for each strain were recorded and analyzed for differences between strains of rats.

5.3.3 Endpoint Measurements

Endpoint measurements that were evaluated consisted of ladder crossing, forelimb support while rearing, and von Frey filament testing. These were conducted for all animals as described in Chapter 4.4.3.

5.3.4 Kinematic Measurements

Kinematic measurements consisted of evaluating distal hind limb abduction angles while locomoting as described in Chapter 4.4.4.

5.3.5 Kinetic Measurements

Kinetic measurements consisted of ground reaction force determination as described in section 4.4.5. Variables of the ground reaction force data examined statistically between strains included those listed and described in section 4.4.5.1 except for peak vertical, propulsive and braking forces for fore and hind limbs bilaterally.

5.3.6 Statistical Analysis

If the data met the criteria of normal distribution and equal variances differences between groups were determined using one-way Analysis of Variance with post-hoc analysis consisting of using Bonferroni's adjusted t-test. If criteria for parametric data was not satisfied differences between groups were determined using Kruskall-Wallis test (Analysis of Variance on Ranks) with post-hoc analysis being completed using Dunn's method.

5.4 Results

5.4.1 Morphometric analysis

Data for long-bone measurements met the criteria for parametric statistical analysis. Measurement of these animals' long bone lengths revealed that

Wistar rats had significantly longer bone lengths compared to many of the other strains of rats (Fig. 5.2). Many of the strains had significantly longer bones than Fischer rats (Fig. 5.2). Wistar and Sprague-Dawley rats had longer femurs than Fischer ($P<0.01$), Lewis ($P<0.01$) and Long-Evans ($P<0.01$) rats. Wistar rats also had longer tibia when compared to Fischer ($P<0.001$), Lewis ($P<0.01$) and Long-Evans ($P<0.01$); whereas, there were no statistical differences determined between the tibia of Sprague-Dawley, Long-Evans, and Lewis rats. Fischer rats had significantly shorter tibia than Sprague-Dawley ($P<0.001$), Long-Evans ($P<0.01$), and Lewis ($P=0.01$) rats. Wistar rats had longer humeri than Fischer ($P<0.001$), Lewis ($P<0.001$), and Long-Evans ($P<0.001$) rats. Sprague-Dawley rats humeri were longer than Fischer ($P<0.001$) and Lewis ($P<0.05$) rats, and Long-Evans rats humeri were longer than the Fischer strain ($P<0.05$). Wistar and Long-Evans rats had longer radii than Fischer rats, but Wistar rats also had longer radii compared to Lewis rats. Last, Wistar and Long-Evans rats had significantly longer ulnae compared to Fischer rats.

Significant differences existed between strain body weight ($H=32.65$, 4 df, $P<0.001$). Post-hoc analysis of maximum body weights demonstrated that Fischer rats were significantly ($P<0.05$) lighter weight (median=187g) than Wistar (median=272g), Long-Evans (median=247g), Sprague-Dawley (median=246), and Lewis (median=238g).

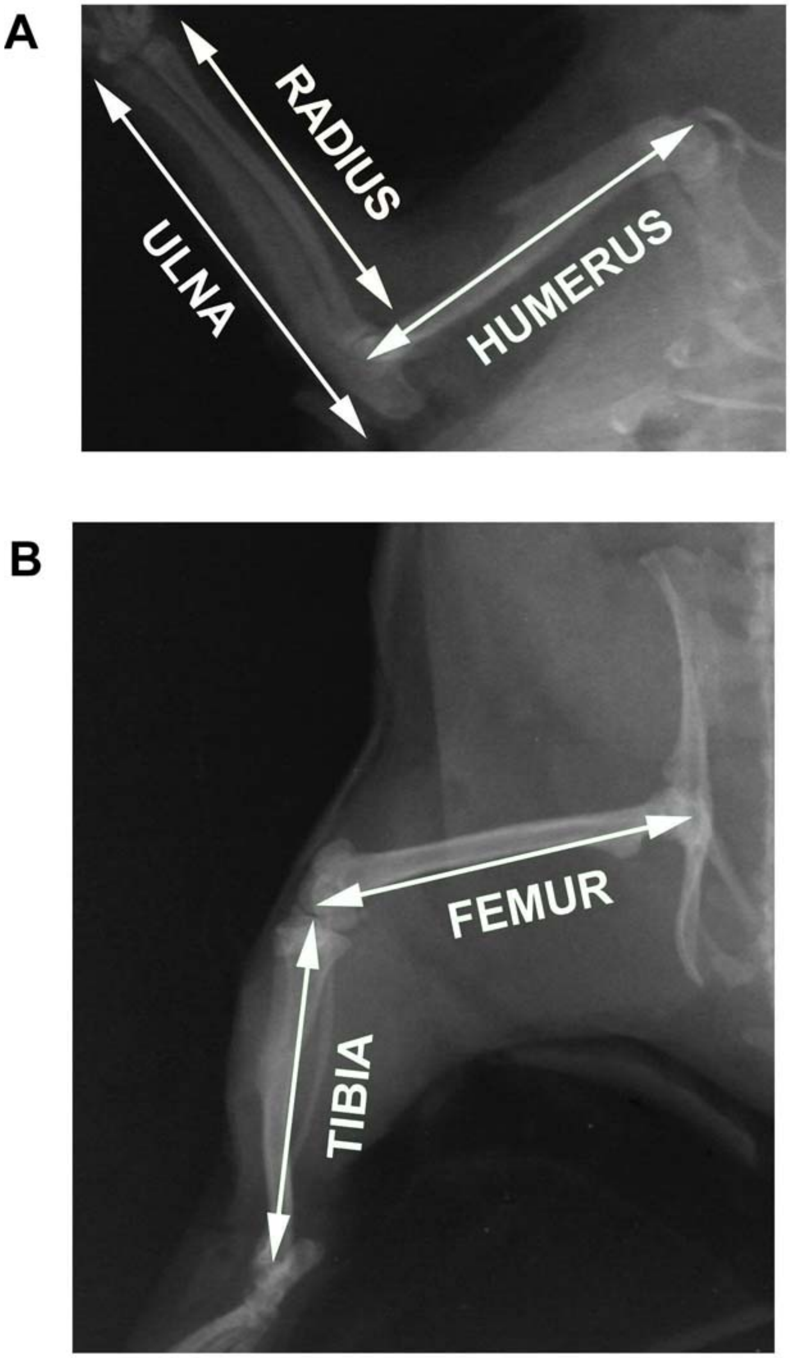


Fig. 5. 1 Radiographs of the long bones of a rat that were measured. The length of each long bone was measured from the most proximal to the most distal aspect of each bone (arrows) from radiographic images.

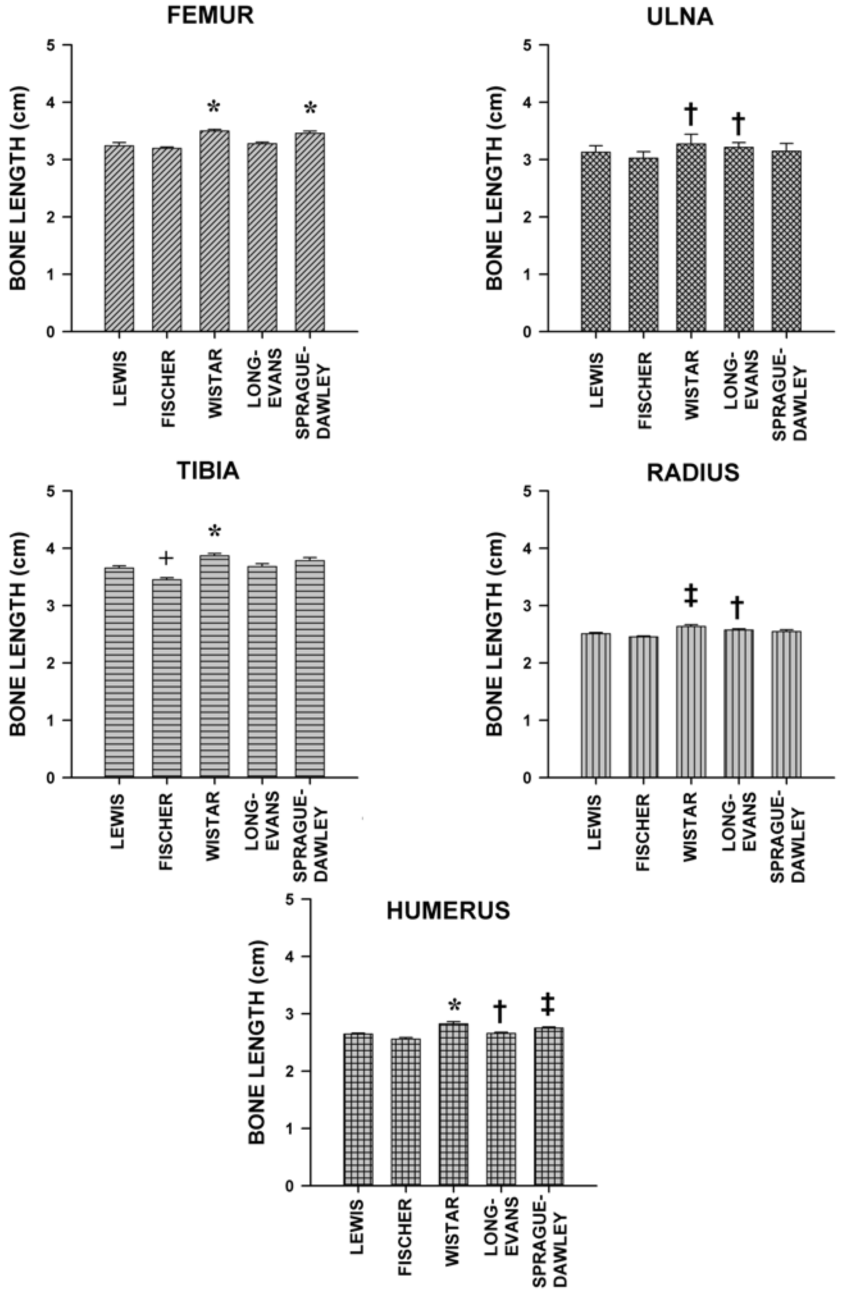


Fig. 5. 2 Bar graphs illustrating long bone length for each strain of rat. Fischer rats had significantly shorter long-bone lengths, whereas Wistar had longer bone lengths compared to many of the other strains. (*significantly different ($P<0.05$) from Fischer, Lewis, and Long-Evans; +significantly different from Sprague-Dawley, Long-Evans and Lewis; ‡significantly different from Fischer and Lewis; †significantly different from Fischer (n=11/strain))

5.4.2 Endpoint Measurements

5.4.2.1 Ladder Crossing

Fischer rats rate of footfall had a median value of 1% compared to 0.4% for Lewis and 0% for the remaining strains. No statistically significant differences were observed between strains of rats (H=6.79, 4 df, P=0.148) for the rate that their paws fell between the rungs of the ladder.

5.4.2.2 Forelimb support while rearing

No significant differences were determined between strains of animals for the percentage of time they used their right (H=4.24; 4 df; P=0.375), left (H=4.28; 4 df; P=0.37) or both limbs together (H=4.52; 4 df; P=0.34) for support while rearing against a plexiglass cylinder (Fig. 5.3). All strains tended to use there right and left limbs approximately 25% of the time while using both limbs together approximately 50% of the time. Examination of the total absolute number of times that each strain used their left, right and both limbs for use while rearing indicated a significant difference between strains ($F_{(4, 54)}$=10.7, P<0.001). Post-hoc analysis indicated that Wistar rats total forelimb activity was greater than Lewis (P<0.001), Fischer (P<0.001), and Sprague-Dawley (P=0.002), while Long-Evans rats used their limbs significantly more while rearing than Lewis (P<0.05) and Fischer (P<0.05) (strain (mean total number of foot placements +/- SEM): Wistar (94 +/- 5); Long-Evans (74 +/- 10); Sprague-Dawley (54 +/- 6); Fischer (42 +/- 7); Lewis (38 +/- 7)).

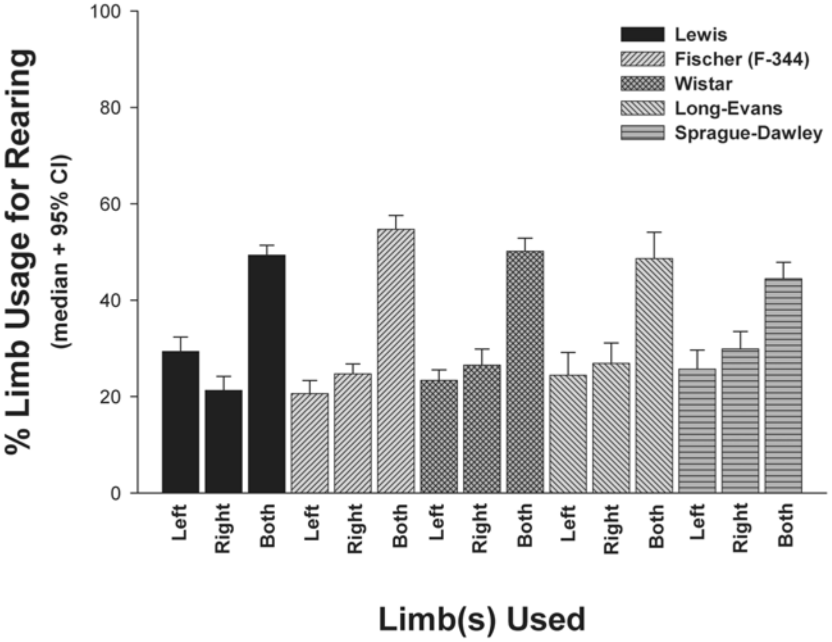


Fig. 5. 3 Percentage limb usage while rearing for each strain of rat. No statistical difference was observed between the different strains for paw preference while rearing against a vertical cylinder. All strains used each of their forelimbs independently approximately 25% of the time while using both limbs together the remaining 50% of the time.

5.4.2.3 Pedal sensitivity to mechanical stimuli

There was a statistical difference observed for thresholds to mechanical sensitivities between strains of rats ($F_{(4, 25)} = 4.74$, $P=0.005$). Fischer rats had significantly lower thresholds for mechanical paw stimulation compared to Lewis and Sprague-Dawley rats ($P<0.05$).

5.4.3 Kinematic Measurements

Kinematic measurements were made by measuring the angle between a line along the length of the 5th metatarsal bone and a line dropped perpendicular from the point of the hock (tibiotarsal joint) to the ground (Fig. 5.5A). Bilateral tibiotarsal angles were significantly larger for Fischer rats when compared to the other four strains ($P<0.001$ bilaterally)(Figs. 5.5A and 5.5B). There were no differences detected for any other strain of rat.

5.4.4 Kinetic Measurements

Ground reaction force determination combined with limb overlap data indicated that all strains of rats moved over the force plates at a trot (Figs.5.6.1 and 5.6.2). Trotting is considered a symmetrical gait where diagonal limb pairs hit the ground at approximately the same time (Figs. 5.6.1 and 5.6.2). These findings are consistent with previous reports of ground reaction forces of

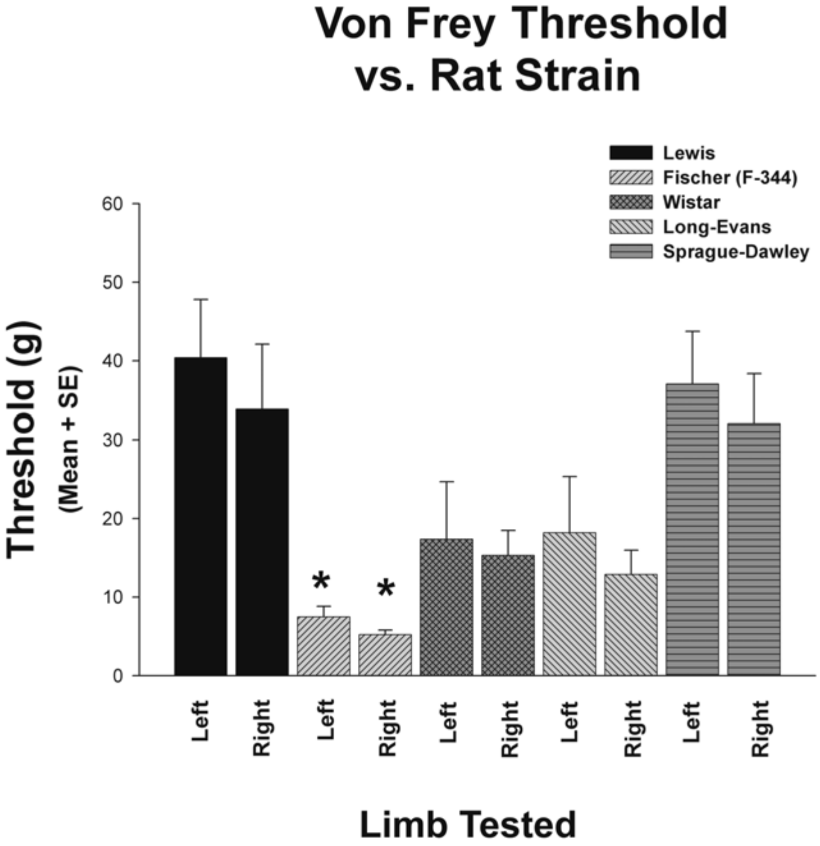


Fig. 5. 4 Threshold in grams of force necessary to elicit consistent foot withdrawal for each strain of rat. Fischer rats had significantly lower thresholds for mechanical paw stimulation compared to Lewis and Sprague-Dawley rats (n= 6/strain; * P<0.05).

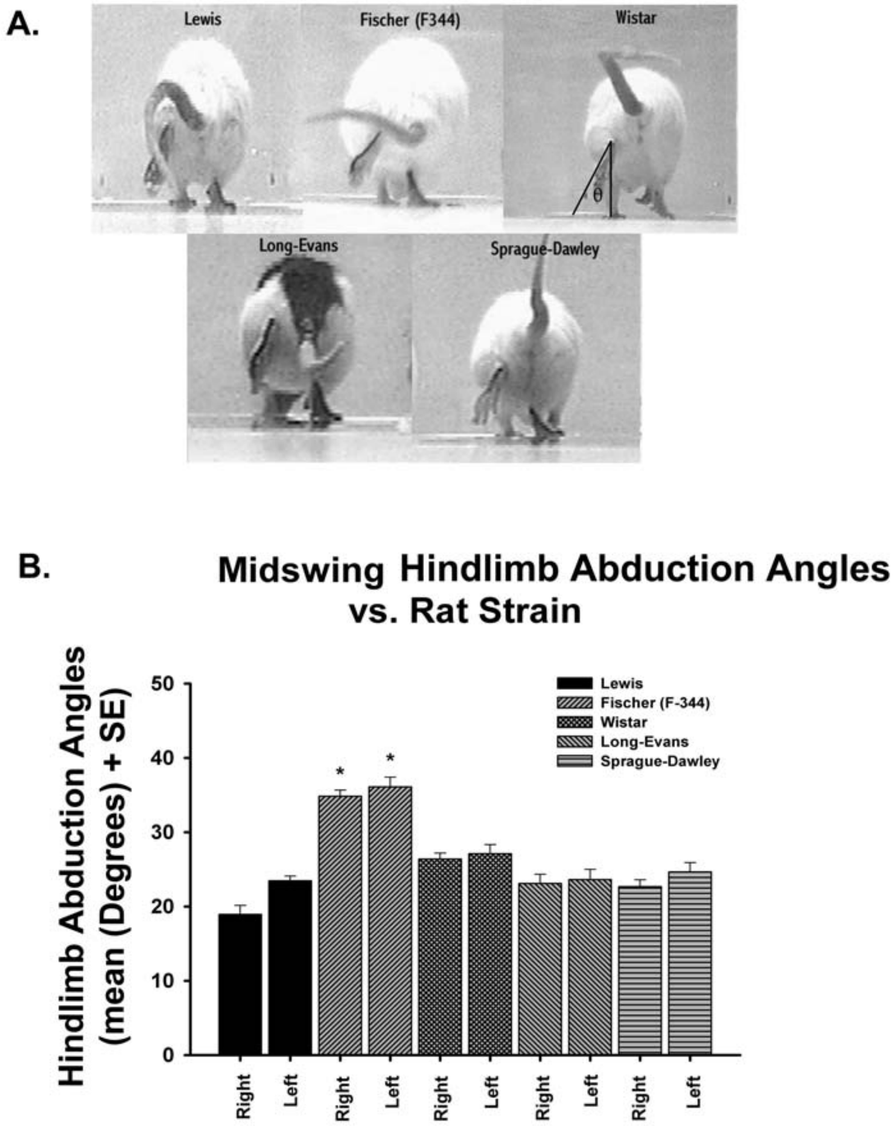


Fig. 5. 5 A) Representative video fields for each strain of rat as viewed from a caudal position. Distal hind limb abduction angles were measured at midswing while rats locomoted unrestrained across a runway for food reward. B) Hind limb abduction angles for each strain of rat. Fischer rats had significantly larger distal hind limb abduction angles compared with any other strain (n=11/strain, * P<0.001).

female Long-Evans rats. Summing the ground reaction forces for right and left limbs provides the total ground reaction forces acting on the body. For trotting animals, this involves summing ground reaction forces for diagonal limb pairs. There were no asymmetries observed from examining the total ground reaction force vs. time graphs (Figs. 5.6.1 and 5.6.2). Left and right limbs were used similarly and symmetrically for both forelimbs and hind limbs within each particular strain (Fig. 5.7).

Statistically significant differences were detected for Individual Limb Peak Vertical Force Symmetry for left ($F_{(4, 52)} = 13.8$; $P<0.001$) and right ($F_{(4,52)} = 11.9$; $P<0.001$) limbs (Fig. 5.7). Post-hoc analysis revealed this difference was due to Lewis rats having statistically higher peak vertical force ratios (forelimb:hind limb), bilaterally, compared to Sprague Dawley ($P<0.001$ left and right), Fischer ($P<0.001$ left and right) and Wistar rats ($P<0.024$ left, $P<0.023$ right). Long-Evans rats had higher peak vertical force ratios compared to Sprague-Dawley ($P<0.001$ left and right) and Fischer ($P<0.001$ left and right). These statistical differences occurred because Lewis and Long-Evans rats generated higher peak vertical forces with their forelimbs compared to their hind limbs, while Fischer and Sprague-Dawley rats generated smaller peak vertical forces with their forelimbs compared to their hind limbs (Fig. 5.7). Wistar (1.01 left ratio, 0.989 right ratio) rats generated similar peak vertical forces with their fore- and hind limbs. Overall, Lewis rats use their forelimbs more than their hind limbs for bearing body weight

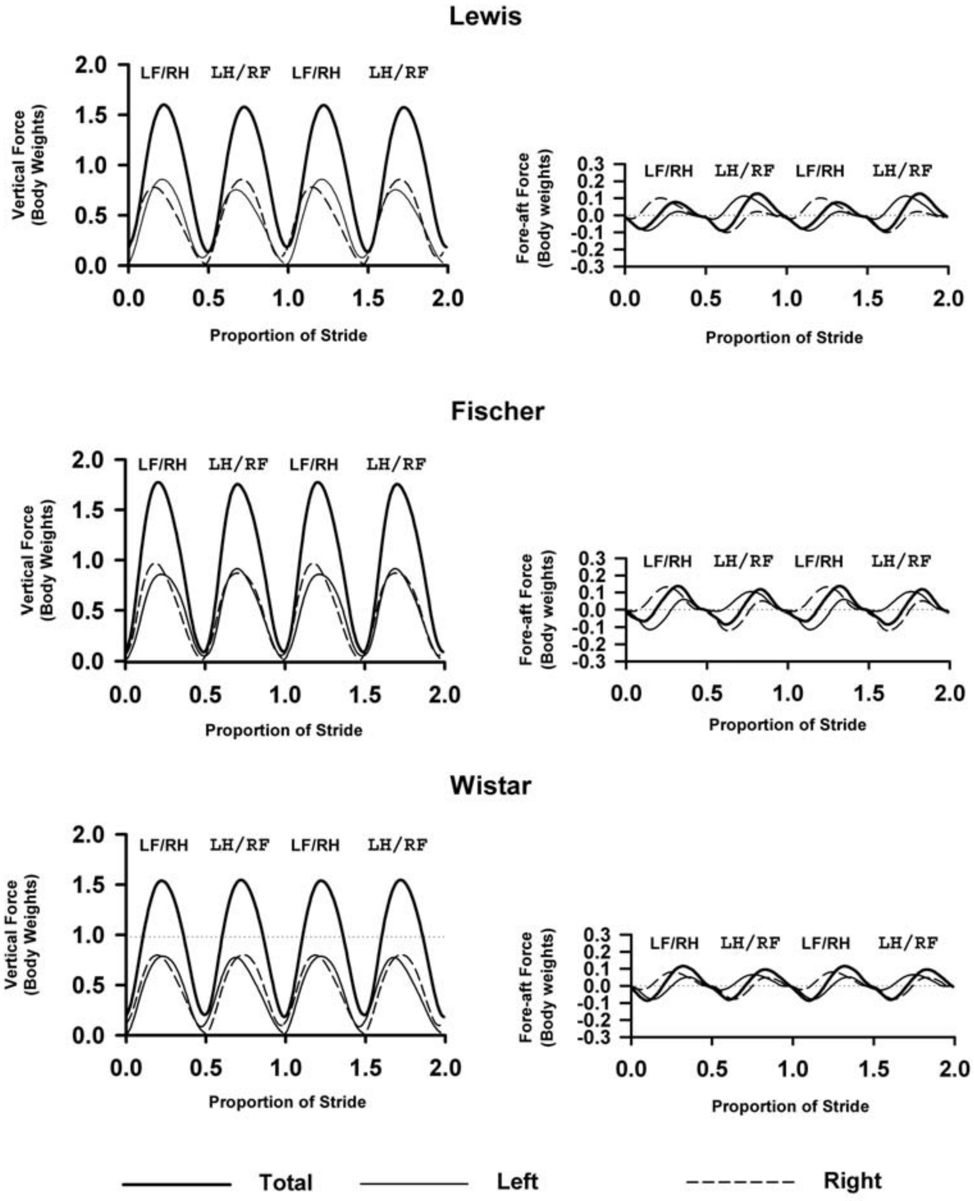


Fig. 5.6. 1 Total ground reaction forces (normalized to body weight) for Lewis, Fischer and Wistar rats. A) Summed ground reaction forces revealed that all strains of rats locomoted symmetrically while at a trot. Peak summed vertical forces were symmetrical between diagonal limb pairs for all strains (n=11 for Lewis, Fischer, Wistar (P=0.09)).

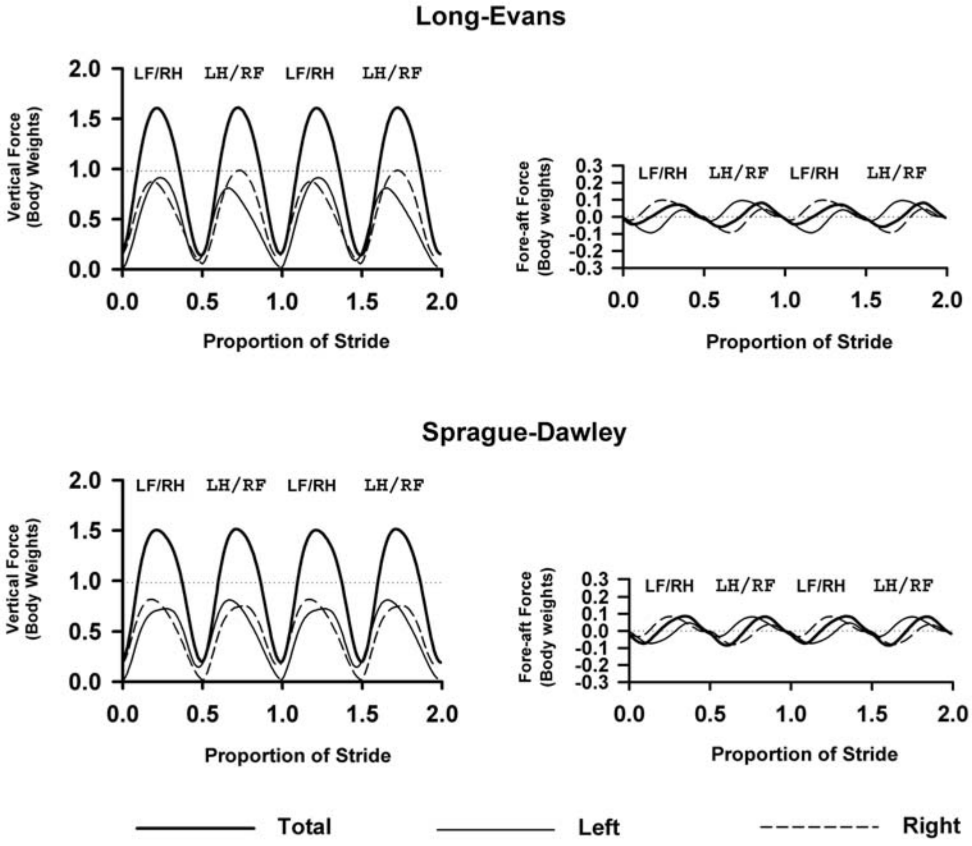


Fig. 5.6. 2 Total ground reaction forces for Long-Evans and Sprague-Dawley rats. B) Summed ground reaction forces revealed that all strains of rats locomoted symmetrically while at a trot. Peak summed vertical forces were symmetrical between diagonal limb pairs for all strains (n=11 for Long-Evans; n=9 for Sprague-Dawley (P=0.09)).

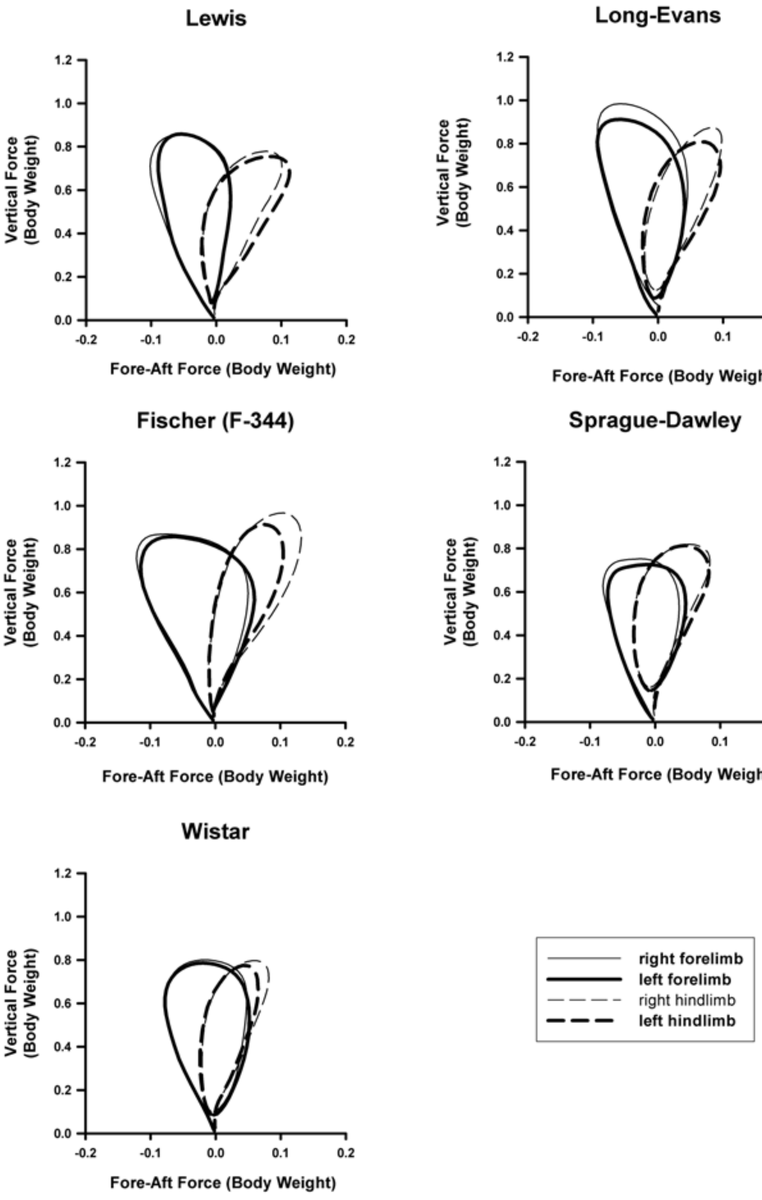


Fig. 5. 7 Vertical force versus fore-aft force for one stride (vector dynamogram) for each limb for each strain. Vector dynamograms for each strain illustrate the contribution of fore and hind limbs to braking and propulsion, and the asymmetry observed between relative weight bearing for fore and hind limbs during trotting. For all strains, forelimbs are used mostly for braking, while hind limbs are used mostly for propulsion. Lewis and Long-Evans bore more weight on their forelimbs while Sprague-Dawley and Fischer rats bore more weight on their hind limbs ($P<0.05$). Wistar rats distributed their weight evenly between the fore and hind limbs (n=11 for Fischer, Lewis, Long-Evans, Wistar; n=9 for Sprague-Dawley).

compared to Sprague-Dawley, Fischer and Wistar rats, and Long-Evans rats bear more weight on their forelimbs compared to their hind limbs than Sprague-Dawley and Fischer rats. Wistar rats tend to bear approximately 50% of their body weight on their forelimbs and 50% on their hind limbs.

Analysis of Individual Limb Peak Braking to Peak Propulsive Forces indicated that all rats have values greater than 1 for forelimbs and less than 1 for hind limbs (Fig. 5.7). Values greater than one indicate greater braking force while values less than one indicate greater propulsive force. Statistical differences were detected for hind limb Peak Braking to Peak Propulsive Forces bilaterally (($H=13.3$; 4 df; $P<0.05$ (left hind)) and ($H=12.8$; 4 df; $P<0.05$ (right hind)). Fischer rats had smaller Peak Braking to Peak Propulsive Forces ratios, and generated smaller braking forces, compared to Wistar and Sprague-Dawley rats (Fig. 5.7). No significant differences were detected between strains of rats for forelimb peak braking to propulsive ratios.

Analysis of Summed Peak Vertical Force Symmetry revealed no statistical difference between strains of animal ($H=1.22$; $df=4$; $P=0.875$). Median values for the strains of rats approached 1, indicating that nearly perfect symmetry was attained between diagonal limb pairs (Lewis = 0.992; Sprague-Dawley=0.983; Wistar=0.987; Fischer=0.991; Long-Evans=0.979).

Analysis of Net Peak Summed Fore-aft Force for one complete stride revealed no statistical difference between strains ($F_{(4,52)} =2.1$; $P=0.09$). Statistical power was lacking (increased likelihood of committing a type 2 statistical error), so there may actually be a difference, although one could not be detected. Mean values for each strain indicated that some rats may have been accelerating more than others (values above zero indicate net propulsive force, below zero indicate net braking force) ((Mean+/-SEM): Fischer=0.11+/-02; Long-Evan=0.07+/-0.04; Wistar=0.04+/-0.02; Lewis=0.03+/-0.03; Sprague-Dawley=0.01+/-0.02).

5.5 Discussion

Several recent papers have examined differences between strains of rats specifically pertaining to visual acuity, reaching, and spatial performance (Prusky et al. 2002; Harker and Whishaw 2002; VandenBerg et al. 2002). This, however, is one of the first studies which describes simultaneously the morphologic, sensorimotor and locomotor differences between different age-matched strains of rats. Fischer rats are much different in their morphology, sensory and locomotor abilities compared to the other four strains examined. Fischer rats are smaller in physical stature, have increased abduction of their distal hind limbs, and are more sensitive to mechanical stimulation of their hind limbs than Lewis and Sprague-Dawley rats. Interestingly, the amount of time each limb is used individually or together for stabilization during rearing appears to be conserved across all strains of rats examined. That is, rats tend to use each individual forelimb approximately 25% of the time while using both forelimbs together the remaining 50% of the time. Wistar and Long-Evans rats, however, tended to place their paws more frequently throughout the five minute testing period. Regardless of strain, rats are able to locomote across a horizontal ladder without slipping between ladder rungs. All strains prefer to use a trotting gait when traveling between 50 and 100 cm/s for food reward. Analysis of ground reaction forces during trotting reveal that Fischer and Sprague-Dawley rats bear more weight on their hind limbs while Long-Evans and Lewis rats tend to bear more weight on their forelimbs. Wistar rats bear weight evenly between their forelimbs and hind limbs during trotting. These morphologic, sensorimotor and locomotor differences have important implications for those evaluating sensorimotor behaviour in laboratory rats.

From simply observing each strain of rat in their cage, it is obvious that Fischer rats are smaller in physical stature compared to the other strains of rats, while Sprague-Dawley and Wistar rats appear to be larger. This observation is confirmed by our long-bone measurements and body weight measurements for each strain. Although body weight alone does not directly indicate the physical stature of an animal, in the present study prevention of obesity was ensured by restricting all rats food consumption so as to maintain a lean body weight. The importance of the observed physical stature differences is relevant to those assessing behaviours requiring apparatuses that are required to “fit” the particular strain of rat. For example, the Montoya staircase test (Montoya et al. 1991) (examines skilled reaching) requires a box to be made in such a way that the rat cannot turn around in the staircase apparatus and must use each limb independently for grasping pellets. A recent study examined the effect of strain on performance using the Montoya staircase test (Nikkhah et al. 1998). This study found that there were indeed differences between the strains of rats examined. Although this study did not determine what factors affected strain performance for the task, they did find that weight alone was not a factor contributing to the differences observed. Physical stature was not evaluated in their study, and considering that only one-size staircase apparatus was used for all strains, it could be that the strain-dependent differences they found were due to mismatched scaling of staircase apparatus to strain size. This study did not examine the reaching abilities of Fischer rats, but one might suspect that this strain would perform differently than other strains given its small physical stature. In another more recent study it has been shown that Fischer rats displayed different abilities in skilled reaching compared to Long-Evans rats (VandenBerg et al. 2002). Although the cage the animals were placed in was relatively large, the slot through which the animals had to grasp the food pellet was narrow and was kept constant for both strains examined. The qualitative and quantitative differences could be due, in part, to a mismatched scaling effect of the apparatus to the strain size.

The results I obtained for footslips while locomoting across a horizontal ladder are similar to those reported for uninjured Wistar (Metz and Whishaw

2002), Long-Evans (Metz and Whishaw 2002), and Sprague-Dawley (Soblosky et al. 2001) rats. Uninjured rats typically fall between ladder rungs less than 2-3% of the time regardless of the strain being used (Soblosky et al. 2001; Metz and Whishaw 2002) Although Metz and Whishaw described the locomotor abilities of Wistar and Long-Evans rats (Metz and Whishaw 2002) while performing locomotion over a horizontally placed ladder, this study did not comment on whether there were significant differences between the two strains of rats. It is interesting, however, that in my study Fischer rats fell through with a median rate of 1% compared to the other strains that fell between ladder rungs 0-0.4% of the time. Although a statistically significant difference was not detected for ladder footfalls, statistical power was lacking and it could be that Fischer rats fall through more often that the other strains. If this is the case rung spacing should be scaled according to the physical stature of the strain being examined. This could be an important consideration if direct comparisons are to be made between results of studies using different varieties of rats especially because these differences might be enhanced following induction of some form of neurological injury or condition.

The finding that rats use each individual limb for rearing approximately 25% for each right and left forelimbs, while using both forelimbs together approximately 50% of the time for rearing is consistent with the findings of others (Liu et al. 1999). This implies that comparisons of results from laboratories using different strains of rats can be made without concern for rat strain contributing to differences observed between laboratories. It appears as though symmetry in limb usage during exploratory behaviour has been preserved amongst different strains of rats. The results also provide evidence that the frequency of total limb usage over the five minute test period varies between strains. Greater frequencies of paw placement occurred in Wistar > Long-Evans > Sprague-Dawley > Fischer > Lewis strains. These differences may, in part, be due to differences in anxiety between the different strains of rats examined. For example, it has been shown that Fischer (F-344) rats behave more anxiously than Wistar rats (Rex et al. 1996; Rex et al. 1999; Bert et al. 2001). In addition, Fischer rats tend to rear less frequently than Wistar rats in an open field, and this difference is exacerbated with habituation (Bert et al. 2002). These differences may influence a researcher's decision of what strain to use when designing an experiment that incorporates this particular paw preference task.

In the present study, von Frey threshold values were significantly lower for Fischer rats when compared to Lewis and Sprague-Dawley rats. This implies that Fischer rats are more sensitive to mechanical stimulation of their hind limbs when compared to these two strains. The Lewis and Sprague-Dawley were less sensitive to von Frey stimulation compared to Fischer, and Wistar and Long-Evans appeared to be intermediate in their response to stimulation. These results are in disagreement with a study which examined percentage of paw withdrawals to a particular force of mechanical stimulation using Long-

Evans, Wistar, and Sprague-Dawley rats (Mills et al. 2001). In this earlier study, it was found that Long-Evans responded less frequently to a given mechanical stimulus than Wistar and Sprague-Dawley, whereas Wistar rats withdrew their limb more frequently than Long-Evans rats. These results imply that Long-Evans rats are less sensitive to mechanical stimulation compared to Sprague-Dawley and Wistar rats. The discrepancies between the present study and that of Mills et al. (Mills et al. 2001) may have resulted from the particular criteria used to evaluate sensitivity to mechanical stimuli. That is, in the present study, the minimum force necessary to elicit consistent paw withdrawal was determined. Mills et al. (Mills et al. 2001) determined the percentage of paw withdrawals to a given amount of force. The discrepancies between these two studies may also reflect differences between similar strains obtained from different sources. Alternatively, the observed von Frey threshold differences may reflect, in part, factors other than the sensory ability of a particular strain. For example, anxiety, freezing behaviour or habituation may contribute to the difference observed in the present study and the discrepancies between our study and Mills et al (Mills et al. 2001).

Examination of von Frey thresholds concurrently with ground reaction forces during trotting provides a possible explanation why Fischer rats are more sensitive to mechanical stimulation than the other strains. The ability to determine changes in an animal's physical environment is essential when considering the adjustments in balance and posture that must be made to meet the needs of traveling on different substrates. Ground reaction forces are similar between strains when comparing vertical force in body weights. However, one must keep in mind that Fischer rats weigh significantly less than the other strains examined. Consequently the forces they produce on the ground during locomotion are smaller. It is possible that Fischer rats require enhanced sensitivity to mechanical stimulation to determine changes in their physical environment. For example, if Fischer rats had equivalent tactile sensitivity to Sprague-Dawley rats, they may not detect changes in environmental substrates as readily and consequently they might be unable to make any necessary locomotor adjustments.

An interesting subjective observation made prior to conducting the current investigation was that Fischer rats standing in their cages had larger distal hind limb abduction angles. These rats do not stand as upright with their hind limbs when compared to the other four strains. It was postulated that this morphological difference would be preserved during locomotion and, indeed, Fischer rats locomote with their distal hind limbs laterally displaced more than the other strains examined. These results could have serious implications for those evaluating recovery following spinal cord injury. A popular method used to evaluate locomotor recovery following spinal cord injury is the Basso, Beattie, Bresnahan locomotor rating scale (BBB scale) (Basso et al. 1995). This scale was designed using Sprague-Dawley and Long-Evans rats. The BBB scale evaluates various components of the hind limbs during the recovery phase of thoracic spinal cord injury in adult rats. One of the features examined is the degree of inward or outward rotation of the paw during the late phases of recovery (Basso et al. 1995). In light of the present results, it is reasonable to consider that lateral deviation of the paw could be enhanced in spinal cord injured Fischer rats compared to other strains of rats. In addition, lateral deviation of the distal hind limb in Fischer rats may actually influence whether these rats will regain the ability to support body weight (another criteria for BBB scoring). Results of BBB scoring from studies using Fischer rats may possibly result in lower BBB scores compared to similar studies using other strains of rats. Lateral deviation of the hind limb of Fischer rats may be one of the factors contributing to the disparity between results in recent studies examining the therapeutic potential of olfactory ensheathing glia (Ramon-Cueto et al. 2000; Takami et al. 2002a) or the anti-inflammatory cytokine IL-10 (Takami et al. 2002b; Bethea et al. 1999).

Ground reaction force determination has been used to describe locomotion in a variety of species (Cavagna et al. 1977; Hodson et al. 2000; Riggs et al. 1993). Recently, this method of evaluation has been successfully used to evaluate locomotor abilities of spinal cord injured rats (Webb and Muir 2002a). Ground reaction force determination is a very sensitive method used to evaluate an animal's locomotor abilities. This form of evaluation could be used to determine the locomotor abilities of different strains of rats following various therapies for a variety of conditions. It is important to determine whether differences exist between different strains of healthy adult rats.

The present study provides evidence that the distribution of weight between fore and hind limbs differs between different strains of rats during trotting. Sprague-Dawley and Fischer rats bore more weight on their hind limbs compared to their forelimbs (implying a more caudally located body center of mass) while Lewis and Long-Evans rats bore more weight on their forelimbs (implying a more cranially located body center of mass) compared to their hind limbs during trotting. Wistar rats bore approximately 50% of their weight on their forelimbs and 50% on their hind limbs. This finding may have an impact on the ability of particular strains of rats to support their body following thoracic spinal cord injury. It has been shown that thoracic spinal cord injured rats bear more weight on their forelimbs compared to sham-operated controls indicating a cranial movement of their center mass (Webb and Muir 2002a). Strains which normally distribute more of their body weight on their forelimbs might compensate more quickly, and potentially locomote more effectively, following thoracic spinal cord injury, than those strains that normally bear more weight on their hind limbs.

Recently, it has been shown that body weight is distributed towards the forelimbs during deceleration and to the hind limbs during acceleration in trotting dogs (Lee et al. 1999). Pitching moments (nose-up or nose-down) due to fore-aft acceleration are balanced by redistributing vertical impulse (product of vertical force and time) and this method of balance seems to be important for cursorial animals such as dogs (Lee et al. 1999). In sprawling or crouched quadrupeds, such as rats, roll moments are equally important in maintaining balance (Lee et al. 1999). It could be argued that the differences observed between the strains of rats with regard to peak vertical forelimb to hind limb ratios is due to excessive acceleration or deceleration. In my study, however, the net peak fore-aft forces generated over one stride confirm that all strains were accelerating similarly, and although statistical power was lacking, there were no differences detected between the groups of animals.

5.6 Conclusions

The findings of the present study are consistent with those of previous studies which examine strain differences in rats. The present report indicates that researchers evaluating sensorimotor abilities of rats should be cognizant of the potential role of strain differences. These differences could account for discrepancies between laboratories and within a laboratory where more than one strain is used. Of importance, Fischer rats appear to be most dissimilar in their morphology, sensory and motor performances when compared to Lewis, Long-Evans, Sprague-Dawley and Wistar rats.

Chapter 6. BEHAVIOURAL RECOVERY AFTER CERVICAL SPINAL CORD HEMISECTION IN LEWIS, LONG-EVANS AND WISTAR RATS

6.1 Abstract

Rats are one of the most commonly used species in spinal cord injury research. Furthermore, many different strains are used. Sensorimotor behaviours, especially locomotion, are evaluated when determining whether a particular therapy is efficacious. Consequently, there is a need to determine whether different strains of rats behave similarly to one another following spinal cord injury. The present study set out to investigate whether adult female Lewis, Long-Evans, and Wistar rats behave similarly following unilateral cervical spinal cord hemisection. Sensorimotor evaluation consisted of a limb usage task, von Frey threshold testing, number of errors made during skilled locomotion, distal hind limb abduction angles during locomotion, and ground reaction force determination while trotting. Histology of the lesion epicenter of spinal cords for all rats of each strain confirmed uniformity of lesion size and tract involvement. Six weeks following spinal cord injury all rats from each strain behaved similarly. Injured animals 1) fell through the rungs of a ladder more often than before surgery, 2) used the forelimb

ipsilateral to the spinal cord injury less for support while rearing while using the contralateral forelimb more often for braking, 3) tended to have higher von Frey thresholds for the hind limb ipsilateral to the spinal cord injury, and 4) used the forelimb ipsilateral to the spinal injury less for braking and 5) developed a characteristic asymmetric gait. In conclusion, Lewis, Long-Evans, and Wistar rats behave similarly to unilateral cervical spinal cord hemisection. It appears that results of studies using these strains for unilateral cervical spinal cord injury research can be compared against one another without bias for the particular strain used.

6.2 Introduction

Rats have been used in laboratory research since the mid 1800s (Lindsey 1979). During this period of time numerous strains have been developed, used, and marketed to meet the specific needs of scientists. Since the introduction of the rat to the laboratory setting, the laboratory rat has become the most commonly used species in spinal cord injury research around the world. In part this has stemmed from economics (rats are cheaper to purchase and maintain than dogs or cats), animal welfare issues (generally more accepted to use rats vs. monkeys), knowledge pertaining to its physiology and neuroanatomy, and similarities that this species shares with humans (eg. manual dexterity).

Regardless of this species' popularity in spinal cord injury research, most of our knowledge pertaining to the neural control of sensorimotor tasks such as locomotion comes from work done using cats or dogs. Furthermore, little information pertaining to the sensorimotor, particularly locomotor, abilities of rats following unilateral spinal cord injury exists (McKenna and Whishaw 1999; Soblosky et al. 2001; Schallert et al. 2000; Ballermann et al. 2001; Webb and Muir 2002b). In the previous chapter it was shown that morphological, sensorimotor and locomotor differences exist between five different strains of rats (Webb et al. 2003). Consequently, the present study was undertaken to determine whether sensorimotor, especially locomotor, abilities of rats are similar between strains of rats with unilateral spinal cord injury. By using a battery of behavioural tests, the sensorimotor and locomotor abilities of rats with unilateral cervical spinal cord hemisection. The results are discussed in light of previously published findings and with respect to the significance for those evaluating behavioural recovery in rats following spinal cord injury.

6.3 Materials and Methods

6.3.1 Subjects

Eleven female rats of each of 5 different age-matched strains were obtained from Charles River Laboratories, Canada (Quebec, Canada). Animals were approximately 5 months of age. The strains used included: Fischer (CDF(F-344)/CrlBR); Lewis (LEW/CrlBR); Long-Evans (Crl:(LE)BR); Sprague-

Dawley (Crl:CD (SD)IGS BR) and Wistar (Crl:(WI)BR). Lewis and Fischer rats were developed as an inbred strain whereas Long-Evans, Sprague-Dawley and Wistar rats were developed as outbred strains. Animals were housed in pairs with 12 h light/12 h dark cycle in the laboratory animal care facility at the Department of Veterinary Biomedical Sciences, Western College of Veterinary Medicine at the University of Saskatchewan. All animals were examined daily, and were deemed healthy by a licensed veterinarian. All animals were cared for according to the standards set out by the Canadian Council on Animal Care.

6.3.2 Anesthesia, Analgesia, and Surgical Procedures

All animals underwent standard anesthesia, analgesia and cervical spinal cord hemisections as described in Sections 4.2 and 4.3 of this thesis.

6.3.3 Behavioural Analysis

All animals were assessed behaviourally using endpoint, kinematic and kinetic (ground reaction force) measurements. Endpoint measurements consisted of von Frey threshold determination, the number of footfalls made while crossing a ladder, and forelimb usage while rearing in a cylinder. Kinematic measurements consisted of determining the degree of hind limb abduction during locomotion. Kinetic measurements were determined by measuring ground reaction forces during locomotion. All measurements, excluding von Frey filament testing, were collected prior to surgery and again at 6 weeks following spinal cord injury. Von Frey thresholds were determined before surgery and again at 2, 4, and 6 weeks after surgery.

6.3.3.1 Endpoint Measurements

Endpoint measurements that were evaluated consisted of ladder crossing, forelimb support while rearing, and von Frey filament testing. These were conducted for all animals as described in Chapter 4.4.3.

6.3.3.2 Kinematic Measurement

Kinematic measurements consisted of evaluating distal hind limb abduction angles while locomoting as described in Chapter 4.4.4.

6.3.3.3 Kinetic Measurements

Kinetic measurements consisted of ground reaction force determination as described in section 4.4.5. Variables of the ground reaction forces that were examined statistically were determined as described in section 4.4.5.1.

6.3.4 Statistical Analysis

Two factor repeated measures analysis of variance was to be employed (using strain and time as factors) to determine differences between strains and to determine any interactions. Most of the data was non-parametric, however, and there is no non-parametric equivalent to use. Consequently, alternative statistical methods were used. These methods were chosen based on their ability to answer the particular questions asked.

To determine whether strains responded similarly to cervical spinal cord hemisection, presurgery data was subtracted from post-surgery data for each strain. If the data met the criteria for parametric data, differences between strains were determined using one-way Analysis of Variance with post-hoc analysis consisting of using Bonferroni's adjusted t-test. If criteria for parametric data was not satisfied, differences between groups were determined using Kruskall-Wallis test (Analysis of Variance on Ranks) with post-hoc analysis being completed using Dunn's method.

The paired t-test was used to determine if differences existed for behaviours before and after surgery within each strain for data obtained from the forelimb usage task, ladder task, distal hindlimb abduction angles, and ground reaction forces. In the case of von Frey filament testing, however, one-way repeated measures analysis of variance was used to determine differences over time for each strain. Differences between left and right limbs, when appropriate, were determined using Student's t-test within each time point for each strain.

6.3.5 Histology

Histological processing and staining was done at 6 weeks following spinal cord injury as described in Section 4.5 of the thesis.

6.4 Results

Unfortunately, insufficient data could be obtained from Fischer (F-344) and Sprague-Dawley rats during the post-operative period. This was in part due to shorter stride length following spinal cord injury (especially in Fischer rats), uncooperative behaviour of these strains, and because many of these animals developed severely impaired ability of the forelimb ipsilateral to the spinal cord injury. These animals held their forelimb backward in extension and were consequently unable to locomote or rear without falling. Electromyograms of the appendicular muscle of some of these animals (when anesthetized) failed to demonstrate fibrillation potentials or positive sharp waves. It remains unknown why these animals such impaired forelimb abilities.

6.4.1 Histology

Only animals with nearly complete unilateral cervical spinal cord hemisection were included in this study. Animals had to have complete damage to their lateral funiculus and at least 50% damage to each of the dorsal and ventral funiculi. All animals included in the subsequent behavioural analysis had similar regions of their left cervical spinal cord injured. Sparing of the left medial dorsal column and left ventral funiculus was consistent amongst all animals within each strain (Fig. 6.1).

6.4.2 Ladder Crossing

All strains of rats fell through the rungs of the ladder significantly more often following cervical spinal cord hemisection ($P<0.05$; Fig. 6.2). No differences in how the strains behaved to cervical spinal cord hemisection were detected.

6.4.3 Forelimb usage

Analysis of forelimb usage while rearing revealed that all strains of rats used their left forelimb significantly less while rearing after left cervical spinal cord hemisection ($P<0.05$ for all strains) (Fig. 6.3). In addition, all strains used the right forelimb more after spinal cord injury ($P<0.05$ for all strains)(Fig. 6.3). Long-Evans rats, however, used both forelimbs less for stabilization while rearing after injury ($P=0.001$). When postsurgery values were subtracted from presurgery values, and strains were compared against each other ($P>0.05$).

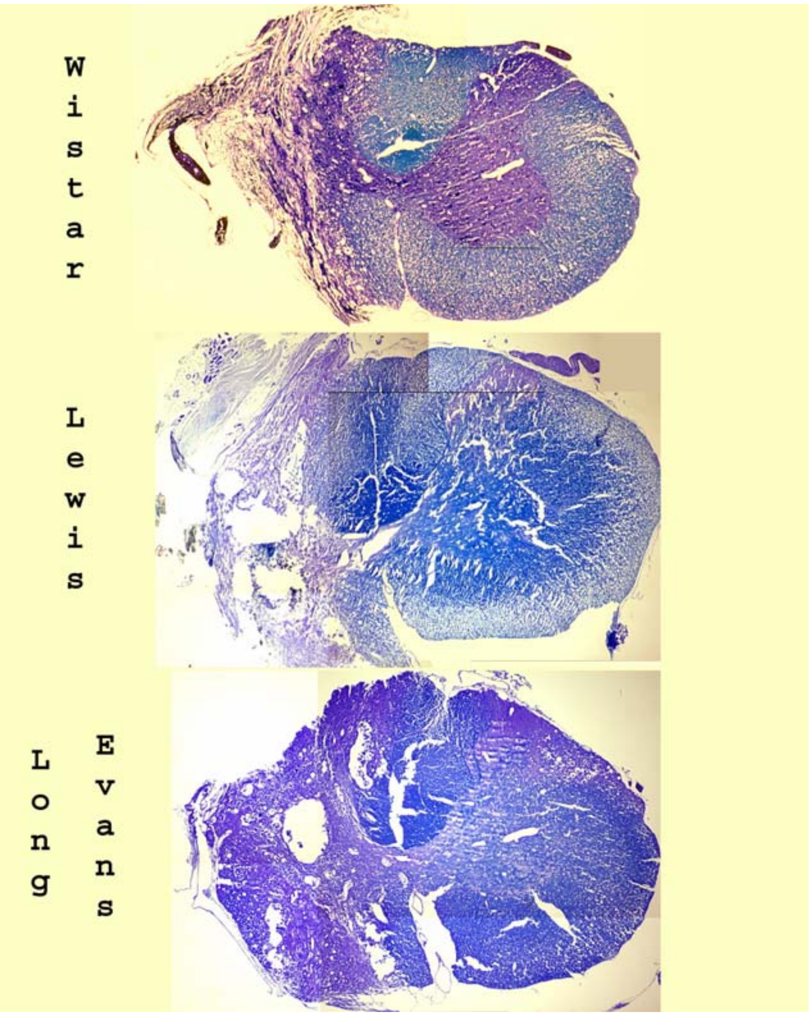


Fig. 6. 1 Photomicrographs of representative lesion epicenters from Wistar, Lewis and Long-Evans rats. Note there was some sparing of the medial dorsal columns and ventral funiculus, although this was similar for animals in each strain.

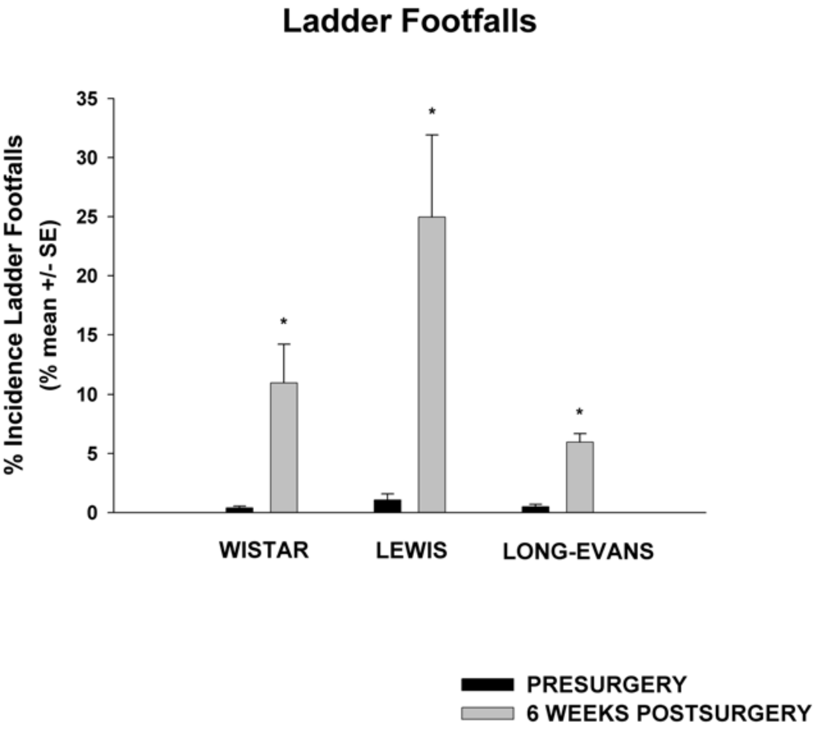


Fig. 6. 2 Percent incidence of footfalls while performing the ladder task. All rats had significantly more footfalls following cervical spinal cord hemisection ($P<0.05$) ($n=11$ for all strains preoperatively; Wistar $n=7$, Lewis $n=11$, Long-Evans $n=8$ postoperatively).

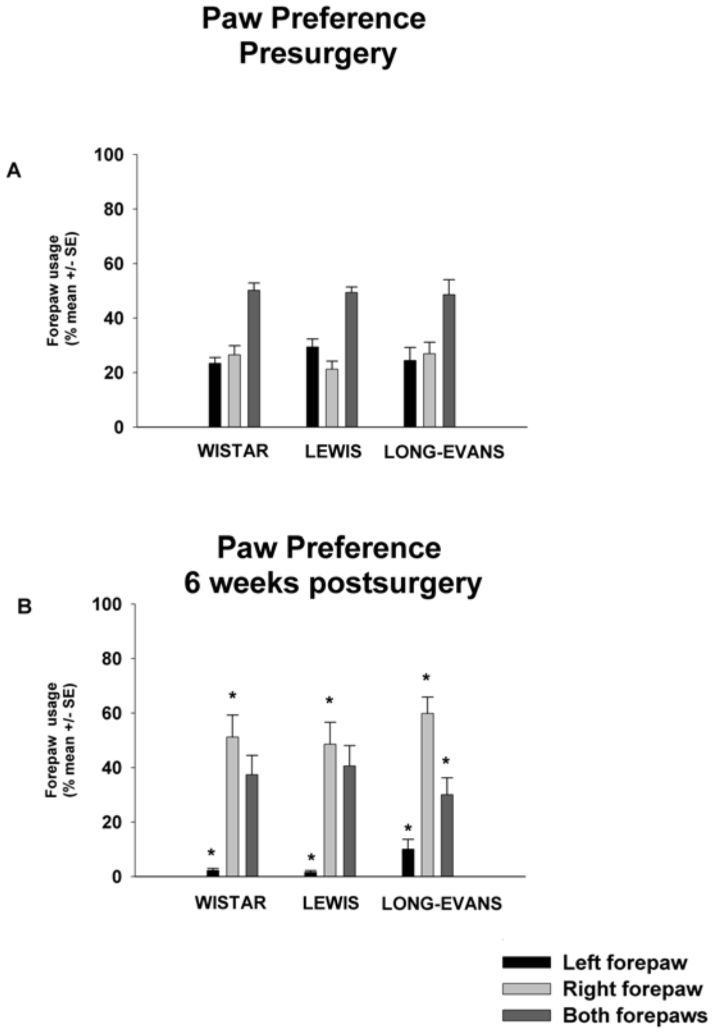
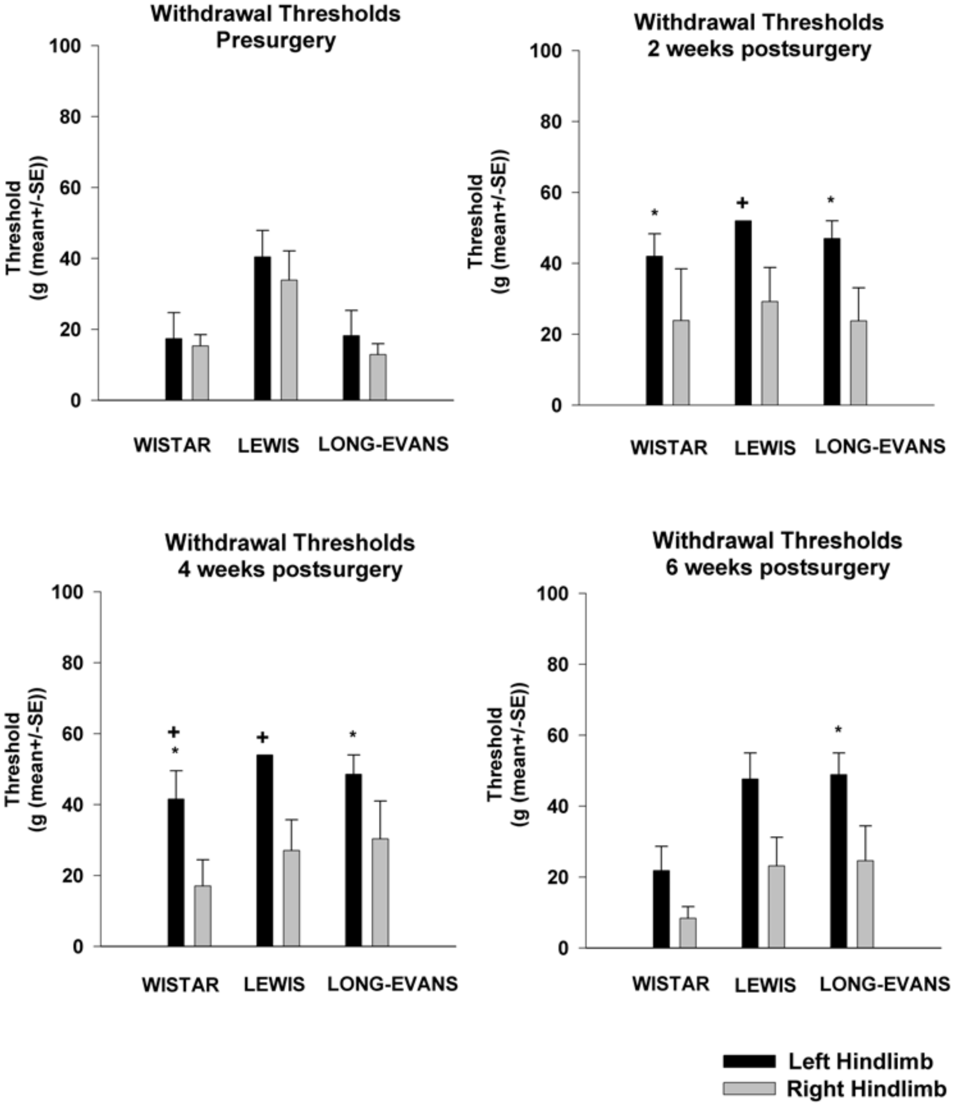


Fig. 6. 3 Paw usage while rearing against a vertical surface before (A) and after (B) spinal cord injury. All rats, regardless of strain, used the left (ipsilateral to injury) forelimb less for rearing and the right (contralateral to injury) forelimb more for rearing following unilateral left cervical spinal cord hemisection. Long-Evans rats used both forelimbs together significantly less while rearing compared to before surgery ((n=11 for all animals before injury; Wistar n=7, Lewis n=11, Long-Evans n=10 postsurgery) (*=significantly different from corresponding presurgery value, $P<0.05$).

Lewis rats were found to use the left forelimb significantly less than either Long-Evans or Wistar following cervical spinal cord hemisection ($F_{2, 27}=4.9$, $P<0.05$; $P<0.05$ post hoc) (Fig. 6.3B).

6.4.4 von Frey Thresholds

Long-Evans and Wistar rats had significantly higher von Frey thresholds for their left hind limb at all time points after spinal cord injury ($P<0.05$). The trend indicated that Lewis rats too had higher thresholds for the left hind limb, although statistical power was lacking. Analysis of left versus right thresholds for each strain within each time period revealed that Lewis rats had significantly higher von Frey thresholds for their left hind limb at 2 and 4 weeks after spinal cord injury, while Wistar rats had significantly higher thresholds for the left (ipsilateral to lesion) hind limb at 4 weeks following injury (Fig. 6.4). The general trend indicated that all strains of rat had typically higher thresholds for the left hind limb at all time points after spinal cord hemisection, although statistical power was lacking (Fig. 6.4). No significant differences were detected between strains when comparing von Frey threshold differences over time (presurgery value minus postsurgery time point value). That is, all strains behaved similarly following cervical spinal cord hemisection.


Withdrawal Thresholds Presurgery

Withdrawal Thresholds 2 weeks postsurgery

Withdrawal Thresholds 4 weeks postsurgery

Withdrawal Thresholds 6 weeks postsurgery

Fig. 6. 4 Von Frey thresholds for different strains of rats presurgery, and at 2, 4, and 6 weeks post-surgery. Animals tended to have significantly higher thresholds for the hind limb ipsilateral to the spinal cord injury (left) (n=6 for all strains pre and post-operatively) (*=significantly different from presurgery values – $P<0.05$; +=significantly different from contralateral limb – $P<0.05$).

6.4.5 Distal Hindlimb Abduction Angles

No statistical differences were detected between right and left hindlimb abduction angles within each strain pre- or postsurgery ($P>0.05$). No differences in how the right and left abduction angles changed following cervical spinal cord hemisection were detected between strains (right hind limb: $F_{2,24}=1.3$, $P>0.05$, left hind limb: $F_{2,25}=2.0$, $P>0.05$).

6.4.6 Ground Reaction Forces

Ground reaction force analysis revealed that all strains of animals used their limbs symmetrically prior to cervical spinal cord hemisection (Fig. 6.5A, 6.6A, 6.7A). In addition, all strains of animals trotted symmetrically before surgery (Fig. 6.8). All strains used their limbs similarly after left cervical spinal cord hemisection, although Wistar rats (Fig. 6.7B) appeared to use their right and left limbs equally for weight bearing compared to Lewis (Fig. 6.5B) and Long-Evans (Fig. 6.6B). Long-Evans rats bore less weight on their left (ipsilateral to injury) forelimb compared to the right forelimb ($P<0.05$). Although not statistically significant (power was lacking), Lewis rats also tended to bear less weight on their left forelimb after spinal cord injury. Lewis rats bore less weight on their left (ipsilateral to injury) hindlimb than the right hind limb ($P<0.05$). Long-Evans rats also tended to behave similarly, although statistical significance was not detected (power was lacking). Lewis and Long-Evans rats used their left (ipsilateral to injury)

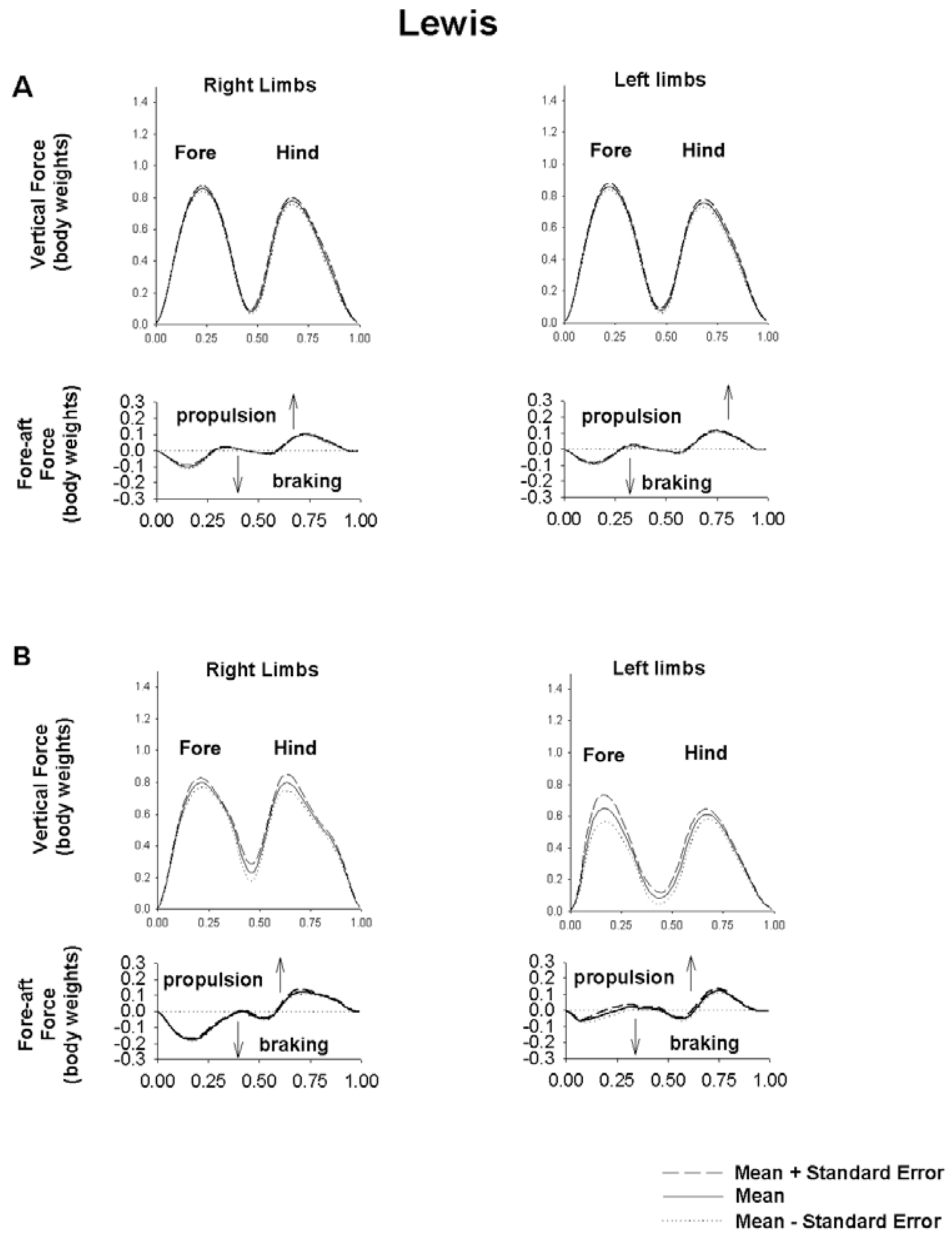


Fig. 6. 5 Individual limb ground reaction forces for Lewis rats A)pre- and B)6 weeks post-operatively. Notice the impaired braking ability of the left (ipsilateral to injury) forelimb and increased braking ability of the right forelimb. Also notice that Lewis rats tend to bear less weight on their left limbs compared to their right limbs post-operatively (n=11 preoperatively; n=6 postoperatively).

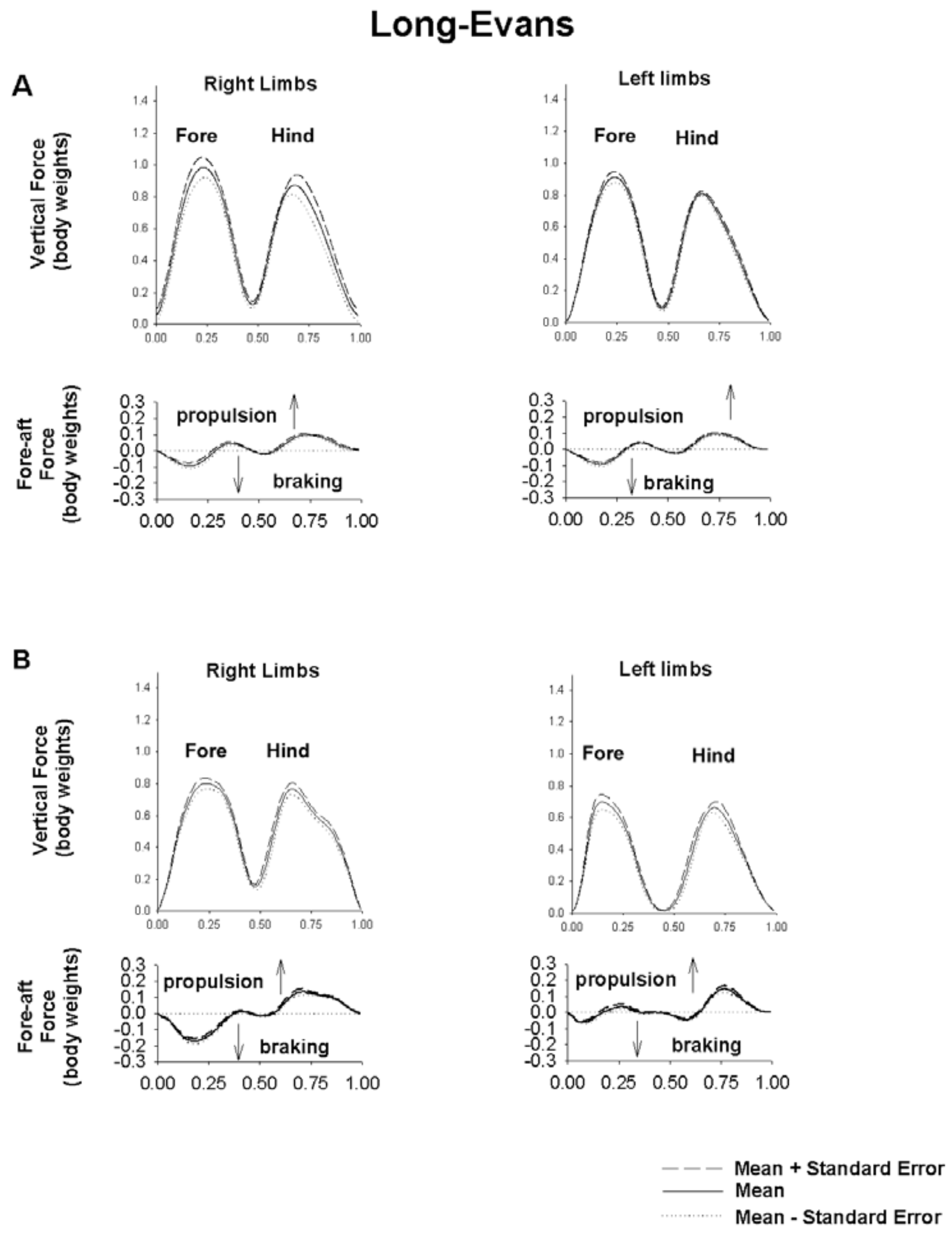


Fig. 6. 6 Individual limb ground reaction forces for Long-Evans rats A)pre and B)6 weeks post-operatively. Notice the impaired braking ability of the left (ipsilateral to injury) forelimb and increased braking ability of the right forelimb. Also notice that Long-Evans rats tend to bear less weight on their left limbs compared to their right limbs post-operatively (n=11 presurgery; n=9 post-operatively).

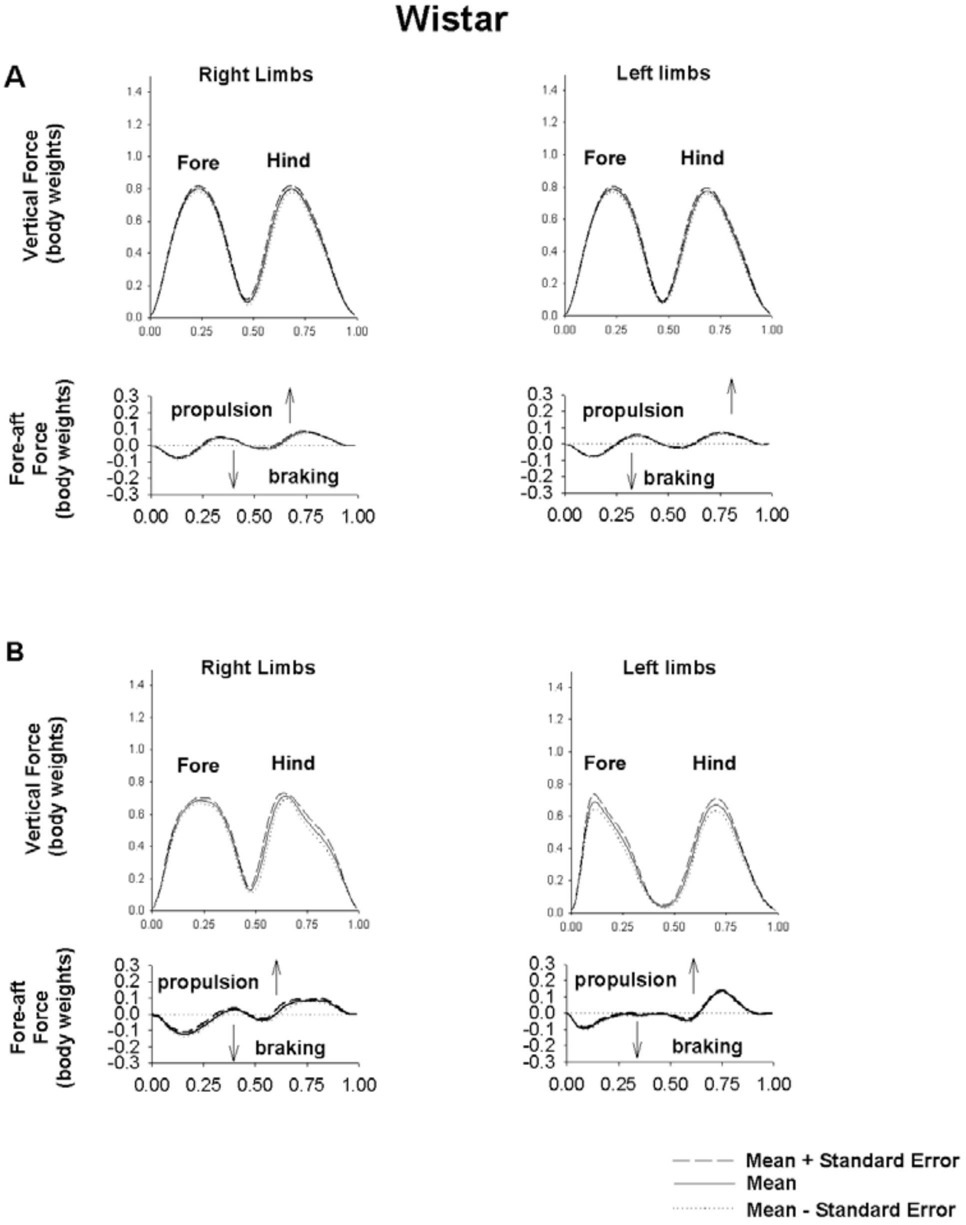


Fig. 6. 7 Individual limb ground reaction forces for Wistar rats A)pre and B)6 weeks post-operatively. Notice the impaired braking ability of the left (ipsilateral to injury) forelimb (n=11 presurgery; n=7 post-operatively).

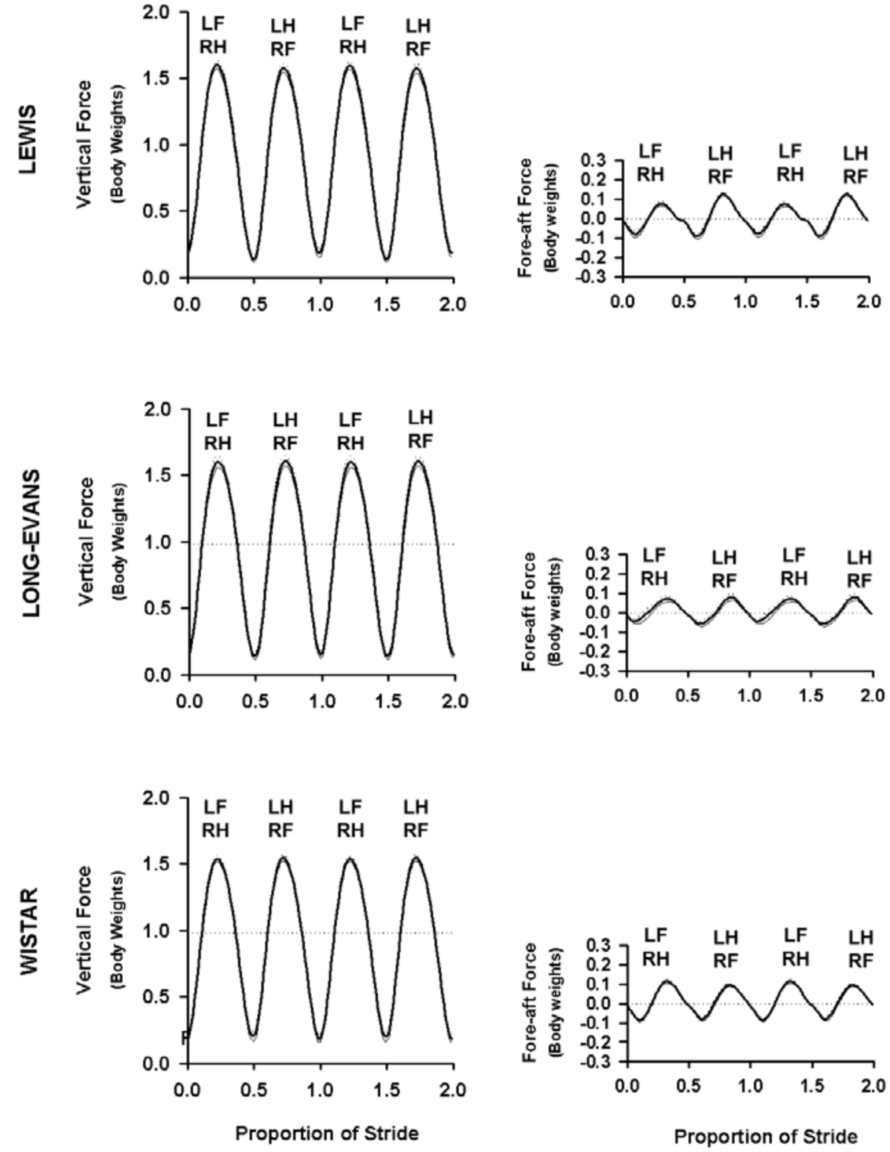


Fig. 6. 8 Summed ground reaction forces for uninjured Lewis, Long-Evans and Wistar rats. Notice that these animals are trotting symmetrically (n=11 for all strains presurgery; Lewis n=6, Long-Evans n=9, Wistar n=7 postoperatively). Fine solid lines = mean - SEM; dotted lines = mean + SEM; bold solid lines = mean.

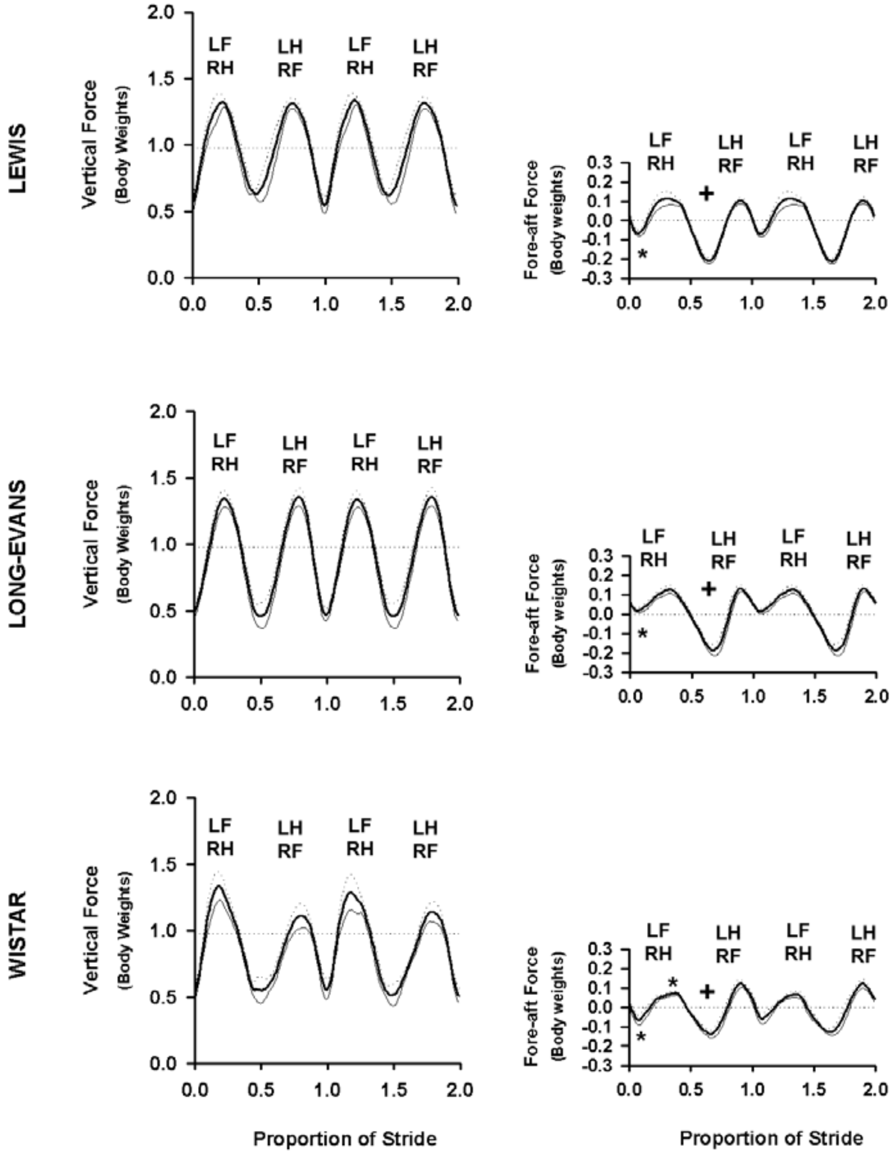


Fig. 6. 9 Summed ground reaction forces for Lewis, Long-Evans, and Wistar rats 6 weeks following cervical spinal cord hemisection. Notice that all strains use the left (ipsilateral to injury) forelimb/right hind limb less for braking (*) than the left (ipsilateral to injury) hind limb/right forelimb. Also note that the left hind limb/right forelimb pair are used more for braking (+) compared to before surgery for all strains (n=11 for all strains presurgery; Lewis n=6, Long-Evans n=9, Wistar n=7 postoperatively). Fine solid lines = mean - SEM; dotted lines = mean + SEM; bold solid lines = mean.

forelimb less for braking than the right forelimb (P<0.05). Long-Evans rats used their left hind limb more for braking than the right hind limb after spinal cord injury (P<0.05).

Analysis of summed ground reaction forces revealed that after spinal cord injury all strains produced more braking force with the left hind/right fore diagonal limb pair after cervical spinal cord hemisection (Fig. 6.9). This alteration was most pronounced in Long-Evans rats compared to the other strains examined. Long-Evans rats produced less braking force with the left fore/right hind diagonal limb pair after injury. All strains used the left fore/right hind diagonal limb pair less for braking than the left hind/right fore diagonal limb pair after cervical spinal cord hemisection (P<0.05 for all strains) (Fig. 6.9). In addition, Wistar rats used the left fore/right hind diagonal limb pair less for propulsion than the left hind/right fore diagonal limb pair (Fig. 6.9).

6.5 Discussion

The present study has demonstrated that Lewis, Long-Evans, and Wistar rats behave similarly following unilateral left-sided cervical spinal cord hemisection. These results are in agreement with a recent study that grossly assessed locomotor recovery in Long-Evans, Sprague-Dawley and Wistar rats with thoracic spinal cord hemisection (Mills et al. 2002). The present study demonstrated that animals fell between rungs of a ladder more often 6 weeks after spinal cord hemisection. Cervical spinal cord hemisection also results in decreased usage of the forelimb ipsilateral to the injury for support while rearing, but increases the usage of the contralateral forelimb while rearing. These animals also tend to have higher von Frey thresholds for the hind limb ipsilateral to the spinal cord injury. In addition, Lewis and Long-Evans rats tended to bear less weight on the fore and hind limbs ipsilateral to the spinal cord injury during locomotion. All strains used the forelimb ipsilateral to the injury less for braking compared to before surgery and developed a characteristic gait while locomoting 6 weeks following spinal cord injury.

No differences have been detected for the number of footfalls made during skilled locomotion in uninjured Lewis, Long-Evans, and Wistar rats. All animals in the present study responded similarly to cervical spinal cord hemisection. Specifically, animals tended to fall through the rungs of a ladder more frequently after cervical spinal cord hemisection. These results are in agreement with a study investigating unilateral cervical spinal cord contusion injury in adult female Sprague-Dawley rats (Soblosky et al. 2001). Soblosky also found that as the extent of spinal tracts injured increase so does the number of footfalls while rats cross a horizontally placed ladder.

Rats typically use their forelimbs for support and stabilization while rearing against a vertical surface. Uninjured rats, regardless of strain, typically use each forelimb individually for support 50% of the time while using both forelimbs together the remaining 50% of the time (Webb et al. 2003). This exploratory behaviour has been used to describe the forelimb motor abilities of rats following various models of cervical spinal cord injury (McKenna and Whishaw 1999; Liu et al. 1999; Soblosky et al. 2001; Webb and Muir 2002b). Like many of these studies, the present study found that unilateral cervical spinal cord injury results in decreased use of the forelimb ipsilateral to the spinal cord injury. Subsequent chapters in this thesis indicate that the rubrospinal tract and ascending dorsal column pathways are important for animals to perform this task, while lesioning of the pathways traveling in the ventrolateral funiculus do not produce long-lasting behavioural changes. In addition, animals typically use the forelimb contralateral to the spinal cord injury more than before surgery. This finding too is in agreement with those of others (Soblosky et al. 2001; Schallert et al. 2000; Liu et al. 1999). Regardless, Lewis, Long-Evans, and Wistar rats appear to behave similarly to cervical spinal cord hemisection with respect to forelimb usage while rearing.

Von Frey filaments are commonly used to evaluate sensory function in rats (Pitcher et al. 1999). In the previous chapter it was shown that not all rats have similar von Frey thresholds. In the present study and in future chapters of this thesis, it has and will be demonstrated that von Frey thresholds tend to be increased for the hind limb ipsilateral to the spinal cord injury both compared to the contralateral hind limb and compared to presurgery values. Although statistical significance was not detected for each strain, the trend indicated a similarity in response to von Frey thresholds between strains. These increased threshold responses are could be a manifestation of impaired sensory and motor function to the limb ipsilateral to the spinal cord injury and not simply because of impaired sensory function to the limb. As previously mentioned in the first chapter of this thesis (see Section 1.2.2), both the post-synaptic dorsal column pathway and the dorsal column medial lemniscus pathway ascend within the ipsilateral spinal cord to synapse on the dorsal column nuclei in the brain. Injury to these pathways may have been partially responsible for sensory loss. The rubrospinal tract, however, is one motor system involved with flexion. Injury to this pathway may have resulted in an impaired flexor response, a motor behaviour required to assess von Frey filament threshold. As is readily apparent, injury to both the sensory and motor systems could explain the increase in von Frey threshold.

Long-Evans rats bore less weight on the limbs ipsilateral to the side of the spinal cord injury. Statistical power was lacking for Lewis rats, however, and it could be that these animals too bore less body weight with the limbs on the ipsilateral side of the spinal cord injury. Ground reaction force determination revealed that animals with unilateral cervical spinal cord hemisection develop a characteristic gait and use the forelimb ipsilateral to the spinal cord less for braking regardless of strain. The lack of braking ability of the forelimb ipsilateral to the spinal cord injury is reminiscent of rats with unilateral pyramidal tract and red nucleus ablation (Muir and Whishaw 1999a; Muir and

Whishaw 2000). Although injury to the corticospinal tract leads to impaired braking early after recovery these animals tend to recover completely by one week following injury Muir and Whishaw 1999a). Consequently, the corticospinal tract seems unlikely to be contributing to the impaired braking seen in the animals of the present study. Red nucleus ablation, however, results in long-lasting impairments in the braking ability of the contralateral forelimb (Muir and Whishaw 2000). This suggests that the rubrospinal tract is important in overground locomotion. Another potentially important pathway that may have resulted in impaired braking ability of the ipsilateral forelimb in rats of the present study could be the vestibulospinal tract. The vestibulospinal tract has been shown to have a net excitatory effect on limb extensors (Orlovsky 1972c). As well, neurons from the lateral vestibular nucleus are most active during the stance phase of locomotion in cats (Matsuyama and Drew 2000a). The lack of excitatory input to limb extensors from the vestibulospinal tract. Further discussions exploring the potential reasons for impaired braking ability of this limb are provided in Chapter 7. The characteristic gait of cervical spinal cord hemisected rats also resulted in impaired braking while the forelimb ipsilateral and the hind limb contralateral to the spinal cord injury were on the ground simultaneously. This was due to the fact that these animals had severely altered braking ability of the forelimb ipsilateral to the spinal cord injury. Conversely, the contralateral forelimb and ipsilateral hind limb produced significantly higher braking forces during locomotion. This is most likely a manifestation of behavioural compensation.

That is, the animals had to brake more with this diagonal limb pair to compensate for the impaired braking ability of the other diagonal limb pair. This would ensure that the animals could travel at a relatively constant velocity. Again, Lewis, Long-Evans and Wistar rats develop a similar gait following cervical spinal cord hemisection.

6.6 Conclusions

The chapter indicates that adult female Lewis, Long-Evans, and Wistar rats develop similar sensorimotor, especially locomotor, abilities following unilateral cervical spinal cord hemisection. These results imply that comparisons between laboratories using these strains, following unilateral cervical spinal cord hemisection, can be made without concern for strain effects.

Chapter 7. COMPENSATORY LOCOMOTOR ADJUSTMENTS OF RATS WITH CERVICAL OR THORACIC SPINAL CORD HEMISECTIONS

7.1 Abstract

The accurate measurement of behavioural compensation after central nervous system trauma, such as spinal cord injury, is important when assessing the functional effects of injury and treatment in animal models. The locomotor abilities of Long-Evans rats with unilateral thoracic or cervical spinal cord injuries were evaluated using a locomotor rating (BBB) scale, reflex tests, and quantitative kinetic measurements. The BBB rating scale indicated that thoracic spinal hemisected rats had more severely affected hindlimbs compared to cervical spinal hemisected and sham-operated animals. Kinetic measurements revealed that cervical and thoracic spinal hemisected animals moved with different ground reaction force patterns which nevertheless shared some similarities with each other and with the gait patterns of rats with different unilateral CNS lesions. Uninjured rats typically had an equal distribution of their body weight over the forelimbs and hind limbs, and used their forelimbs predominantly for braking while using their hind limbs mostly for propulsion. Cervical spinal hemisected rats bore more weight on their hind limbs than their forelimbs while thoracic spinal hemisected animals bore more weight on their forelimbs than their hind limbs. Neither cervical nor thoracic spinal cord hemisected rats used the forelimb ipsilateral to the spinal hemisection for net braking or propulsion. The hindlimb contralateral to the

hemisection was placed on the ground prematurely during the stride cycle for both cervical and thoracic spinal hemisected animals. The altered kinetics of the locomotor pattern in hemisected animals resulted in changes in the oscillations of total body potential and kinetic energies. These two forms of energy oscillate synchronously in intact locomoting rats, but were asynchronous during parts of the stride cycle in spinal hemisected animals. It is concluded that rats develop locomotor adjustments in response to unilateral spinal cord injury, regardless of the rostro-caudal location, which may help stabilize the animal during locomotion.

7.2 Introduction

Traumatic spinal cord injury afflicts 17-50 per million people annually (Chen et al. 1997; Karamehmetoglu et al. 1997; Hoque et al. 1999; National Spinal Cord Injury Statistical Center 2000; Surkin et al. 2000). Although the incidence is low, many people are afflicted at less than 40 years of age (Chen et al. 1997; Karamehmetoglu et al. 1997; Hoque et al. 1999; National Spinal Cord Injury Statistical Center 2000; Surkin et al. 2000), and consequently the prevalence of spinal cord injury is high. There are approximately 250 000 people living with spinal cord injury in the United States alone (Ergas 1985; National Spinal Cord Injury Statistical Center 2000). Because of the high demand for a cure and for better treatments for spinal cord injury, there has been a great amount of effort to assess the benefits of treatments on behavioural motor recovery, including locomotor recovery. Most fundamental knowledge regarding the neural circuitry controlling overground locomotion has been obtained from a variety of cat preparations. Only recently has there been an interest for a more complete understanding and integration of knowledge concerning neural control mechanisms and responses to particular spinal cord injuries in locomoting rats, as these are popular animals used by researchers studying spinal cord injury. Much of the knowledge gained from research in cat models of locomotion has been adopted and applied to spinal cord injury in the rat. These assumptions may not be valid for a variety of reasons which include 1) species differences in neuroanatomy; and 2) morphometric differences that exist between rats and more cursorial animals such as cats. Consequently, there is a demand for novel findings regarding the locomotor abilities in spinal cord injured rats which may provide valuable insight into the roles of different neuroanatomical pathways during overground locomotion in the rat and their response to spinal cord injury.

Terrestrial locomotion of quadrupedal species involves both a swing phase (when the foot is not in contact with the ground) and a stance phase (when the foot is in contact with the ground). There are very few studies investigating the compensations rats make during the stance phase of the locomotor cycle (Kunkel-Bagden et al. 1992; Muir and Whishaw 1999a; Muir and Whishaw 2000). Importantly, the forces that the animal exerts on the ground during the stance phase provide unique, objective and quantitative information regarding compensations animals make for CNS injury (Muir and Whishaw 1999b; Muir and Whishaw 1999a; Muir and Whishaw 2000). Herein, the behavioural adjustments during the stance phase of freely behaving unilateral cervical or thoracic spinal hemisected rats are described and compared with sham-operated controls. It has previously been demonstrated that rats make similar locomotor compensations for a variety of unilateral central nervous system (CNS) injuries. The current investigation was conducted in order to determine whether similar behavioural compensations would be made for spinal cord injury at different levels of the spinal cord, i.e. thoracic vs. cervical. The results indicate that behavioural compensations made following unilateral spinal cord injury are similar to other unilateral lesions produced in the CNS. These compensations may result from the constraints imposed upon terrestrial locomotion (Muir and Whishaw 2000) or may be the consequence of innate, plastic changes that occur within the central nervous system. These results, taken together with previously published information, lend novel insights into the response of rats to unilateral central nervous system lesions, which has direct impact on those investigating repair strategies for central nervous system, especially spinal cord, injuries. These results are interpreted and discussed in light of phylogenetic differences in neuroanatomy seen between rats and other species, the neurophysiological basis for locomotion, and what is known about locomotor changes following other unilateral central nervous system lesions in rats and cats.

7.3 Materials and Methods

7.3.1 Subjects

Thirteen adult female Long-Evans rats (Charles River Canada, Quebec, Canada) weighing between 250-320 grams were used. Rats were fed restricted amounts of food to maintain constant body weights and to ensure they were motivated to perform the locomotor task (see Training below). All rats were housed as pairs or trios in cages in the laboratory animal care facility, with 12 hours light/12 hours dark controlled lighting, at the Western College of Veterinary Medicine at the University of Saskatchewan. All animals were examined daily by a veterinarian and were cared for according to the standards set out by the Canadian Council on Animal Care.

7.3.2 Training

As described in section 4.1.

7.3.3 Anesthesia and analgesia

As described in section 4.2.

7.3.4 Surgery

As described in section 4.3. Five rats underwent left lateral cervical hemisection at vertebra C3. Four rats underwent left lateral thoracic spinal cord hemisection at vertebra level T9–10. Four animals were sham-operated (2 cervical, 2 thoracic).

7.3.5 Behavioural assessments

7.3.5.1 BBB scoring

A commonly used method for categorizing locomotor abilities known as the Basso, Beattie and Bresnahan (BBB) scoring system (Basso et al. 1995) was employed as part of the methods to describe behavioural adjustments. All rats were assessed weekly using the BBB scoring system as previously described (Basso et al. 1995). Ground reaction forces and proprioceptive/reflex tests (see below) were not performed until the rat scored at least 15 (for the lowest scoring hindlimb) on the BBB scoring system, with no further change in their score for at least one week. This score was chosen because animals that score 15 could occasionally locomote without dragging their toes on the ground (a prerequisite for ground reaction force determination). Rats also had to be able to consistently locomote, within a runway, for food reward without dragging their paws. Some animals met this criterion earlier than others, but all animals had met the criterion by 40 days following surgery.

7.3.5.2 Reflex testing

Several proprioceptive and reflex tests were performed by an observer blinded to whether an animal had undergone hemisection or not. Reflexes were tested following ground reaction force data collection for all rats. Reflex testing consisted of lateral tactile placing, hopping, and pedal withdrawal (Bradley et al. 1983; Sechzer et al. 1984; Wolgin 1985; Kunkel-Bagden et al. 1992). Each limb of each rat was tested separately for each of the reflexes. Briefly, lateral tactile placing was assessed by covering the animal's eyes and moving the animal toward a table top until the lateral surface of the paw touched the edge of the table. A normal response for this reflex was to place the paw on the surface of the table. This test assesses sensory and motor function (Bradley et al. 1983; Metz et al. 2000b).

Hopping involved holding the animal above a hard surface and allowing only one paw to touch the surface and bear weight. The animal was moved laterally to the weight bearing paw. The normal response was to pick up and place the paw down again as the body passed over the paw. This test requires intact proprioception and motor function (Wolgin 1985; Little et al. 1988; Kunkel-Bagden et al. 1992). This test was performed for each limb of each rat.

Pedal withdrawal responses were tested by manually applying pressure to a single digit on each limb. The normal response is flexion of all the joints of the limb.

All animals were videotaped while performing the reflex tests so as to provide a permanent record, and in the event that further evaluation was required. Animals were scored qualitatively using a scale of 0-3 where:

3 = exaggerated response (eg. hypermetria for hopping; clonic response for withdrawal)

2 = normal response (compared to normal animals)

1 = decreased/inconsistent response (increase in latency or number of times to complete withdrawal, placing, or hopping)

0 = absent response (unable to complete task)

Data were not normally distributed and was analyzed using analysis of variance on ranked data (SigmaStat, Chicago, IL).

7.3.5.3 Ground reaction forces

As described in section 4.4.5.

7.3.6 Data Analysis

7.3.6.1 Histologic evaluation of lesion site

As described in section 4.5.

7.3.6.2 BBB scoring

BBB scores were assigned to each animal based on previously published scoring criteria (Basso et al. 1995). Both right and left hind limbs were scored independently.

7.3.6.3 Ground reaction forces

Components of the ground reaction force data analyzed consisted of: (1) rate of increases and decreases in limb loading for individual limbs; (2) peak vertical, propulsive, and braking forces for individual limbs and diagonal limb pairs; (3) vertical impulse for individual limbs, and (4) net fore-aft impulse for individual limbs. Briefly, rates of limb loading and unloading for individual limbs were determined by measuring slopes for four components of the vertical force/time curve (Fig. 7.1). Slopes were calculated for initial limb loading , i.e. from 0 to 0.5 body weights (BW), (Fig. 1A), for limb loading after 0.5 BW to peak vertical force (Fig. 1B), limb unloading from peak vertical force to 0.5 BW (Fig. 1C), and limb unloading from 0.5 BW to 0 (Fig. 1D). Peak forces generated by individual limbs and diagonal limb pairs were taken directly from the force/time curves for the vertical and fore-aft curves. Vertical impulse was determined by calculating the area under the vertical

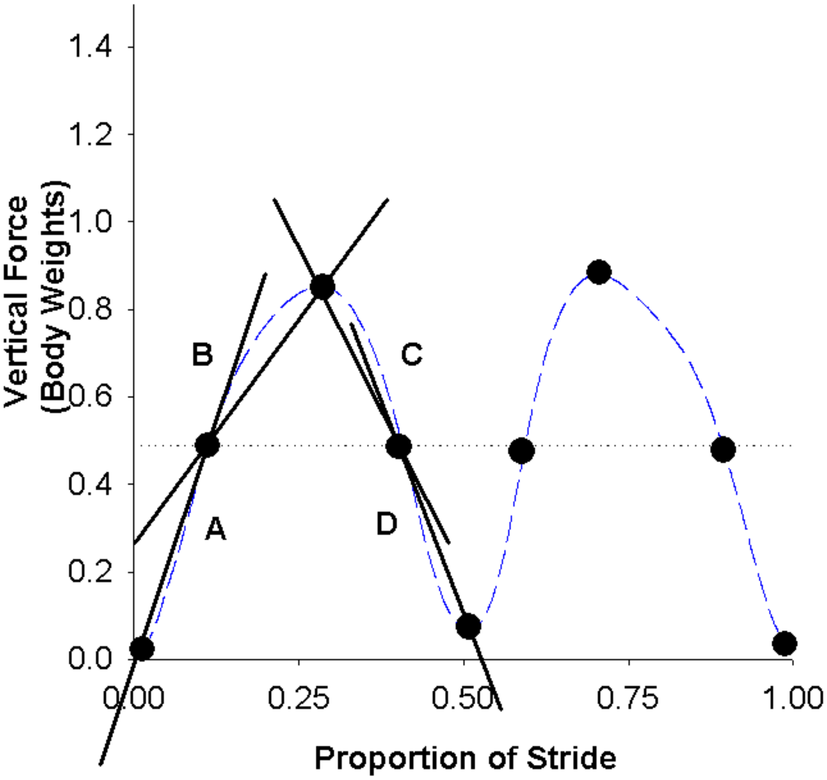


Fig. 7. 1 Diagram showing calculation of rates of loading and unloading of limbs using vertical ground reaction forces. A, initial rate of loading = slope of straight line from 0.0-0.5 body weights; B, final rate of loading = slope of straight line from 0.5 to peak body weight support; C, initial rate of unloading = slope of straight line from peak body weight support to 0.5 body weights; D, final rate of unloading = slope of straight line from 0.5-0.0 body weights.

force/proportion of stride curve. Net fore-aft impulse was determined by summing the area under the curve in the braking direction with the area under the curve for the propulsive direction, thereby yielding the net fore-aft impulse which indicates the relative contribution of the limb for propulsing or braking. Averaged total vertical and fore-aft ground reaction forces were used to determine potential and kinetic energy fluctuations throughout a stride, respectively. Potential and kinetic energy changes were calculated using custom written software (Visual Basic, Microsoft Corp, San Francisco, CA) after the method of Cavagna (Cavagna 1975). Briefly, total vertical forces and fore-aft forces (expressed in body weights) were converted to acceleration (m/s^2). Acceleration due to gravity ($9.8\ m/s^2$) was subtracted from total vertical acceleration, the difference was then integrated twice with respect to time to yield vertical displacement of the body's center of mass. The integration constant for the first integration of total vertical force was assumed to be zero because the average change in height of the body's center of gravity is zero over a number of steps while moving over a horizontal surface. Potential energy changes were calculated over time using the following equation:

$$E_p = mgP \tag{7.1}$$

where:

Ep = potential energy (J)
m = body mass (kg)
g = acceleration due to gravity (m/s^2)
P = vertical displacement (m) of the body's center of mass

Velocity in the forward direction was calculated by integrating total fore-aft acceleration once with respect to time using the mean group velocity as the integration constant. Kinetic energy in the forward direction was then calculated over time using:

$$E_k = {}^1/_2\, mv^2 \tag{7.2}$$

where:

m = body mass (kg)
v = velocity in the forward direction (m/s)

Kinetic and potential energies were indirectly derived from ground reaction force data and as such were not analyzed statistically. These energy changes were calculated and examined to provide descriptive information of the movement of the center of mass for each group of animals during locomotion.

7.3.6.4 Statistical Analysis

If the data met the criteria of normal distribution and equal variances, differences between groups were determined using one-way Analysis of Variance and post-hoc comparisons were made using Bonferroni's adjusted t-test. If criteria for parametric data were not satisfied, differences between groups were determined using Kruskal-Wallis test (Analysis of Variance on Ranks) with post-hoc analysis being completed using Dunn's method.

7.4 Results

7.4.1 Histology

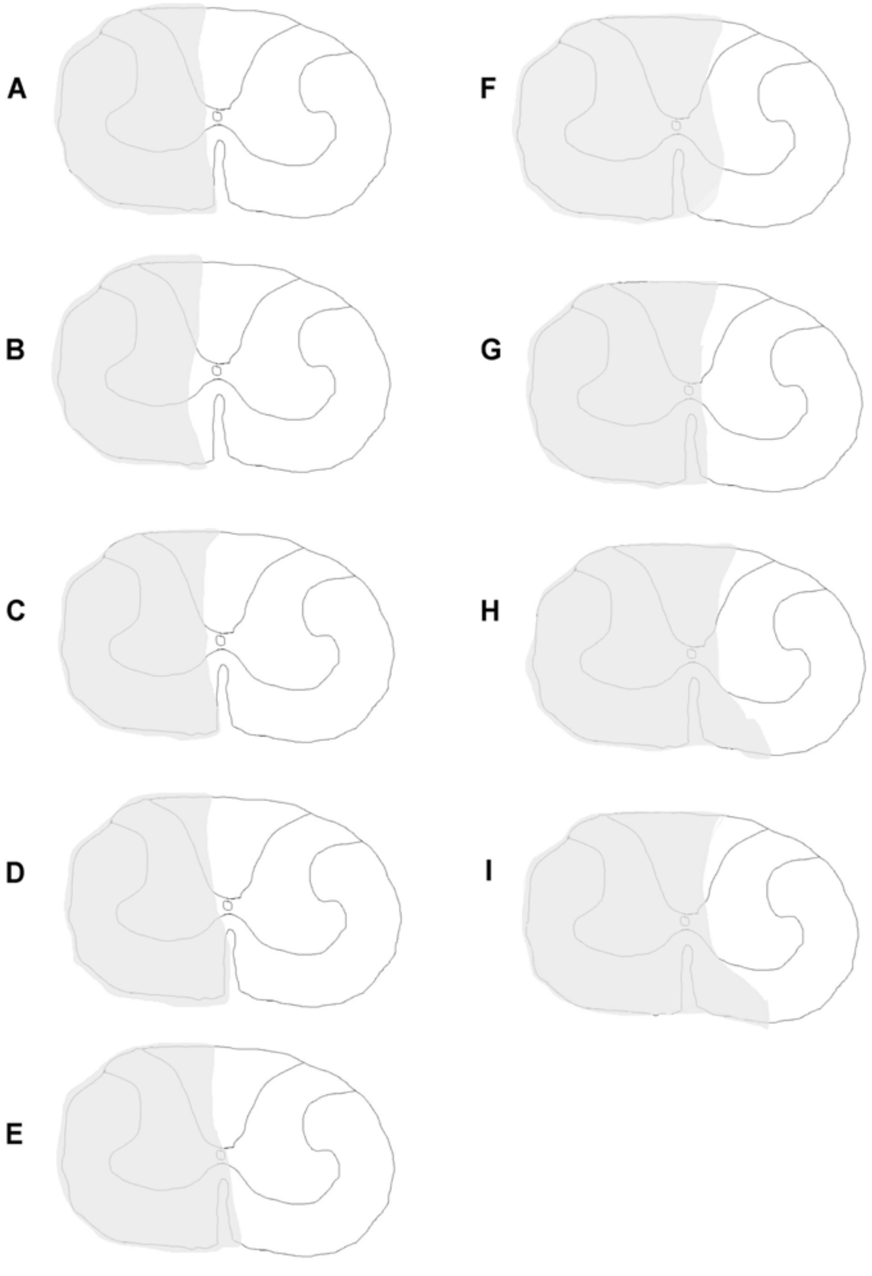


Fig. 7. 2 Schematic drawings depicting lesion epicenters (shaded) for 5 cervical (A-E) and 4 thoracic (F-I) spinal hemisected rats. Cervical (A-E) spinal hemisections typically had some sparing of the left (ipsilateral) dorsal and ventral funiculi. Thoracic (F-I) spinal hemisections were complete and included variable amounts of right (contralateral) dorsal and ventral funiculi.

Sham-operated controls had no evidence of intraparenchymal inflammation or tissue destruction and had very few dural adhesions present. Cervical spinal hemisections (Fig. 7.2A-E) typically spared a small amount of the ipsilateral dorsal and ventral funiculi. Thoracic spinal cord hemisections (Fig. 7.2F-I) were 100% complete and included some of the contralateral dorsal and ventral funiculi. The overlying dura was adherent to the spinal cord throughout the level of the injury site for both cervical and thoracic spinal hemisected animals. All animals with spinal hemisection were included in the study.

7.4.2 BBB scores

7.4.2.1 Left hindlimb

Three cervical spinal hemisected (CH) rats achieved a BBB score of at least 15 for the left (bad) hind limb by the end of the first week post-operatively. All CH rats (n=5) had achieved and surpassed a score of 15 by 2 weeks post-operatively. Only two thoracic spinal hemisected (TH) animals had achieved a score of at least 15 by 2 weeks post-operatively, one TH rat achieved a score of at least 15 by three weeks post-operatively, and the final TH rat achieved a score of 15 during the 6th post-operative week. No TH rats received a score higher than 16 for the duration of the study. Although, statistically, TH rats did not take longer to recover compared to CH rats they did take longer than sham-operated controls ($F_{(2, 10)} = 4.9$, $P = 0.033$). CH rats did not take statistically longer to recover compared to sham-operated control rats. Statistical power was lacking when comparing CH rats with TH rats, therefore one cannot discount the possibility that CH rats generally take less time to reach a BBB score of 15 compared to TH rats. Because of the small sample sizes, frequency of BBB score determination, and the relatively speedy rate of recovery for cervical spinal hemisected rats, I was unable to conclude that cervical spinal hemisected rats recover more of their hind limb locomotor abilities in a shorter amount of time compared to thoracic spinal hemisected animals. Future studies investigating the rapidity of recovery for cervical and thoracic spinal hemisected rats will need to score animals daily using the BBB scoring system, as recovery is generally very rapid with these partial lesions.

7.4.2.2 Right hindlimb

The right (good) hind limb received a score of 21 for all cervical spinal hemisected animals and for three of the four thoracic spinal hemisected animals at post surgical day 40, while one thoracic spinal hemisected rat received a score of 16 for the right (good) hind limb.

7.4.2.3 General

There were no significant differences between BBB scores received by sham and cervical hemisection groups (Fig. 7.3). Cervical spinal hemisected animals achieved a score of 21 for both hind limbs by 40 days post-operatively. Several behaviours were altered in cervical spinal hemisected animals that were noted during BBB scoring but could not be documented using the BBB-scoring system itself. Cervical spinal hemisected (CH) animals

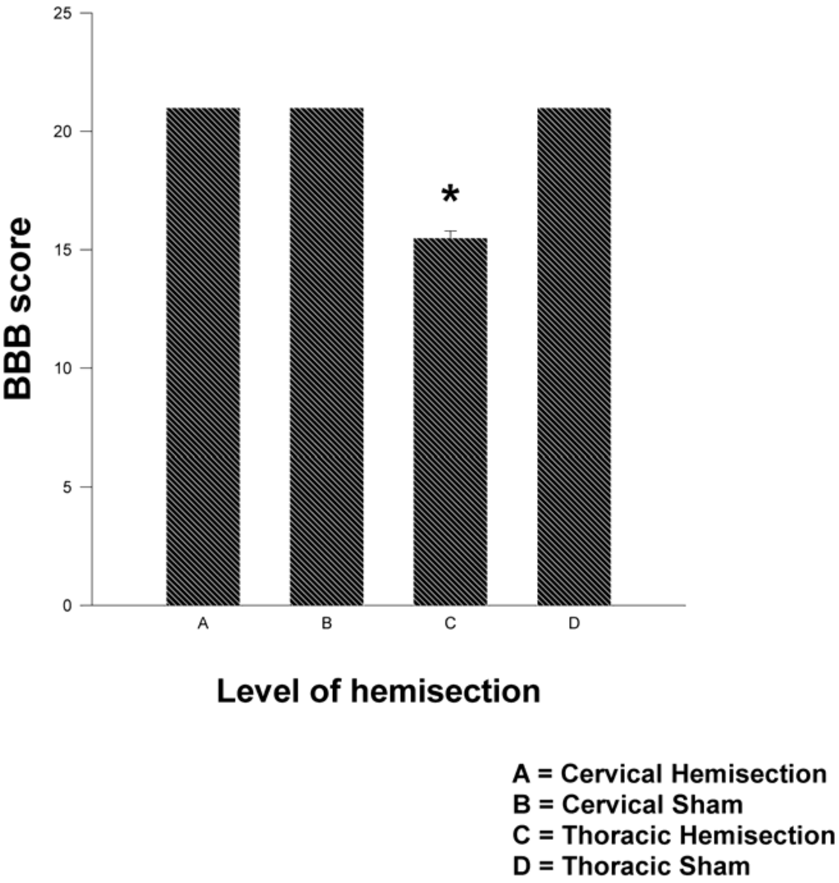


Fig. 7. 3 BBB score for left (bad) hind limb versus level of hemisection, at 40 days post-operative. Only rats with thoracic hemisections (n=4) were significantly different from control animals (n=4) (Mean BBB scores +/- Std. Error; *=P<0.05).

held their left (bad) forelimb flexed during rest and appeared to not fully extend it during locomotion or at rest. Generally, CH rats would lie in sternal recumbancy or stood on four limbs, and occasionally on three limbs, with the affected forelimb being raised off the ground. These rats commonly held the paw of the affected (left) forelimb closed during locomotion while in the open field. Occasionally, their paws appeared to open during the stance phase of locomotion. CH rats would preferentially balance themselves with their right forelimb during rearing-up on the side of the open field. Cervical spinal hemisected animals groomed with only the right (good) forelimb whereas thoracic and sham animals did not appear to have any forelimb preference for grooming. TH animals would stand on all four limbs while at rest and would rear up onto the side of the open field appearing to put more weight on the right side of their bodies. TH rats did not show any preference for balancing themselves with their right or left forelimbs while rearing-up on the side of the open field. TH animals BBB scores were significantly decreased (H = 11.6, 2 df, P = 0.01) compared to sham (Fig. 7.3).

7.4.3 Description of locomoting rats during the runway task

Careful review of videotaped records showed that sham-operated rats did not have any asymmetries between their forelimbs or hindlimbs locomoting for a food reward while in the runway. Cervical spinal hemisected animals, however, consistently placed their left (bad) forelimb down almost perpendicular with their bodies at the onset of stance. That is, CH animals did not extend their left (bad) shoulder compared to sham-operated animals. Consequently, the left (bad) forelimb step length appeared to be shorter than that for the right limb. CH animals did, however, open the paw of the left forelimb prior to the onset of stance during the runway task and consistently used plantigrade paw placement during runway locomotion with paw drag. This is in contrast to their behaviour during the BBB scoring sessions.

TH animals generally held their tails high in the air, except for one animal who held its tail parallel with the body and occasionally touched down on the ground with the end of its tail. As mentioned previously, only trotting data was collected for all rats for the assessment of ground reaction forces. One thoracic spinal hemisected rat, however, occasionally used a pacing gait to locomote while in the runway (not used for forceplate analysis). A pace occurs when the ipsilateral limbs hit the ground at the same time. This gait is seen in more cursorial animals such as camels, horses, dogs and cats, so it was quite unusual to observe pacing in this animal. There did not appear to be obvious asymmetries for the forelimbs in TH rats. The left (bad) hind limbs of these animals appeared to move in an exaggerated fashion. That is, the left hind limb would be lifted higher than the right limb and the range of extension and flexion appeared increased in the left limb. None of the TH rats dragged their paws while locomoting in the runway.

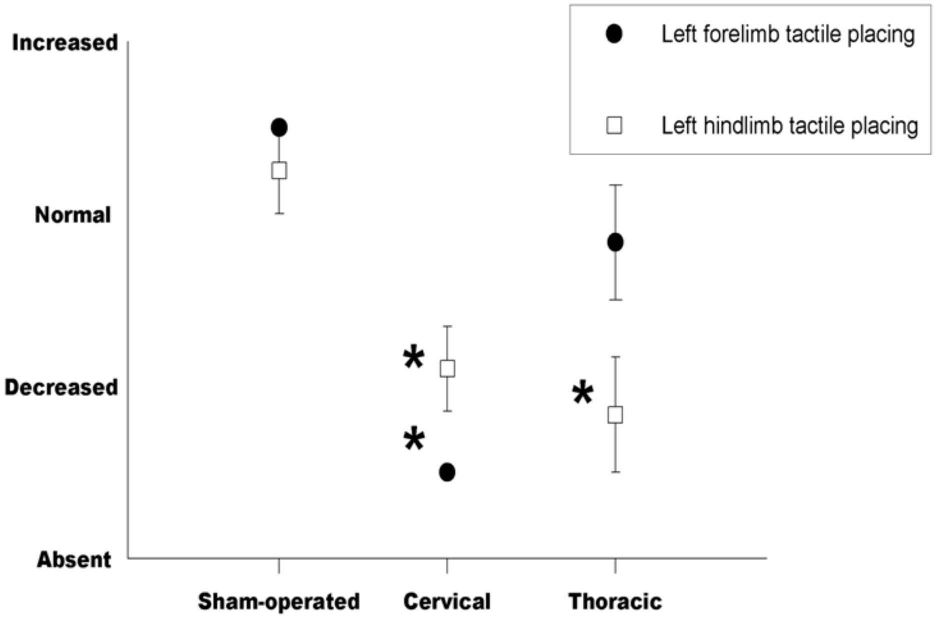


Fig. 7. 4 Tactile placing responses vs. level of hemisection for the left limbs of either left cervical or left thoracic hemisected groups of rats. Responses of both the left forelimb and hind limb of cervcal hemisected rats (n=5), and of only the left hind limb of thoracic hemisected rats (n=4) were significantly different from control animals (n=4) (mean+/- std error, * = P<0.05). Tactile placing responses for right limbs did not differ from controls (not shown).

7.4.4 Reflex testing

Of the reflexes assessed, only lateral tactile placing was able to discriminate CH and TH animals from control animals (Fig. 7.4). CH animals had impaired or absent lateral tactile placing responses in both the left fore (ipsilateral to lesion) ($H=10.3$, 2 df, $P<0.001$) and left hind (ipsilateral to lesion) ($H=6.65$, 2 df, $P=0.039$) limbs. TH animals had impaired or absent responses in the left hind limb only ($H=6.65$, 2 df, $P=0.039$). There were no differences between groups of animals with respect to pedal withdrawal responses and the hopping reactions.

7.4.5 Ground Reaction forces

7.4.5.1 Control Animals

Prior to surgery, the ground reaction forces of the three groups of animals (CH, TH, and sham-operated) could not be distinguished from one another. Post-surgery, no differences were detected for cervical sham and thoracic sham-operated animals. Consequently, data for both cervical and thoracic sham animals were combined into a single control group. The most common gait employed by sham-operated rats before and after surgery was trotting. No walking gaits were observed even at speeds less than 50 cm/s. Analysis of vertical impulse for individual limbs revealed that trotting rats (Fig. 7.5A) bear approximately 0.25 body weights (BW) on each limb over one single trotting stride. Thus 50% of their body weight is supported by the forelimbs and 50% is supported by the hind limbs. These findings are also supported by

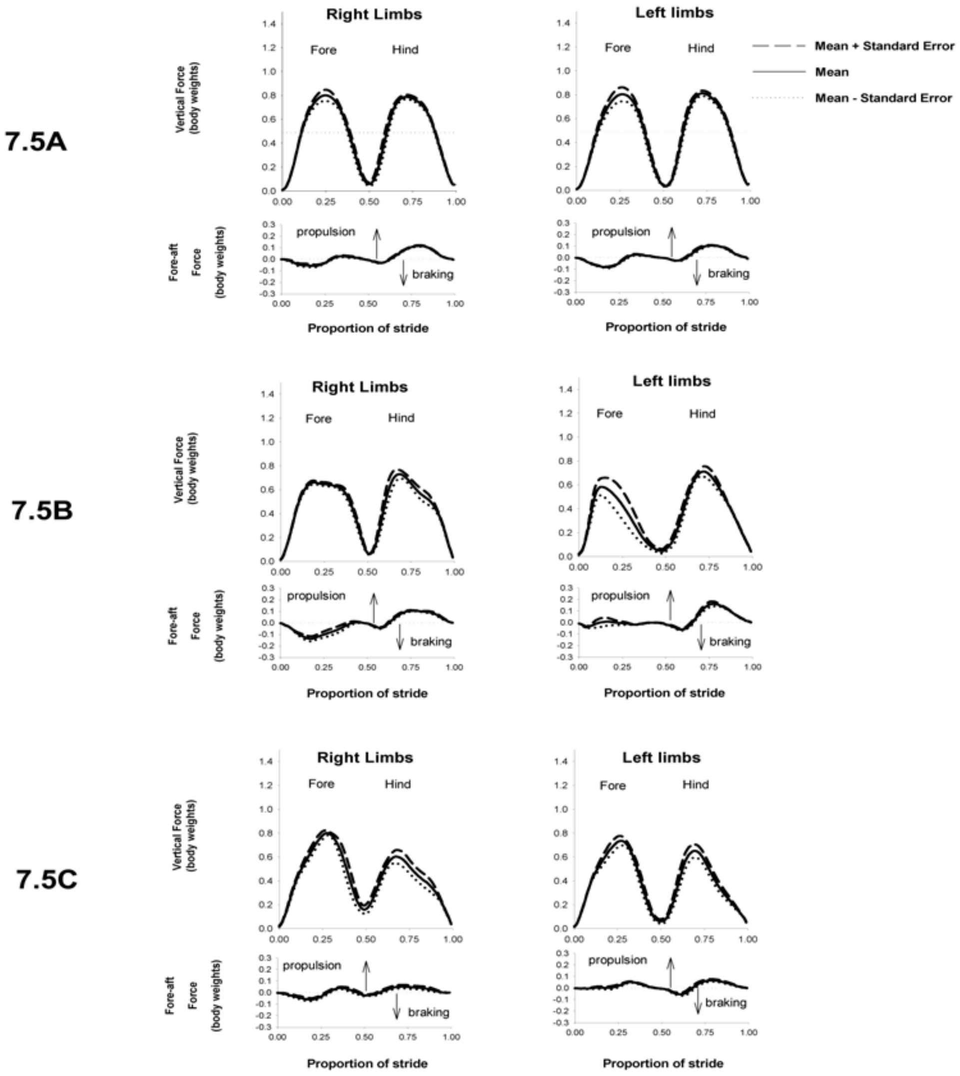


Fig. 7. 5 A-C. Averaged ground reaction forces vs. proportion of stride for individual limbs in the fore-aft and vertical directions. Solid lines represent mean curves for each group of animals. (A) Sham operated animals (n=4) demonstrated no asymmetries in either the vertical or fore-aft direction between fore/hind limbs and between right and left sides. (B) Cervical hemisected rats (n=5) did not brake with their left forelimb and produced more vertical force with both hind limbs compared to forelimbs. (C) Thoracic hemisected animals (n=4) also did not brake with their left forelimb but produced higher peak vertical forces with both forelimbs compared to the hind limbs.

the fact that peak vertical forces were not different for forelimbs compared to hind limbs. Analysis of net fore-aft impulse revealed that sham-operated rats use their forelimbs more for braking than propulsion, and their hind limbs more for propulsion than braking over one stride (Fig. 7.5A).

7.4.5.2 Cervical Spinal Hemisected Animals

Peak vertical forces generated by both forelimbs of CH animals were significantly smaller than control animals (Fig. 7.5B) (left fore $F_{(2, 10)} = 9.5$, $P<0.005$; right fore $F_{(2,10)} = 8.54$, $P=0.007$). CH rats initially unloaded their right forelimb more slowly until 0.5 BW was met ($F_{(2, 10)} = 8.76$, $P=0.006$) (see Fig. 1 for description). These rats initially unloaded their right hind limb more slowly than sham-operated controls ($F_{(2, 10)} = 89.7$, $P<0.001$). The left (bad) forelimb was loaded normally ($F_{(2,10)} = 3.35$, $P=0.08$). However, this limb was unloaded more slowly (initial phase $F_{(2, 10)} = 5.11$, $P=0.03$) (late phase $F_{(2, 10)} = 13.49$, $P=0.001$). The left (bad) hind limb was loaded more slowly throughout the loading period for CH animals (initial phase $F_{(2, 10)} = 5.43$, $P=0.025$) (late phase $F_{(2, 10)} = 4.95$, $P=0.032$). This same limb was unloaded more slowly only after 0.5 BW was met ($F=56.09$, $df=12$, $P<0.001$). CH animals were able to generate a significantly higher peak propulsive force with their left (bad) hind limb than the control animals' left hind limb ($F_{(2, 10)} = 11.79$, $P<0.005$). These rats generated a higher peak braking force with their right (good) forelimb compared to the forelimbs of control animals ($F_{(2, 10)} = 6.91$, $P=0.013$). CH animals essentially did not use their left (bad) forelimb for braking or propulsion. However, the right (good) forelimb was used significantly more for braking compared to controls ($F_{(2, 10)} = 10.62$, P=0.003) and the right (good) hind limb was used similar to controls ($F_{(2, 10)} = 2.02$, P=0.18).

7.4.5.3 Thoracic Spinal Hemisected Animals

Thoracic spinal hemisected (TH) animals generated significantly smaller peak vertical forces with the right hind limb, and nearly significant smaller forces with the left hind limb, compared to sham-operated control animals (left hind $F_{(2, 10)} = 3.57$, P=0.067; right hind $F_{(2, 10)} = 5.97$, P=0.02) (Fig. 7.5C). However, analysis of total vertical forces revealed that the left fore/right hind (bad fore/good hind) had statistically smaller peak vertical forces compared against control animals ($F_{(2, 10)} = 4.7$, P=0.04) (Fig. 7.6C). These animals initially loaded their right forelimb more slowly ($F_{(2, 10)} = 11.3$, $p<0.003$) than controls and unloaded their right hind limb more slowly throughout the unloading period (initial phase $F_{(2, 10)} = 89.7$, $P<0.001$) (late phase $F_{(2, 10)} = 18.08$, $P<0.001$). The left forelimb was loaded similarly to controls. However, the left hind limb was loaded more slowly during the late phase of loading ($F_{(2, 10)} = 4.95$, P=0.03). The left hind limb was also unloaded more slowly during the late phase of unloading compared to controls ($F_{(2, 10)} = 56.09$, $P<0.001$)(Fig. 7.5C). The peak propulsive force produced by TH rats was significantly reduced for the right (good) hind limb ($F_{(2, 10)} = 16.2$, $P<0.001$) but not the left (bad) hind limb compared to control animals. This group also had significantly reduced braking force generated by the left forelimb compared to forelimbs of the control animals ($F_{(2, 10)} = 19.14$, $P<0.001$)(Fig. 7.5C). The left (bad) forelimb of TH animals was used more for propulsion than control animals ($F_{(2, 10)} = 41.7$, $P<0.001$). They used the right (good) hind limb similar to control animals for braking and propusion ($F_{(2, 10)} = 2.02$, $P = 0.18$) (Fig. 7.5C). However, these rats use the left (bad) hind limb significantly less for braking compared to control animals ($F_{(2, 10)} = 23.3$, $P<0.001$)(Fig. 7.5C).

7.4.6 Energetics

In the present study, kinetic and potential energy was determined from integrating the summed vertical and fore-aft ground reaction forces twice with respect to time so as to provide insight into the efficiency by which hemisected animals locomote. Potential energy changes indicate the vertical position of the body whereas kinetic energy changes reflect the fore-aft movement of the body. During walking, kinetic and potential energies are approximately 180 degrees "out-of-phase" with each other, such that energy could be exchanged between the two forms, thereby reducing total energy expenditure by as much as 70% (Cavagna et al. 1977). During normal trotting in quadrupeds, kinetic and potential energies are almost completely in-phase with each other and energy is conserved not by exchange between the two forms but by conversion to elastic energy which is stored in tendons and muscles during stance (Cavagna et al. 1977).

7.4.6.1 Control Animals

Sham-operated rats demonstrate both footfall placement patterns and energetics which confirm that they are in fact trotting (Fig 7.7A). Diagonal limb pairs landed almost simultaneously on the ground together in an alternating pattern and potential and kinetic energies were in-phase with each other (see bar diagram, Fig. 7.7A). Overall, kinetic and potential energies oscillated twice per stride and there was little exchange between potential and kinetic energy.

7.4.6.2 Cervical Spinal Hemisected Animals

Cervical spinal hemisected (CH) animals displayed slightly abnormal trotting footfall patterns and energetically appeared to have a gait somewhere between walking and trotting. The right (good) forelimb hit the ground slightly before the left (bad) hind limb, then the right (good) hind limb hit the ground while the left (bad) hind was on the ground and before the left (bad) forelimb contacted the ground (see bar diagram, Fig. 7.7B). The potential and kinetic energies of this group of rats were not completely in-phase with each other. There were two instances during the stride where kinetic and potential energies were out of phase with each other. The first instance occurred at 0.65 to 0.8 proportion of the stride (Fig. 7.7B). As the potential energy rose, kinetic energy fell, indicating that the rats were rising and slowing down during this portion of the stride. The second instance occurred at 0.9 to 1.1

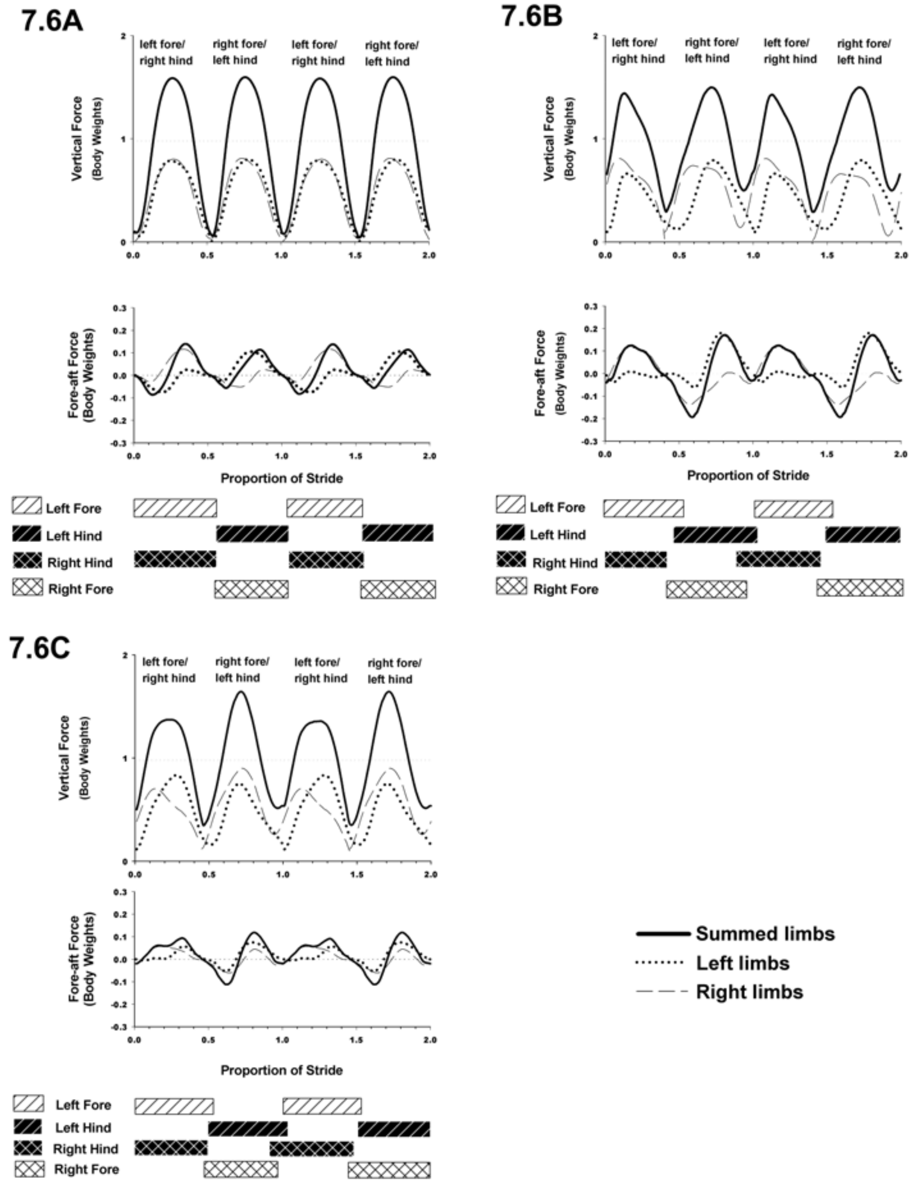


Fig. 7. 6 A-C. Total ground reaction forces vs. proportion of stride for (A) sham operated (n=4), (B) cervical hemisected, and (C) thoracic hemisected rats. Both cervical (n=5) and thoracic hemisected (n=4) rats do not use their left fore/right hind limbs for braking or propulsion (fore-aft force B and C). Thoracic hemisected animals produce less total vertical force on the left fore/right hind limb pair (vertical force C). Cervical and thoracic hemisected rats place their right hind limb down before their left fore (horizontal bars). Horizontal bars indicate the stance phase for each limb.

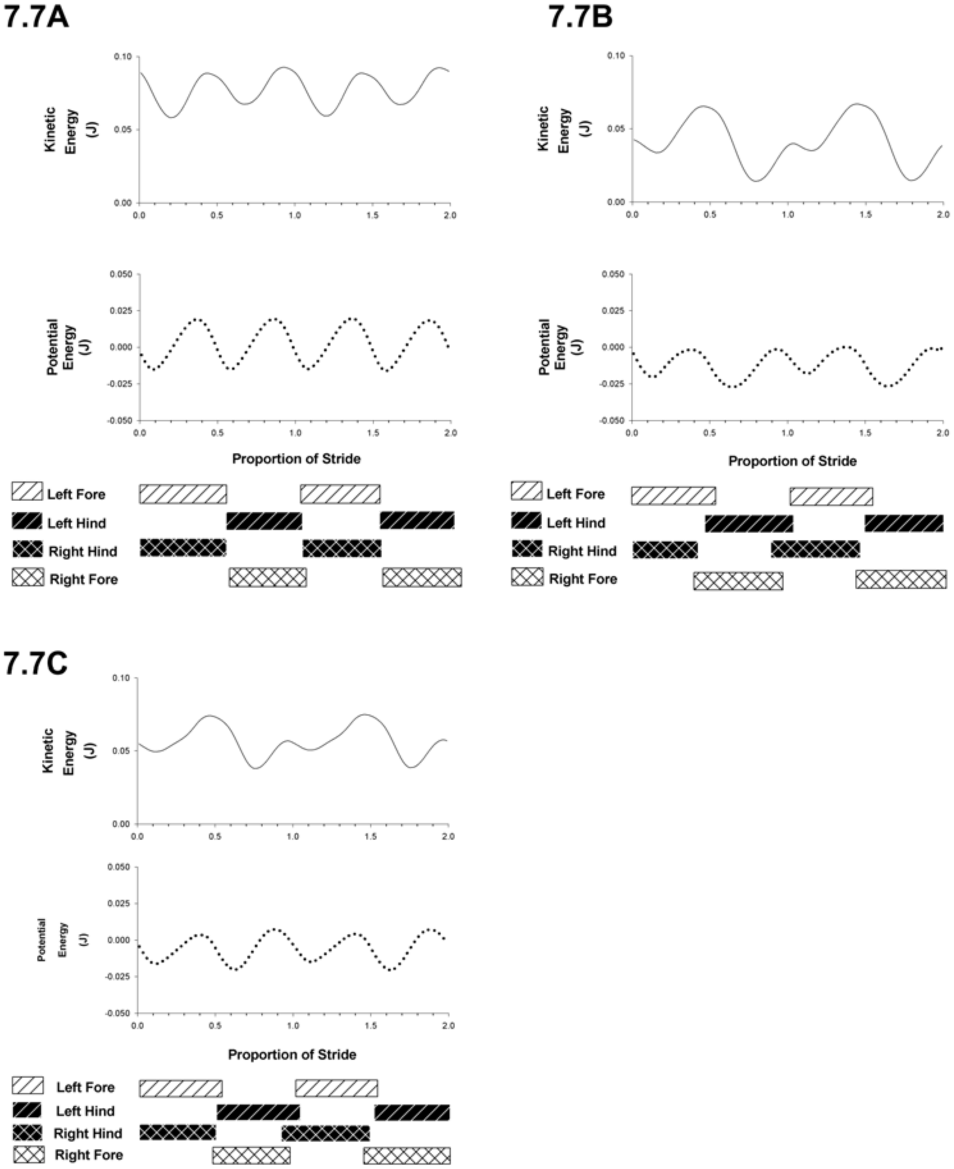


Fig. 7. 7 A-C. Mean potential energy and mean forward kinetic energy produced by (A) sham operated rats (n=4), (B) cervical hemisected rats (n=5), and (C) thoracic hemisected rats (n=4) vs. proportion of stride. Values for potential energy indicate changes relative to the potential energy of a non-locomoting animal (arbitrarily set at zero). Both the potential energy and kinetic energy of sham-operated rats oscillated in-phase twice per stride. For cervical and thoracic hemisected rats, potential energy oscillated twice per stride but is asymmetric. Kinetic energy in these rats oscillated only once per stride. Horizontal bars indicate the stance phase for each limb.

proportion of stride, when the right (good) hind limb was being placed on the ground just before the left forelimb contacted the ground (Fig. 7.7B). During this phase, kinetic energy rose and potential energy fell, implying that the rats were falling onto their left (bad) forelimb and were speeding up. Potential energy oscillated twice per stride whereas kinetic energy oscillated less than twice per stride (Fig. 7.7B).

7.4.6.3 Thoracic Spinal Hemisected Animals

Interestingly, thoracic spinal hemisected (TH) rats demonstrated similar foot-fall and energetic patterns as cervical spinal hemisected rats. This group of animals also had two phases during the stride where there was an exchange between kinetic and potential energies. Kinetic and potential energies were being exchanged from 0.65 to 0.8 proportion of stride (Fig. 7.7C). As potential energy rose kinetic energy fell. Again, this indicated that the center of mass was rising and slowing down at this point in the stride, similar to that of the CH group. There was also a brief period of time when the right (good) hind limb was on the ground where kinetic and potential energies were out-of-phase with each other, indicating that an exchange between potential and kinetic energy occur. However, as in the cervical spinal hemisected rats, this energy exchange lasted only until the left (bad) forelimb was placed on the ground (ie. 0.9-1.1 proportion of stride). Similar to the CH group of animals, these animals were also falling down and speeding up while landing on the left forelimb (Fig. 7.7C).

7.5 Discussion

The results demonstrated that, overall, rats with cervical or thoracic spinal hemisections make similar behavioural compensations during locomotion. In particular, the forelimb ipsilateral to either lesion produced no effective braking or propulsive forces and the hind limb contralateral to the lesion contacted the ground early in the stride cycle compared to sham-operated controls. Nevertheless, kinetic measurements revealed some differences between the two lesion models, eg. CH rats bore more weight on their hind limbs whereas TH rats bore more weight on their forelimbs. Interestingly, the BBB hind limb locomotor rating scale revealed that the hind limbs of CH animals were much less impaired than those of TH animals, and in fact could not be distinguished from those of sham-operated animals after 40 days post surgery. CH rats also displayed deficits in tactile placing of the ipsilateral hind limb as well as the ipsilateral forelimb, whereas TH rats had tactile placing deficits only in the ipsilateral hind limb.

7.5.1 BBB Scoring

The BBB scores that TH animals obtained for the limb ipsilateral to the hemisection was similar to that for other Long-Evans rats examined at 35 days following thoracic spinal hemisection (Mills et al. 2001). The similarity between CH and TH rats revealed by ground reaction force measurements was not detected using the Basso, Beattie, Bresnahan (BBB) locomotor rating scale. Also interesting is my finding that the BBB scoring system did not distinguish cervical spinal hemisected animals from sham-operated control animals upon the animals developing maximum recovery. This probably was the result of the fact that the BBB scoring system was used for a lesion for which it was not originally designed. The BBB scoring system was designed for contusion models of thoracic spinal cord injury in rats (Basso et al. 1995) and not for cervical hemisection models of spinal cord injury. The BBB scoring system evaluates the hind limbs, not the forelimbs, of rats during locomotion. The BBB scoring system did, however, determine that thoracic spinal hemisected rats had more severely altered use of their hind limb than did cervical spinal hemisected animals. This may be in part due to the relative sparing of white matter tracts in cervical spinal hemisected animals and the involvement of additional contralateral spinal tracts in thoracic spinal hemisected rats. Additionally, the left hind limb of TH animals may have received less supraspinal input compared to the left hind limb of CH animals simply because of the differences in the distance between the lesion and the left hind limb motoneuron pools in these two groups of animals. In short, these results emphasize the importance of using methods for assessing behavioural recovery only in experimental models where they have been shown to be useful.

An additional observation was that the BBB scoring system did not assess the maximum behavioural abilities of rats following spinal cord injury. In this study, rats receiving 15 on the BBB scale, i.e. occasional toe clearance, did not drag their paws while moving along the runway for a food reward. This finding emphasizes the difference in motor performance when animals are carrying out a task in which they are motivated i.e. locomoting in the runway, compared to when they are not, i.e. open field (exploratory) locomotion. This is important and relevant when assessing the maximum beneficial effects of therapies for spinal cord injuries, and when addressing discrepancies in the results obtained between laboratories.

7.5.2 Tactile placing

Both cervical and thoracic spinal hemisected animals demonstrated absent tactile placing responses on the side ipsilateral to the lesion. These findings are consistent with those of others (Bradley et al. 1983; Kunkel-Bagden et al. 1992). Absence of the placing response for a given limb was related to the level of the lesion. Cervical spinal hemisected animals lacked tactile placing in both limbs ipsilateral to the hemisection whereas thoracic spinal hemisected animals lacked tactile placing only in the hind limb ipsilateral to the lesion. The exact neural control pathways for the tactile placing response are still unknown. There is some evidence that the reflex is: (1) cortically mediated (Bard 1933; Brooks 1933; Zappella 1966; Donatelle 1977; Bregman and Goldberger 1983b; Bregman and Goldberger 1983a; Bregman and Goldberger 1983c; Barth and Stanfield 1990) ; (2) spinally mediated (Forssberg et al.

1974); and (3) dependent upon white matter tracts running within the ventrolateral spinal cord (Metz et al. 2000b).

The discrepancies in neural control of the tactile placing response may be explained by the phylogenetic differences in neuroanatomy. Cerebral cortical inputs may be more important in primates, as corticospinal tract axons make more projections to ventral gray matter in primates compared to other mammals (Armand 1982). For example, humans and monkeys have more projections within the gray matter containing motoneurons for limb muscles than rats, and marsupials have no projections to these areas whatsoever (Kuypers 1982). It should be mentioned, however, that marsupials inconsistently demonstrate tactile placing (Cassidy et al. 1994), which might imply that a corticospinal tract is indeed necessary for tactile placing.

Taken together, the findings of studies investigating the neural control of tactile placing imply that the absence of tactile placing in CH and TH rats is most likely due to 1) the disruption of tracts running in the ventral portion of the hemisected spinal cord and/or 2) the disruption of the corticospinal tracts (Kunkel-Bagden et al. 1992). Variable amounts of the major dorsal corticospinal tract were spared in the cervical spinal hemisected rats. This implies that the dorsal corticospinal tract is not solely responsible for tactile placing in cervical spinal hemisected rats.

7.5.3 Ground reaction forces

Limb timing and ground reaction force data were similar between cervical and thoracic spinal hemisected rats but were different compared to sham-operated control animals. The most obvious similarities between cervical and thoracic spinal hemisected animals were seen in the fore-aft ground reaction force pattern which was in part due to abnormal interlimb coordination (Fig. 7.6B and 7.6C). In both thoracic and cervical spinal hemisected animals, the right (good) hind limb hit the ground during left (bad) hind limb stance but prior to left (bad) forelimb stance. In intact animals, the left forelimb normally contacted the ground at the same time as the right hind limb. In addition, the left (bad) forelimb was not used for braking in either the thoracic or cervical hemisection groups. Normally, rats use their forelimbs primarily for braking and their hindlimbs for net propulsion (Fig. 7.6A).

It is interesting that cervical and thoracic spinal hemisected animals use their left forelimb similarly. Reasons for these similarities differ between the two groups. Cervical spinal hemisected animals lack a large amount of supraspinal input to the motor neurons controlling the left forelimb. For example, the forelimb impairment may be the result of an interruption of the vestibulospinal tract in cervical spinal hemisected rats. The vestibulospinal tract is known to have a net excitatory effect on limb extensors (Orlovsky 1972b). Animals with lesions of their vestibulospinal tract walk with a crouched gait and slightly abnormal interlimb coordination (Matsuyama and Drew 2000a). Additionally, the ascending proprioceptive and exteroceptive input from this limb was interrupted, as the ipsilateral dorsal column and spinocerebellar tracts were disrupted. Possible reasons explaining why cervical spinal hemisected animals used their left (bad) forelimb less for braking could be that 1) these animals lacked important proprioceptive or exteroceptive tracts or, 2) because they lacked motor control and/or significant inputs necessary for initiation of this limb's central pattern generator. The left forelimb must therefore rely on afferent input from the limb to stimulate the local central pattern generator and/or rely on input from the central pattern generator of the contralateral forelimb.

It has been shown that the red nucleus plays an important role for normal locomotion in rats (Muir and Whishaw 2000). Similar to the findings presented here, rats with unilateral red nucleus ablation use their contralateral (bad) forelimb less for braking than control animals (Muir and Whishaw 2000). In the present study, the left (bad) forelimb/right (good) hind limbs were not used together for braking but were used predominantly for propulsion. This occurred because the left forelimb was not used for braking. In unilateral red nucleus lesioned rats, however, the equivalent limb pair was not used for braking or propulsion because the timing of the forces generated by each limb negated one another (Muir and Whishaw 2000). Rats have many projections from the red nucleus to cervical and lumbar enlargements (Huisman et al. 1982). Disruption of descending inputs from the red nucleus may account for some of the similarities seen in braking and propulsion in CH and red nucleus lesioned rats. Nevertheless, because the timing of forces differs between red nucleus lesioned rats and CH rats, there must be other pathways that are important, and/or working in concert, to account for the differences in these two groups of animals.

Although rats can recover complete locomotor abilities following corticospinal tract lesions (Muir and Whishaw 1999a) this recovery may be the consequence of plastic changes occurring within the central nervous system. Rats with unilateral corticospinal tract lesions do not recover immediately following surgery. Two days after surgery, ground reaction force patterns are very similar to both CH and TH rats (Muir and Whishaw 1999a). As was observed in CH rats in the present study, the bad forelimb and good hind limb of acutely corticospinal injured rats are together used predominantly for propulsion and not for braking (Muir and Whishaw 1999a) and the impaired forelimb is used less for braking compared to control animals. This indicates that the corticospinal tract may be used in locomotion, but in cases where the corticospinal tract is the only injured tract, rats are able to regain full locomotor recovery. This indicates that the corticospinal tract is not absolutely necessary for overground locomotion in rats. However, this does not rule out the possibility that descending corticospinal input is necessary and important for locomotion when other tracts have been abolished. There was histological evidence that the major corticospinal tract was injured with variable sparing between thoracic and cervical spinal hemisected rats. The right (contralateral to hemisection) corticospinal tract was partially injured in the thoracic spinal hemisected rats whereas the cervical spinal hemisected rats sometimes had an incomplete injury of the left (ipsilateral to hemisection) major corticospinal tract. Direct corticospinal input would be more important in the cervical injured group, because corticospinal fibers terminate only on motor neurons in cervicothoracic spinal cord in the rat (Armand 1982). In light of the substantial amount of sparing of the corticospinal tract in cervical spinal hemisected rats, the dorsal corticospinal tract probably cannot account for the deficits seen in these animals.

Thoracic spinal hemisected rats use the left (bad) forelimb less for braking compared to control animals (Fig. 7.5C). Why do TH animals use this limb much the same as CH rats? Descending inputs onto hind limb motor neurons are affected but this does not account for the similar ground reaction force pattern seen for the left (bad) forelimb. Propriospinal inputs from the ipsilateral (bad) hind limb are interrupted following thoracic spinal hemisection. Long ascending propriospinal neurons project from the lumbar spinal cord and connect the cervical and lumbar enlargements and may influence central pattern generating networks (English et al. 1985). Long ascending propriospinal neurons are thought to play a role in interlimb coordination (English 1989); however, it is not definitely known how these neurons contribute to interlimb coordination. It has been hypothesized that they probably synapse on high cervical interneurons and/or indirectly onto motor neurons of the ipsilateral forelimb (English et al. 1985). It may be that propriospinal neurons relay information, directly and/or indirectly, about the position of the hind limbs to the forelimbs. Although the specific role of propriospinal fibers was not assessed in this study, thoracic spinal hemisection has interrupted the connection from the left hind limb to the ipsilateral forelimb which could prevent local spinal networks from generating normal ground reaction force timing. Abnormal limb timing could then prevent normal braking as the animal moves down onto the left forelimb during locomotion.

7.5.4 Energetics

TH animals prematurely placed their right (good) hind limb on the ground, similar to CH animals. This may be a general compensatory response used by both groups of animals in an attempt to stabilize the body and conserve energy during the transition period when the left (bad) forelimb is being placed on the ground. Kinetic and potential energy exchange occurs briefly during the placement of the right (good) hind limb (Fig. 7.7B&C). I also found that potential and kinetic energy was exchanged during 0.65-0.8 proportion of stride (Figs. 7.7B and 7.7C). In this instance the animals are rising up and slowing down. The left (bad) hind limb and right (good) forelimb are on the ground during this exchange between energies. The rats may be slowing down and raising their bodies in an attempt to place their right (good) hind limb up and under their body in anticipation of having to fall quickly onto their left (bad) forelimb. This energy exchange is also seen in normal walking gaits, in both quadrupeds and bipeds, and can help to recover up to 70% of the energy in a given stride thereby leaving only 30% to be generated by muscles (Cavagna et al. 1977).

It is also interesting to consider the changes in the position of the body center of gravity between each group of animals. CH animals bore more weight on their hind limbs compared to controls, and TH animals bore more weight on their forelimbs compared to control animals. This indicates that the center of gravity of CH rats was more caudally located whereas the center of gravity of TH rats was located more cranially. These findings may be a reflection of the relative impairments of the fore and hind limbs in CH and TH animals. Gross observations made during BBB scoring indicated that in CH animals, the left forelimb was more severely affected than their hind limbs. Because BBB scores were significantly lower in the TH rats, these animals had more severely affected left hind limbs compared to CH rats. CH animals may have shifted their weight caudally in an attempt to use their most severely impaired limb most effectively.

7.5.5 Methodological considerations

A variety of spinal cord injury rat models exist and are used for many purposes (Khan and Griebel 1983; Black et al. 1986; Black et al. 1988; Kunkel-Bagden et al. 1992; Basso et al. 1996a; Muir et al. 1998; Metz et al.

2000a; Metz et al. 2000b). The various paradigms used for spinal cord injury investigations involve either complete or partial injuries. Partial spinal cord injury models lend insight into the importance of different neuroanatomical structures for regulating, modifying, and controlling terrestrial locomotion (for review see, (Rossignol et al. 1999)) which may provide information useful for studying regeneration within the spinal cord. It has been suggested that bilateral lesions might produce symmetrical changes making it difficult to detect specific alterations in an animal's gait (Muir and Whishaw 2000). Unilateral lesion paradigms may be more sensitive for detecting differences from normal, symmetrical locomotion (Muir and Whishaw 2000). Few studies have investigated the effects of unilateral spinal cord injuries on overground locomotion (Bregman and Goldberger 1983b; Bregman and Goldberger 1983a; Bregman and Goldberger 1983c; Kato 1992; Muir et al. 1998; Soblosky et al. 2001). This may result from the difficulty of detecting quantitative differences following such lesions and because many animals recover a substantial amount of their locomotor abilities following unilateral spinal cord injury (Bregman and Goldberger 1983b; Muir et al. 1998). Using a variety of behavioural testing procedures makes it possible to more completely describe the locomotor abilities of animals having undergone partial spinal cord injury.

It has been recommended that a variety of tests should be used when assessing the locomotor abilities of rats following spinal cord injury (Muir and Webb

2000; Metz et al. 2000b). Each method used to assess locomotor recovery in rats has it own innate limitations and benefits (Muir and Webb 2000). The results obtained from cervical and thoracic spinal hemisected rats demonstrated the limitations and benefits of various testing procedures and demonstrate how the benefits of one testing procedure can be used to compensate for the limitations of another. For example, the BBB scoring system provides qualitative kinematic data that was used to indicate the motor capabilities of the hind limbs of both cervical and thoracic spinal hemisected animals and allowed us to screen animals before proceeding to ground reaction force determination. In addition, the information obtained from each testing procedure provides useful and unique information about the locomotor abilities of spinal cord injured animals. By employing various methods of assessing behavioural recovery it was possible to maximize the information obtained concerning the locomotor abilities of spinal cord injured rats following either cervical or thoracic spinal hemisection.

This study provides obvious examples of how employing different methods of assessing motor recovery following spinal cord injury is able to maximize the description of the motor recovery and how one testing procedure complements another. The BBB locomotor rating scale was originally designed to assess the progression of hind limb functional recovery following thoracic spinal cord injury in rats. Some studies, however, have used the final score at the end of the study to assess therapeutic benefits of a given treatment (Kamencic et al. 2001). In the present study animals had to have regained a satisfactory amount of recovery following spinal hemisection in order for ground reaction force determination to be performed (a limitation of ground reaction force determination). In this instance the BBB locomotor rating scale acted as a screen by which animals could be tested using ground reaction force determination and provided a general description of how the animals behaved from simple observations. Ground reaction force determination, although unable to be used during the recovery period, provided specific, objective, quantitative information regarding the locomotor adjustments rats made during stance.

7.6 Conclusions

In conclusion, it appears that a general compensatory response is adopted for unilateral lesions at different levels of the spinal cord. This response also shares similarities with locomotor behaviour observed after unilateral red nucleus ablation and corticospinal tract lesions in rats. The function of this compensation is to possibly provide extra stabilization of the animal during locomotion, which also brings about an exchange between potential and kinetic energies. The similarity in ground reaction force patterns seen between the two groups of animals in this study is probably the result of an interruption of descending supraspinal input for both groups, and of disruption of the ascending and descending propriospinal neurons connecting the fore- and hind- limbs in TH animals. The present findings provide novel and useful information regarding the locomotor abilities of rats following unilateral cervical or thoracic spinal cord hemisection. These findings provide insight into the neural control of interlimb coordination that may not otherwise be detected in rats using more conventional methods.

Chapter 8. UNILATERAL DORSAL COLUMN AND RUBROSPINAL TRACT INJURIES AFFECT OVERGROUND LOCOMOTION IN THE UNRESTRAINED RAT

8.1 Abstract

The purpose of this study was to determine the importance of the rubrospinal pathway and the ascending components of the dorsal column for overground locomotion in adult, freely behaving rats. The dorsal column (excluding the corticospinal tract), the rubrospinal tract, or both were damaged unilaterally in rats at the level of the upper cervical spinal cord. Behavioural analysis consisted of skilled locomotion (an evaluation of footslips during ladder walking), a paw usage task, and the assessment of ground reaction forces during unrestrained locomotion. All lesioned animals used the forepaw ipsilateral to the lesions less while rearing. Animals with dorsal column injuries used their contralateral forelimb significantly more while rearing compared to uninjured animals. All lesioned animals tended to produce more foot falls while crossing the ladder compared to uninjured animals. All injuries, regardless of the pathway affected, resulted in significant alterations in body weight support and reduced braking forces from the forelimb ipsilateral to the injury during overground locomotion. Animals typically

bore less weight on the hindlimb ipsilateral to the lesion compared to the hindlimb contralateral to the spinal injury. Taken together with previously published work, this data indicates that the rubrospinal and dorsal column pathways are important for forelimb support while rearing and for skilled locomotion. Additionally, the ascending dorsal column pathways and the rubrospinal tract play a role during flat surface overground locomotion. Combined damage to these pathways does not alter the acquired gait.

8.2 Introduction

Rats are commonly used to assess the efficacy of treatments for experimentally induced spinal cord injuries. In order to effectively evaluate the functional contribution of regenerating fibres, it is important to understand the role of particular pathways during overground locomotion. Much of our knowledge regarding locomotor contributions of spinal pathways has been attained through research on cats and it is not known how much of this information applies to laboratory rodents, such as the rat. Many of the injury paradigms used today create relatively large, bilateral damage to ascending and descending pathways of the thoracic spinal cord (Metz et al. 2000b; Basso et al. 1996a; Kamencic et al. 2001; Ramon-Cueto et al. 2000). Little is known, however, about the locomotor adjustments rats make following unilateral spinal cord injury, particularly injuries made at the level of the upper cervical spinal cord (Mills et al. 2001; Soblosky et al. 2001; Webb and Muir 2002a).

It has previously been shown that unilateral hemisection of the cervical spinal cord in rats results in a characteristic asymmetry during locomotion (Webb and Muir 2002a). This gait shares similarities with the gait adopted by rats with unilateral ablation of the red nucleus (Muir and Whishaw 2000). In the latter study, however, it was not determined whether the locomotor asymmetry was due to loss of the spinal projections of the red nucleus, ie. the rubrospinal tract, or was due to the lack of red nucleus input to the cerebellum, via the rubro-olivary tract. Similarities in the gait adopted by animals with cervical hemisection, which would include a lesion of the rubrospinal tract, suggests that the rubrospinal tract is involved in ongoing locomotion in the rat. This issue is addressed more directly in the present study, by assessing the locomotor abilities of rats with unilateral lesions of the rubrospinal tract.

In addition to lesions involving the rubrospinal tract, lesions of the dorsal columns of the spinal cord were performed. The dorsal columns in the rat contain the pyramidal tract, a pathway which is of much interest in spinal cord research because of its importance in human motor control. The ventral location of the pyramidal tract within the dorsal columns in the rat means that the pyramidal tract cannot be lesioned without damage to the entire dorsal column, such that resulting loss of function cannot be attributed to the pyramidal tract alone (McKenna and Whishaw 1999). Indeed, lesions of the pyramidal tract in the medulla, without damage to the spinal dorsal columns, do not result in permanent locomotor deficits in the rat (Muir and Whishaw 1999a). Thus, it is important to determine whether lesions of the dorsal columns, without damage to the pyramidal tract, do affect overground locomotion in the rat.

The present study investigates the motor abilities of rats following unilateral rubrospinal tract, dorsal column (excluding corticospinal tract) or combined rubrospinal/dorsal column injuries at C3 in the adult rat. This study demonstrates that these pathways are important for skilled and unskilled locomotion, and for stabilization while rearing against a vertical surface.

8.3 Materials and methods

8.3.1 Experimental Subjects

Twenty-five adult female Long-Evans rats were obtained from Charles River Laboratories, Canada (Quebec, Canada). Animals were housed in pairs with 12 h light/12 h dark in the laboratory animal care facility at the Department of Veterinary Biomedical Sciences, Western College of Veterinary Medicine at the University of Saskatchewan. All animals were examined daily and deemed healthy by a licensed veterinarian. All procedures were approved by the University of Saskatchewan's University Committee on Animal Care and Supply. All animals were cared for according to the standards set out by the Canadian Council on Animal Care.

8.3.2 Training

As described in section 4.1.

8.3.3 Behavioural data collection

Behavioural data was collected for all rats prior to surgery and again at six weeks following spinal cord injury. It has previously been shown that rats with cervical spinal cord hemisections do not change their gross hindlimb locomotor behaviour beyond 34 days following injury (Webb and Muir 2002a).

8.3.4 Anesthesia and Analgesia

Animals were randomly assigned to groups receiving either unilateral dorsal column (n=7) or rubrospinal tract (n=18) injuries. As shown previously (Webb and Muir 2002a), the locomotion of sham-operated animals does not differ from that of unoperated animals. To minimize the number of animals used, no sham group was included and instead, injured animals were compared with their own preoperative behavioural data. Anesthesia and analgesic methods are described in section 4.2.

8.3.5 Surgery

As described in section 4.3

8.3.6 Forelimb support while rearing

As described in section 4.4.2.

8.3.7 Ladder Crossing

As described in section 4.4.1.

8.3.8 Ground Reaction Force Determination:

Ground reaction forces were determined as described in section 4.4.5. Variables of the ground reaction forces that were examined statistically were determined as described in section 4.4.5.1. Mediolateral forces were provided for descriptive purposes only and were not analyzed statistically.

8.3.9 Retrograde tracing and histology

As described in section 4.5 and 4.6.

8.3.10 Statistical Analysis

Differences between injured and uninjured animals were determined for the paw preference and ladder crossing tasks using either one-way analysis of variance or Kruskal-Wallis analysis of variance for ranked data depending on whether data met the criteria for equal variances and normal distribution (SigmaStat, Chicogo, IL). Post-hoc analysis was performed using Bonferroni adjusted t-test for parametric data and Dunn's method for determining differences for ranked data (SigmaStat, Chicogo, IL). All animals had ground reaction force determination before and after surgery. Animals within each group served as their own control. Statistical differences were determined for ground reaction force variables using the paired t-test (SigmaStat, Chicogo, IL).

8.4 Results

8.4.1 Histology and retrograde tracing

Eight animals met the criteria for left rubrospinal tract injury (Fig. 8.1A), 4 animals had left dorsal column injuries with sparing of the corticospinal tract (Fig.8.1B), and 5 animals had combined injury involving the left rubrospinal tract and left dorsal column pathways (Fig. 8.1C). Animals with left rubrospinal tract, or combined rubrospinal tract/dorsal column injuries did not have uptake of Fluorogold into the right red nucleus (Fig. 8.2A), while those with left dorsal column injuries had Fluorogold present in both red nuclei (Fig. 8.2B)

8.4.2 Forelimb support

Uninjured rats typically used their individual left and right forelimbs less frequently than using both together while rearing against the wall of the cylinder (Fig. 8.3). There was no significant difference between any of the injured groups compared to the uninjured group when using both forepaws together while rearing ($F_{(3, 26)}$ =1.923; P=0.152). Injured animals used the left forelimb less than did uninjured animals ($F_{(3, 26)}$ =5.35; P=0.005) and less than

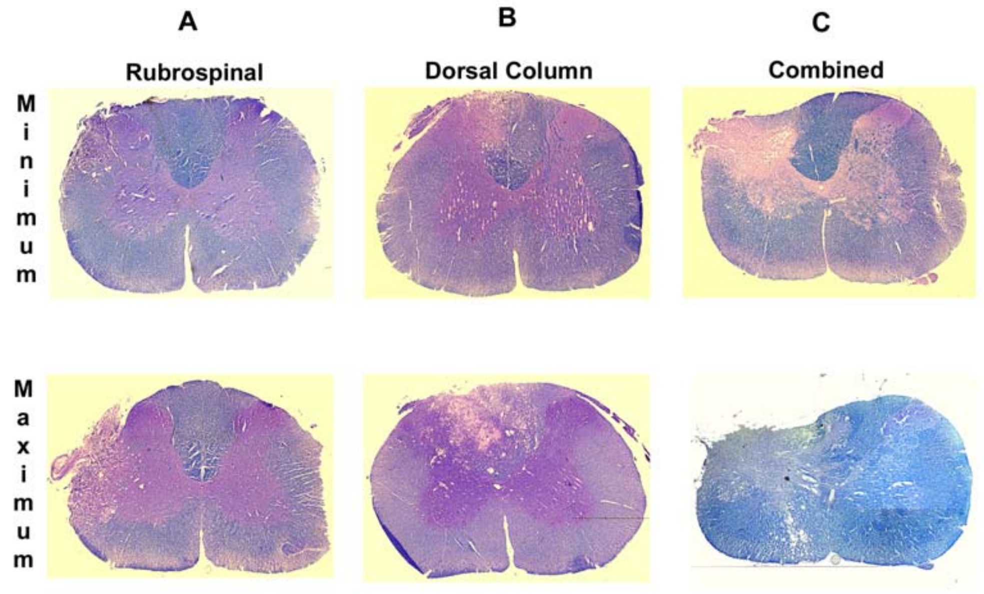


Fig. 8. 1 Photomicrographs of spinal cords from rubrospinal (A), dorsal column (B), and combined rubrospinal/dorsal column (C) injured rats. These images represent the minimum and maximum extent of the lesion in each of the groups of animals. All sections stained with Luxol Fast Blue and Cresyl Violet.

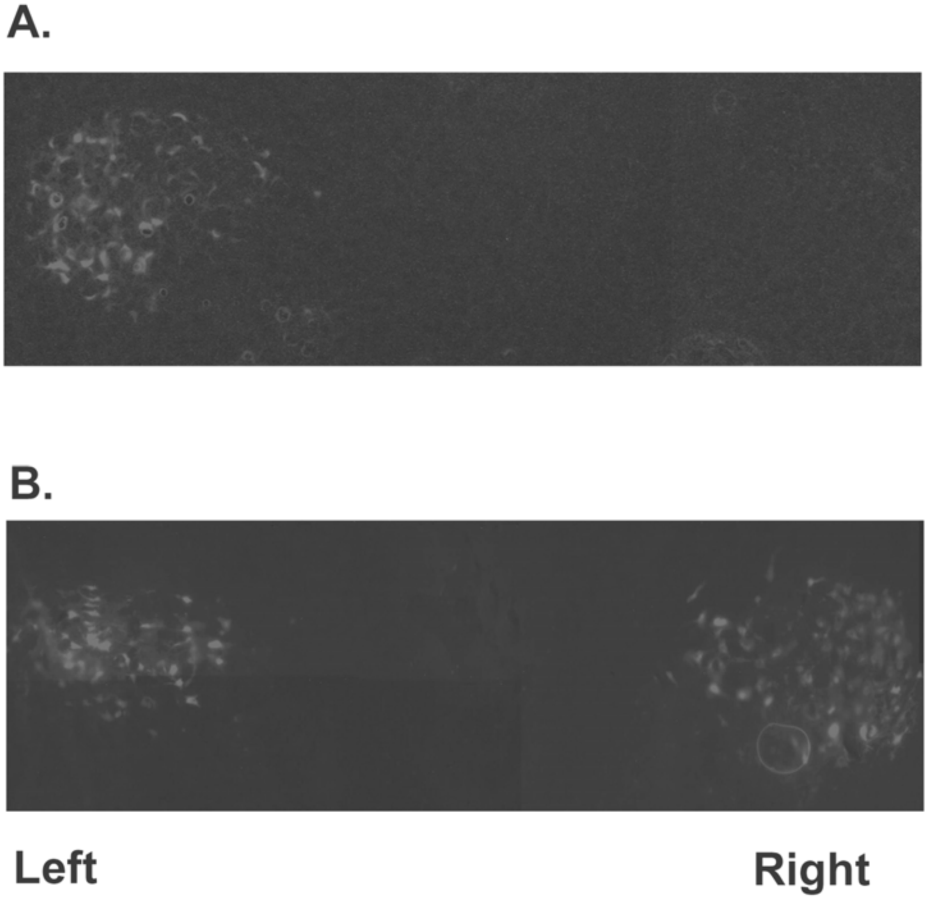


Fig. 8. 2 Fluorescent photomicrographs of representative red nuclei following Fluorogold tracing. A) Animals with unilateral left rubrospinal or combined rubrospinal/dorsal column injuries did not have uptake of Fluorogold into the right red nucleus. B) Rats with unilateral left dorsal column injury had uptake of Fluorogold by both red nuclei.

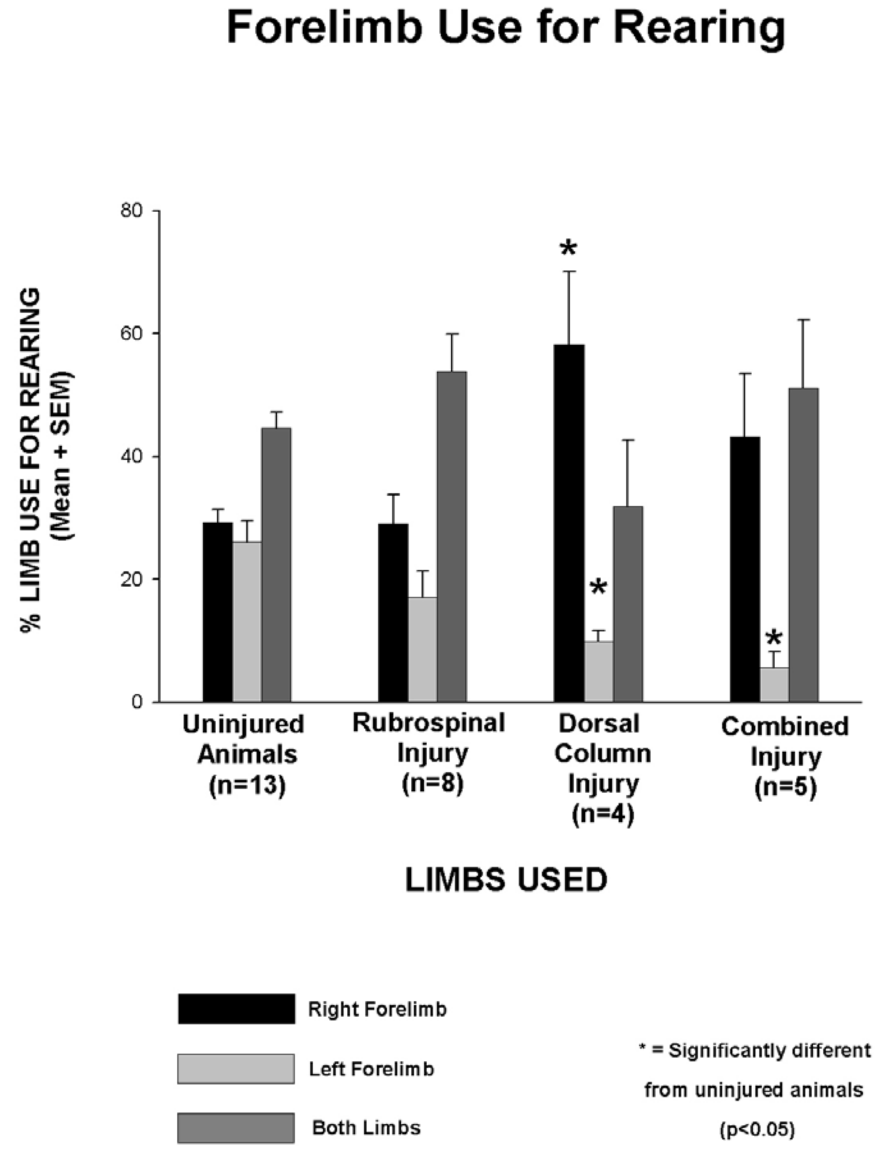


Fig. 8. 3 Pecentage limb usage while rearing for uninjured, rubrospinal, dorsal column, and combined rubrospinal-dorsal column injured animals. Rubrospinal tract injured animals tended to use their left (ipsilateral to injury) forelimb less while rearing compared to uninjured animals. All dorsal column and dorsal column/rubrospinal tract injured animals used the left (ipsilateral to injury) forelimb significantly less than uninjured animals. Animals with dorsal column injuries used the forelimb contralateral to the spinal cord injury significantly more than did uninjured animals.

they used the right forelimb ($P<0.05$) (Fig. 8.3). Post-hoc analysis revealed that dorsal column and dorsal column/rubrospinal tract injured animals used the left limb significantly less than uninjured animals. Although post-hoc analysis did not indicate that rubrospinal tract injured animals used their left forelimb less than uninjured animals statistical power was lacking. Additionally, one of the eight rubrospinal tract injured animals used their left forelimb more than their right forelimb and this contributed to the lack of statistical significance observed for this group. When this animal was removed from the analysis, however, rubrospinal tract injured rats also used their left forelimb significantly less than uninjured animals. Post-hoc analysis for right forelimb usage revealed that animals with dorsal column injuries used the right forelimb more than did uninjured animals while rearing in the plexiglass cylinder ($P<0.01$) (Fig. 8.3).

8.4.3 Ladder crossing

Uninjured rats typically fell through the ladder with a median incidence of 0.8%. Injured animals fell through with median incidences of 2.2% for left rubrospinal tract group, 3.2% for left dorsal column group, and 2.4 % for combined left rubrospinal/dorsal column injured animals. Statistical analysis showed that, after the amount of training in the ladder task was controlled for, all injured animals fell through the ladder rungs more frequently than uninjured animals ($H=11.36$, 3 df, $P<0.05$) (Fig. 8.4A). Closer examination revealed that there was no difference for the incidence of right limbs falling

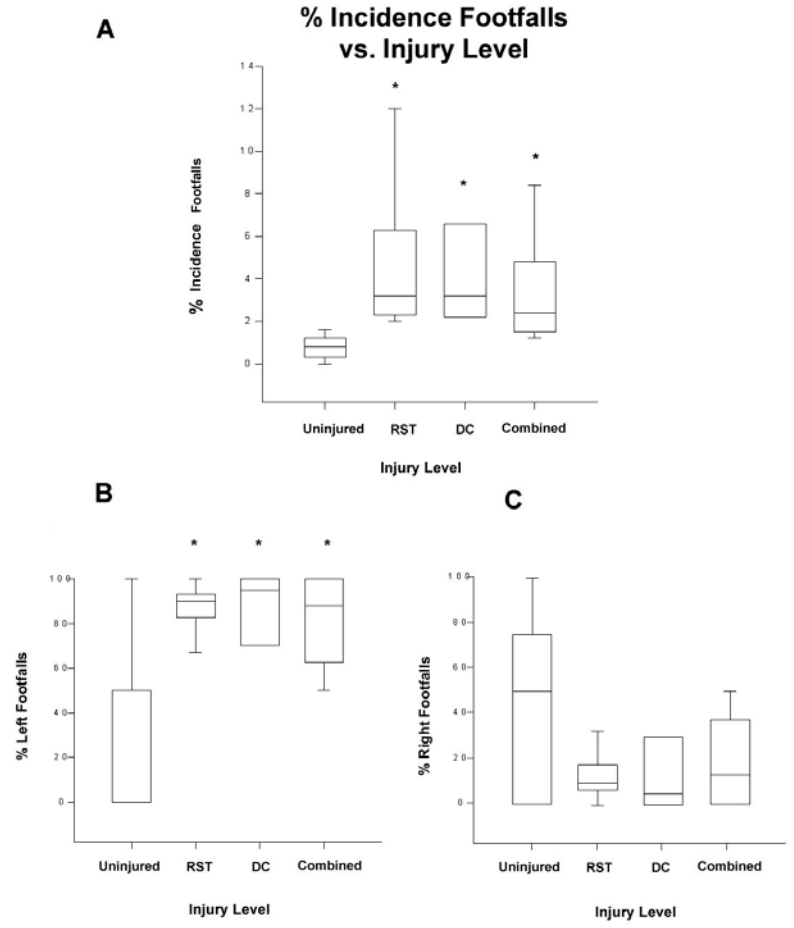


Fig. 8. 4 Percentage footfalls while ladder walking for uninjured, rubrospinal, dorsal column, and combined rubrospinal-dorsal column injured animals A) Rubrospinal (RST) (n=5), dorsal column (DC) (n=4) and combined rubrospinal/dorsal column (combined) (n=5) injured animals fell more frequently than uninjured animals while crossing the ladder. B) These animals' left limbs fell through significantly more than uninjured animals' left limbs. C) No statistical difference was detected between uninjured and injured animals right limbs. Box plot interpretation: Median line within box, 25th and 75th percentiles represented by top and bottom of box, 5th and 95th percentiles represented by error bars. Note median value for uninjured (4B) = zero. * = significantly different from uninjured animals (P<0.05).

between ladder rungs when comparing injured and uninjured animals (H=2.11, 3 df, $P=0.55$) (Fig. 8.4C). A statistically significant difference was determined, however, for the left limbs (H=10.45, 3 df, $P<0.05$). The left limbs of left rubrospinal injured ($P<0.05$), left dorsal column injured $P<0.05$) and combined left rubrospinal/dorsal column injured animals ($P<0.05$) fell through more frequently than uninjured animals (Fig. 8.4B).

8.4.4 Ground reaction forces

8.4.4.1 Left rubrospinal injured animals

Analysis of individual limb ground reaction force parameters revealed significantly decreased braking by the left forelimb ($P=0.007$) and increased braking by the left hindlimb ($P<0.05$) compared to before surgery for these animals (Fig. 8.5). The right hindlimb was used significantly less for braking following spinal cord injury ($P=0.007$) (Fig. 8.5). Analysis of Individual Limb Peak Vertical Force Left to Right Symmetry for fore and hindlimbs revealed that the left hindlimb was used less for weight support than the right hindlimb (reduced ratios for hindlimbs after surgery, $P=0.008$) (Fig. 8.5). Inspection of summed ground reaction forces showed significantly impaired braking forces when the left forelimb and right hindlimb were on the ground ($P< 0.05$, Fig. 8.6 and 8.7). Meanwhile, the left hindlimb and right forelimb generated significantly higher braking while on the ground ($P<0.001$, Fig. 8.6 and 8.7). All animals tended to produce asymmetric lateral forces (Fig. 8.6 and 8.7). Specifically, higher right lateral forces were generated when the left

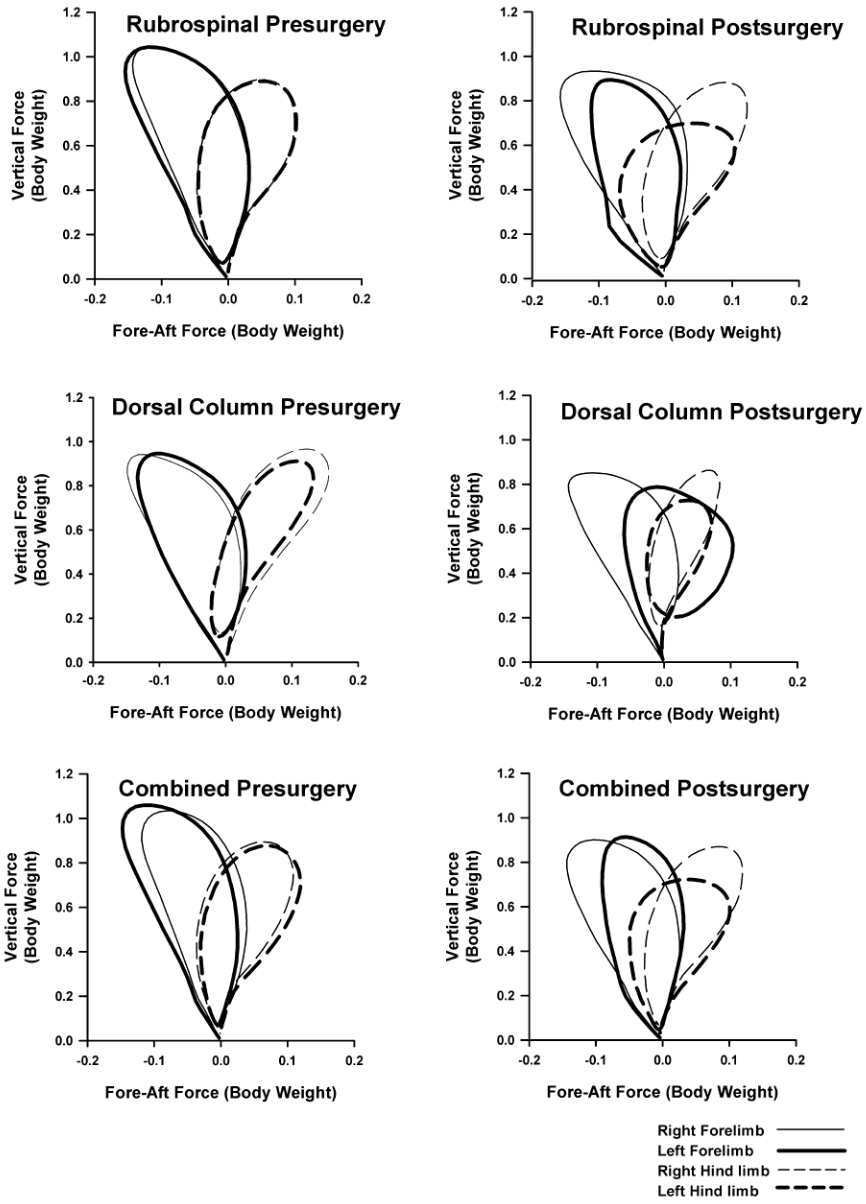


Fig. 8. 5 Vector dynamograms demonstrating limb symmetries. All animals used the forelimb ipsilateral to the spinal cord injury significantly (P<0.05) less for braking after surgery. Rats with unilateral spinal cord injuries used the hindlimb ipsilateral to the injury significantly less for weight bearing after surgery.

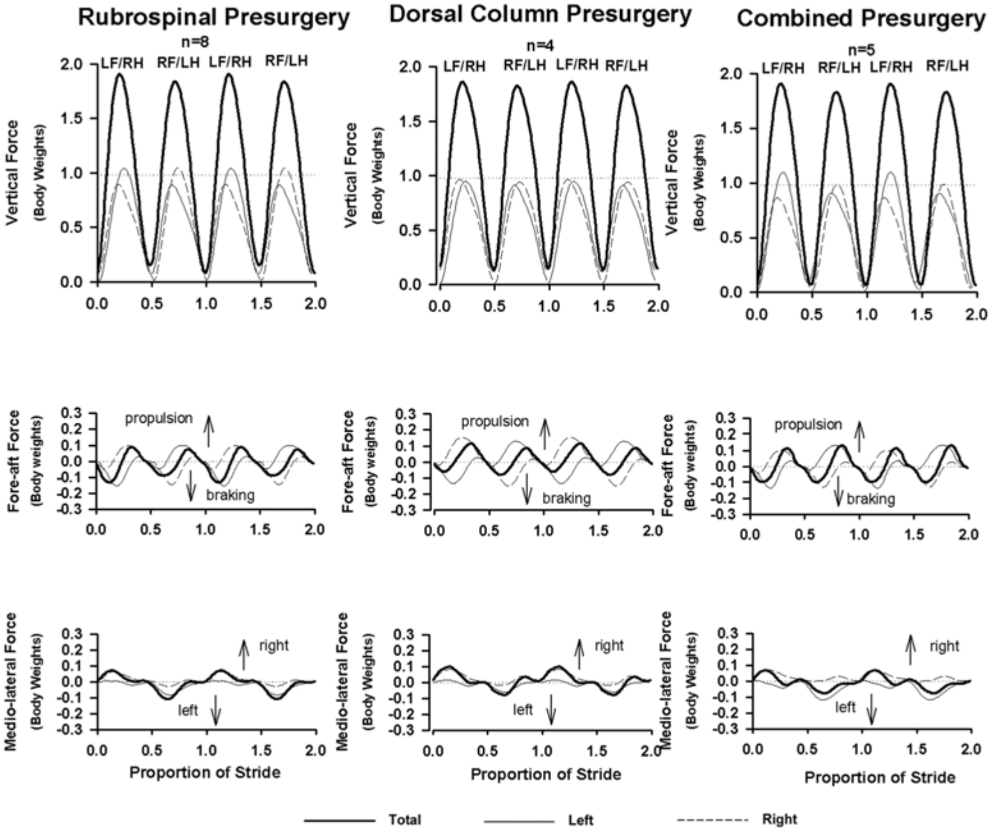


Fig. 8. 6 Summed and individual limb ground reaction forces for each group of animals prior to surgery. All groups of animals demonstrated an alternating placement of diagonal limb pairs indicating that the animals were in fact trotting. Animals were traveling at a relatively constant velocity and along a straight path as indicated by symmetrical fore-aft and medio-lateral forces respectively.

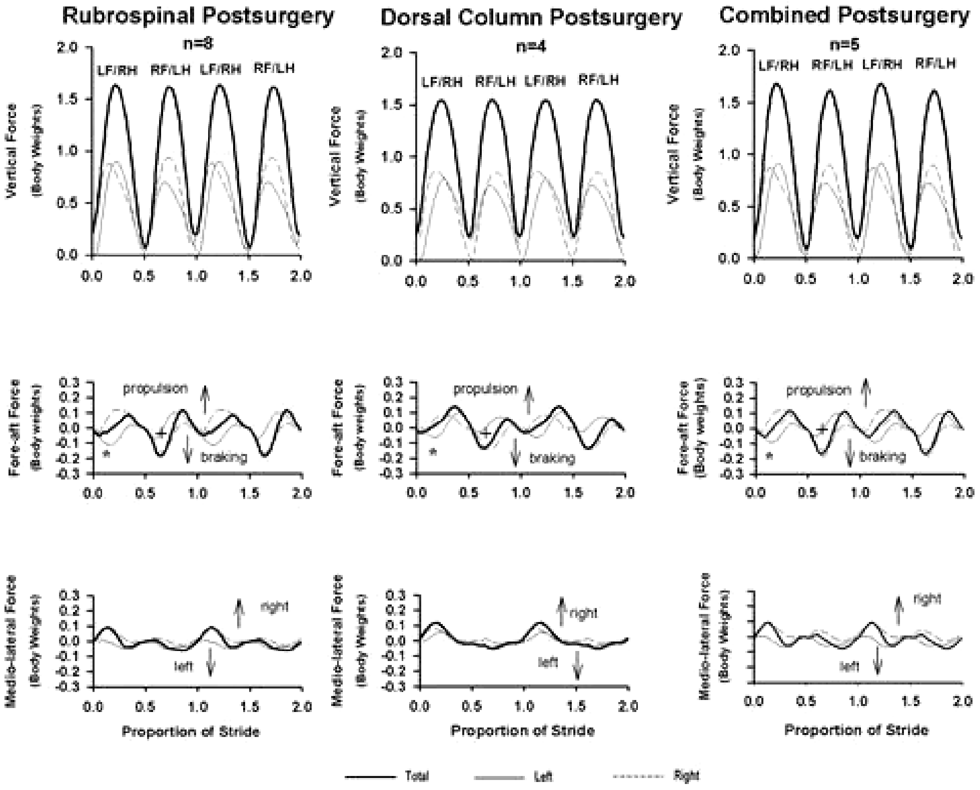


Fig. 8. 7 Summed and individual limb ground reaction forces for each group of animals after left-sided spinal cord injury. Each group of animals adopted a similar gait following spinal cord injury. This gait was characterized by the right hindlimb being placed on the ground prior to the left forelimb. Note the impaired braking forces generated by the left forelimb/right hindlimb pair (*) and the increased braking forces generated by the right forelimb/left (ipsilateral to injury) hindlimb pair (+). All groups tended to have asymmetric mediolateral forces with increased lateral forces generated by the left forelimb/right hindlimb pair.

forelimb/right hindlimb were on the ground simultaneously. These lateral forces indicated that animals' bodies were angled to the left during locomotion.

8.4.4.2 Left dorsal column injured animals

Left dorsal column injured animals generated significantly reduced braking forces ($P<0.05$) and increased propulsive forces ($P<0.001$) with their left forelimb following surgery (Fig. 8.5). These animal's left and right hindlimbs, however, were used significantly less for propulsion ($P<0.05$, Fig. 8.5). Animals with dorsal column injuries also used their left hindlimb less for weight support compared to the right (Individual Limb Peak Vertical Force Left to Right Symmetry was significantly reduced for hindlimbs following surgery, $P=0.005$). Examination of summed ground reaction forces revealed that, like the rubrospinal injured animals, these animals produced smaller braking forces with the left forelimb and right hindlimb pair, although this was not detected statistically (statistical power was low) (Fig. 8.6 and 8.7). These animals also produced increased braking forces when the right forelimb and left hindlimb were on the ground ($P<0.05$) (Fig. 8.6 and 8.7). Like rubrospinal injured animals, all dorsal column injured animals produced asymmetric lateral forces (Fig. 8.6 and 8.7). Again, this asymmetry was characterized by higher right lateral forces generated during the first half of stance when the left forelimb/right hindlimb were on the ground simultaneously.

8.4.4.3 Left combined rubrospinal/dorsal column injured animals

Animals with combined spinal cord injury were not behaviourally different from animals with rubrospinal or dorsal column injuries. Unilateral injury to both the rubrospinal and dorsal column pathways resulted in significantly impaired braking ability by the ipsilateral (left) forelimb ($P<0.05$) (Fig. 8.5). Additionally, combined injury to these pathways resulted in reduced Individual Limb Peak Vertical Force Left to Right symmetry for their hindlimbs. This indicates that animals with combined injuries bore more weight on their right hind limb compared to the left hind limb ($P<0.05$) (Fig. 8.5). Although no statistical difference was found for summed ground reaction force variables, the trend of the summed ground reaction force tracing indicates that these animals too had reduced braking by the left forelimb and right hindlimb compared to uninjured animals (Fig. 8.6 and 8.7). These animals, too, had asymmetric lateral forces nearly identical to those for rubrospinal tract and dorsal column injured animals (Fig.8.6 and 8.7).

8.5 Discussion

The results indicate that unilateral transection of the rubrospinal tract, the dorsal columns or both, at the cervical level, affect forepaw usage and locomotor abilities in the freely behaving adult rat. Rats with these injuries use the forepaw on the lesioned side less for stabilization compared to that of uninjured rats, and animals with dorsal column lesions especially rely more on the forepaw opposite to the lesioned side. Similarly, rats with these injuries produce more footslips with the paws ipsilateral to the lesion during skilled locomotion on a horizontal ladder. Overground, injured rats developed an asymmetric gait, characterized by impaired braking with the forelimb ipsilateral to the injury and reduced weight support by the hindlimb ipsilateral to the injury. Interestingly, rats with combined rubrospinal/dorsal column injuries do not appear to have more severe locomotor and paw preference deficits. These results further our understanding of the importance of the ascending dorsal column pathways and the descending rubrospinal tract for unrestrained overground locomotion in rats.

While it has been shown that ventrolateral pathways are absolutely necessary for mesencephalic locomotor region-induced locomotion (Noga et al. 1991; Steeves and Jordan 1980), severe injury to these pathways and to ventral spinal pathways do not prevent locomotion in the behaving animal (Gorska et al. 1990; Gorska et al. 1993b; Gorska et al. 1993a; Brustein and Rossignol 1998; Loy et al. 2002a). The locomotor role of pathways running in the dorsal part of the spinal cord have been less well investigated, although the importance of the dorsolateral funiculus during locomotion has been recently demonstrated indirectly by combined demyelination of the dorsolateral funiculus and the ventrolateral spinal pathways (Loy et al. 2002a; Loy et al. 2002b). Bilateral demyelination of pathways within the dorsolateral spinal white matter enhances locomotor deficits when combined with bilateral demyelinating lesions of the ventrolateral spinal pathways for up to four weeks following injury (Loy et al. 2002b). Demyelination of the ascending and descending pathways within the dorsal column resulted in only transient enhancement of locomotor deficits when combined with bilateral ventrolateral spinal cord demyelination (Loy et al. 2002b). These results imply that the tracts running within the dorsolateral spinal cord but not the dorsal columns are important for locomotion when the ventrolateral white matter has been injured. In contrast, the present results demonstrate that specific injury to the dorsal column and/or dorsolateral funiculus results in altered locomotor abilities as well as impairments of the forelimb ipsilateral to the spinal cord injury during stabilization while rearing against a vertical surface.

8.5.1 Damage to the rubrospinal tract alters locomotion and forelimb usage

The rubrospinal tract arises from the red nucleus which is located in the mesencephlon. The rubrospinal tract decussates at the level of the tegmentum of the midbrain and extends, predominantly, contralateral to the level of the lumbar spinal cord (Brown 1974; ten Donkelaar 1988). It has been estimated, however, that 10-28% of rubrospinal axon terminals arise from a very small percentage of ipsilaterally projecting axons (Antal et al. 1992). Retrograde tracing of the red nucleus, as shown in the present study, is a reliable indicator of completeness of rubrospinal tract injury. The rubrospinal tract has been described in many terrestrial vertebrates including amphibians, reptiles, birds, and mammals (ten Donkelaar 1988). The rubrospinal tract has been shown to be more prominent in species such as rats and oppussums (ten Donkelaar 1988). This most likely indicates that the rubrospinal tract is more important for locomotion in these species compared to species that have a more pronounced corticospinal tract such as primates (Kuypers 1982). Recently, it has been shown that rubrospinal axons make connections with distal and intermediate appendicular motor neurons but not proximal motor neurons (Kuchler et al. 2002). The rubrospinal tract has also been shown to have predominantly polysynaptic excitatory effects on flexor motor neurons and inhibitory effects on extensors (Hongo et al. 1969a; Hongo et al. 1969b).

The rubrospinal tract has traditionally been thought to be responsible for exciting contralaterally located appendicular distal flexor muscles. Flexion of the contralateral forelimb during stimulation of the dorsal red nucleus and flexion of the contralateral hind limb during stimulation of the ventral red nucleus has been observed using cats (Pompeiano and Brodal 1957). This work also provided evidence for somatotopy of the red nucleus, demonstrated anatomically and using antidromic activation of the rubrospinal axons (Pompeiano and Brodal 1957; Nyberg-Hansen and Brodal 1964; Padel et al. 1972). Increased flexion of distal appendicular hind limb muscles during swing has also been demonstrated in thalamic cats during stimulation of the contralateral red nucleus (Orlovsky 1972c). The rubrospinal tract has also been thought to be inhibitory to extensor muscles. This belief stems from work where rubrospinal neuron destruction resulted in decerebrate rigidity in a precollicular animal (for review see (Massion 1967)). The role of the red nucleus and rubrospinal tract is much less clear in the cortically intact and/or freely behaving animal.

Recently, red nucleus stimulation in cortically intact cats has been conducted and supports the idea of Orlovsky that the red nucleus is important for flexor modification during the swing phase of locomotion (Rho et al. 1999). Furthermore, it has been shown that red nucleus stimulation in cortically intact cats causes facilitation of extensor muscles during the swing phase of the step cycle (Rho et al. 1999). Red nucleus recordings from cortically intact cats further support the role of the rubrospinal tract for flexor excitation during swing, although coincident activity of red nucleus neurons was also seen during stance and extensor muscle activity (Lavoie and Drew 2002).

The effects of rubrospinal tract injury on limb preference for rearing in the present study are consistent with those of others (Liu et al. 1999). Further investigation, using tools and techniques that have been used to identify rubrospinal neuron activity during locomotion in cats (Lavoie and Drew 2002), is needed to further elucidate the role of the rubrospinal tract in performing this behaviour.

Recently, it has been shown that unilateral red nucleus ablation produces permanent locomotor changes in adult rats (Muir and Whishaw 2000). This implies that the red nucleus and rubrospinal tract are important for locomotion. It was shown in the present study, using retrograde tracing, that the rubrospinal tract was indeed injured in animals with dorsolateral funiculus injury, and that these animals have an asymmetric gait similar to that described for unilateral red nucleus lesioned rats (Muir and Whishaw 2000). In addition, it was shown that some rubrospinal tract injured animals fell through the rungs of a ladder during the ladder task more often than uninjured animals. This is consistent with the idea that the rubrospinal tract is important in skilled movements in rats (Whishaw et al. 1998). Single-unit recordings of rubral neurons from cortically intact cats during voluntary gait modifications show that red nucleus neurons increase their discharge activity when the animal's contralateral limb is required to step over an obstacle (Lavoie and Drew 2002). In addition, many of these neurons were found to exhibit multiple periods of activity during both swing and stance for both flexor and extensor muscles (Lavoie and Drew 2002). These results imply that the red nucleus and rubrospinal tract provide necessary input to both flexor and extensor motor neurons during more skilled locomotor activity.

Another potentially important pathway that would have been damaged in the animals with dorsolateral funiculus injuries is the dorsal spinocerebellar tract. Few studies have investigated the role of the dorsal spinocerebellar tract in cortically intact, freely behaving animals. One study, in particular, has shown that injury to the thoracic dorsal spinocerebellar tract does not produce altered interlimb coordination in adult cats (English 1985) providing evidence that the dorsal spinocerebellar tract is unimportant in maintaining normal, overground, locomotion. The present study was not intended to provide evidence of a particular role for the rubrospinal tract on overground locomotion. Rather, the present results taken together with results of unilateral red nucleus ablation in freely behaving adult rats (Muir and Whishaw 2000), provides evidence that the rubrospinal tract is indeed important for normal overground locomotion. Furthermore, the damage to the rubrospinal system produces changes in gait reminiscent of those observed in cervical spinal cord hemisected rats, implying that at least some of the impairment in hemisected animals was due to rubrospinal tract injury.

8.5.2 Dorsal column injured animals have deficits similar to rubrospinal injured animals

In the rat, the dorsal columns contain both descending pathways (corticospinal tract) and ascending pathways (eg. dorsal column-medial lemniscal tracts). Unilateral injury to the pyramidal tract, causing disruption of the crossed and uncrossed corticospinal tract does not produce permanent locomotor deficits (Metz et al. 1998; Muir and Whishaw 1999a). Furthermore, the initial deficits observed following unilateral pyramidal tract injury may have been the result of possible involvement of nearby ascending and descending pathways through inflammation. Regardless, these results imply that the corticospinal tract is not necessary for normal, unrestrained overground locomotion in rats when injured by itself. Histological results of the present study demonstrate complete to nearly complete sparing of the main corticospinal tract; therefore, the behavioural changes observed in these animals can be attributed to the damage incurred by the fasciculus cuneatus and gracilis.

Unilateral injury to the cervical fasciculus cuneatus and gracilis has not been shown to affect paw preference while rearing (Ballermann et al. 2001), contrary to the present study. The discrepancies between the present study and that of Ballerman's could be that lesions of the rat's spinal cord in the present study appear to be larger, and more completely abolish the fasciculus cuneatus and gracilus. Regardless, my results indicate that the limb ipsilateral to the injured fasciculus cuneatus and gracilis is used significantly less while rearing against a vertical surface. Also interesting, is the finding that unilateral dorsal column injured rats used the forelimb contralateral to the spinal injury significantly more while rearing compared to uninjured animals. This may be the result of reduced usage of the ipsilateral limb, when either used alone or in combination with the opposite limb.

Surprisingly, little information concerning the importance of the fasciculus cuneatus and gracilis on overground quadrupedal locomotion exists. The ascending dorsal column system has, however, been shown to be important for skilled reaching in rats (McKenna and Whishaw 1999; Ballermann et al. 2001). Normally rats can discriminate between two texturally distinct food and non-food items (Ballermann et al. 2001). Following unilateral injury to the fasciculus cuneatus and gracilis, rats are unable to discriminate between these food and non-food items (Ballermann et al. 2001). These results demonstrate that the fasciculus cuneatus and gracilis are indeed necessary for hapsis (sense of active touch). The increased incidence of left limb footfalls by the dorsal column injured rats, while locomoting across a ladder, may be the result of altered sensory abilities. The loss of hapsis may also account for the resulting gait characterized by ground reaction forces.

8.5.3 Animals with unilateral spinal cord injury adopt similar gaits

Animals in the present study developed a similar gait regardless of the pathway affected. This characteristic response does not appear to worsen when the injury involves the dorsal and dorsolateral funiculi together. Furthermore, this response tends to be similar to that following cervical spinal cord hemisection (Webb and Muir 2002a) except that animals with cervical spinal cord hemisection tend to bear more weight on their hind limbs than their forelimbs. So what might be some reasons why animals adopt a similar strategy following unilateral spinal cord injury? It could be that this is the most biomechanically efficient way by which these animals can locomote. Another possibility, although not exclusive of the first, is that this gait may be the only way that unilaterally spinal cord injured rats can locomote at a relatively constant velocity without falling or stumbling. If these reasons are indeed valid there could be important consequences for researchers studying recovery of function following experimentally induced spinal cord injury. For example, after developing a gait that is efficient for locomotion following partial spinal cord injury there may not be impetus for the animal to return to using a “normal” gait, regardless of whether treatment-induced regeneration, or sparing of spinal parenchyma has occurred. If a pathway is regenerated, it may not be used until some stimulus “awakens” them - a phenomenon observed in a model of respiratory plasticity (crossed phrenic phenomenon) following cervical spinal hemisection (Sperry and Goshgarian 1993). Therefore, rats may not return to using their preoperative gait even following potential repair of a spinal cord injury.

8.6 Conclusions

Both the dorsal column and rubrospinal tract appear to be important for forelimb support while rearing against a vertical surface. Animals in the present study also adopt an altered gait following rubrospinal tract and/or dorsal column injury. These pathways are also important for skilled locomotion, as injured rats fall more often while crossing a horizontal ladder. Rubrospinal tract injured animals have more footfalls during ladder walking. Consequently, it appears as though the rubrospinal tract is also important for skilled overground locomotion. Taken together with data previously published and presented in this thesis, these results demonstrate that, following unilateral spinal cord injury, rats develop a characteristic strategy to locomote overground. This strategy may be adopted because it is the most practical way that animals can maintain balance and posture during locomotion following unilateral spinal cord injury. The induction of this compensatory response following rubrospinal tract and dorsal column injuries implies that these pathways are important for locomotion.

Chapter 9. COURSE OF MOTOR RECOVERY FOLLOWING VENTROLATERAL SPINAL CORD INJURY IN THE RAT

9.1 Abstract

The purpose of this study was to determine the importance of the pathways running in the ventrolateral spinal funiculus for overground locomotion in adult, freely behaving rats. Left-sided ventrolateral cervical spinal cord injury was performed in adult female Long-Evans rats. The behavioural abilities of these animals were analyzed at 2 days, and at 1.5, 2.5, 3.5, 4.5 and 5.5 weeks following spinal cord injury. Behavioural testing consisted of von Frey filament testing, ladder walking, a paw usage task, and the assessment of ground reaction forces during unrestrained trotting. Animals with injury to the left ventrolateral cervical spinal cord did not develop enhanced sensitivity to pedal mechanical stimulation at 5.5 weeks following injury. At 2 days following injury, animals had impaired skilled locomotion as indicated by increased number of footslips during ladder walking. At 2 days, these animals also used both limbs together more often for support while rearing while using the forelimb ipsilateral to the injury less than did uninjured animals. Ground reaction force determination revealed that animals tend to bear less weight on the forelimb ipsilateral to the spinal cord injury and have substantially

different footfall patterns during locomotion 2 days after injury. All animals returned to normal or near normal sensorimotor, including locomotor, abilities by 5.5 weeks following spinal cord injury. Only very subtle alterations in ground reaction forces were determined by 5.5 weeks following spinal cord injury. These results support the current dogma that there is substantial functional redundancy of pathways traveling in the ventral spinal cord locomotor pathways.

9.2 Introduction

Electrical stimulation of the mesencephalic locomotor regions (MLR) in decerebrate cats results in walking. Interestingly, increasing the intensity of stimulation results in a change in the animal's gait (walking to trotting to galloping) (Shik et al. 1969). The MLR affects the central pattern generators (CPG) for the limbs indirectly by synapsing on neurons located in the pontomedullary reticular formation (RF) (Steeves and Jordan 1980). The RF sends axons to the CPGs for the limbs. These axons are arranged within the ventrolateral and ventral funiculi of the spinal cord (reticulospinal tract) (Houle and Jin 2001). Consequently, the reticulospinal tract has long been thought to be essential in the initiation of locomotion (Steeves and Jordan 1980). Little is known, however, about the importance of pathways traveling in the ventral and ventrolateral funiculi for the maintenance of overground locomotion in the freely behaving adult rat.

Recently, it was shown that bilateral demyelinating lesions of the ventral or ventrolateral funiculi of the spinal cord resulted in subtle changes in the locomotor abilities of rats (Loy et al. 2002a). The changes in locomotor abilities were, however, mild and appeared to recover over a four-week period. When demyelinating lesions were extended to include both ventrolateral and ventral funiculi together, however, severe long-lasting locomotor deficits were observed during open-field behaviour. The results of the study concluded that there is a substantial amount of functional redundancy within the ventral and ventrolateral funiculus. Given that locomotion was grossly assessed during exploratory behaviour in the study by Loy, it is possible that other forms of long-lasting behavioural changes were overlooked. The working hypothesis of the work presented herein was that tracts running within the ventrolateral spinal cord are important for overground locomotion and injury to these pathways produce subtle, long-lasting changes in locomotor behaviour.

Unilateral transection of the ventrolateral funiculus in rats was performed. These animal's behaviours were evaluated using a number of sensorimotor tasks including both endpoint and kinetic measurements. The findings are discussed in light of previous work evaluating recovery following ventrolateral spinal cord injuries, and with respect to the importance of spinal pathways found within the ventrolateral funiculus of rats.

9.3 Materials and Methods

9.3.1 Animals

Eight adult female Long-Evans rats were obtained from Charles River Laboratories, Canada (Quebec, Canada). Animals were housed in 12 hours light/12 hours dark photoperiod within the animal care facility in the Department of Veterinary Biomedical Sciences at the University of Saskatchewan. Work for this project was approved by the University of Saskatchewan's University Committee on Animal Care and Supply. Animals were examined daily and deemed healthy by a licensed veterinarian and were cared for according to the standards set out by the Canadian Council on Animal Care.

9.3.2 Training

As described in section 4.1.

9.3.3 Anesthesia and analgesia

As described in section 4.2.

9.3.4 Surgery

As described in section 4.3.

9.3.5 Behavioural assessment

All animals were assessed behaviourally using endpoint and kinetic (ground reaction force) measurements. Endpoint measurements consisted of von Frey threshold determination, the number of footfalls made while crossing a ladder, and forelimb usage while rearing in a cylinder. Kinetic measurements were determined by measuring ground reaction forces during locomotion. All measurements excluding vonFrey filament testing were collected prior to surgery and again after surgery at 2 days and at 1.5, 2.5, 3.5, 4.5 and 5.5 weeks. Von Frey thresholds were determined before surgery and again at 5.5 weeks after surgery.

9.3.5.1 von Frey Thresholds

As described in section 4.4.3.

9.3.5.2 Ladder Crossing

A ladder used in previous studies evaluating the locomotor abilities of various strains of animals was used (Webb et al. 2003). The ladder crossing task was conducted as described in section 4.4.1.

9.3.5.3 Forelimb support while rearing

The method used to determine forelimb preference for body support while rearing was conducted as previously described (see section 4.4.5.1). Criteria from other studies was used to determine whether right, left or both limbs were being used for support while rearing (Liu et al. 1999; Webb et al. 2003). This task was conducted as described in section 4.4.2.

9.3.5.4 Ground Reaction Forces

Data was only analyzed from animals that were 1) trotting, 2) appearing to travel at a constant velocity, and 3) traveling between 50 and 100 cm/s. Variables of the ground reaction forces that were examined statistically were determined as described in section 4.4.5.1.

9.3.6 Histology

As described in section 4.5.

9.3.7 Behavioural and Statistical assessments

Many of the behavioural tests used have been shown not to be affected by cervical laminectomy alone (Soblosky et al. 2001; Webb and Muir 2002a). Thus, in order to minimize the number of animals used, no sham-operated group was included. Paired t-tests were used to evaluate von Frey filament thresholds because these measurements were made only twice. Statistical assessments of data obtained from all other behaviours were made using Repeated Measures Analysis of Variance (RM-ANOVA) (Sigma Stat, Chicago, IL, USA) for normally distributed data with homogeneous variances, while Friedman's Repeated Measures Analysis of Variance for Ranked data was used for non-parametric data (Sigma Stat, Chicago, IL, USA). Multiple comparisons were made using Bonferroni's t-test for parametric data and Dunn's method for non-parametric data (Sigma Stat, Chicago, IL, USA). In addition, statistical differences were determined between peak forces for right versus left sides at each time point using Student's t-test.

9.4 Results

9.4.1 Histology

Histologic examination of the lesion epicenters from each of the animals demonstrated consistent spinal cord injury to the ventrolateral funiculus (Fig. 9.1). Ascending pathways partially found within this portion of the spinal cord include the medial and lateral spinothalamic, spinomesencephalic, spinohypothalamic, and ventral spinocerebellar tracts (Fig.9.2). Descending pathways partially found within this area include the coeruleospinal,vestibulospinal, and reticulospinal tracts (Fig 9.2). All lesioned animals were included in the study because all animals had a lesion within the ventrolateral funiculus.

9.4.2 Von Frey filament testing

Von Frey filament threshold testing revealed no significant differences ($P>0.05$) (Fig. 9.3). Statistical power was lacking, however, and it may be that the hind limb ipsilateral to the lesion had a higher threshold than before surgery.

Fig. 9. 1 Representative photomicrograph of a spinal cord from one of the rats with left unilateral ventrolateral (ULVL) funiculus injuries. This image represents the lesion epicenter (stained with Luxol Fast Blue and Cresyl Violet).

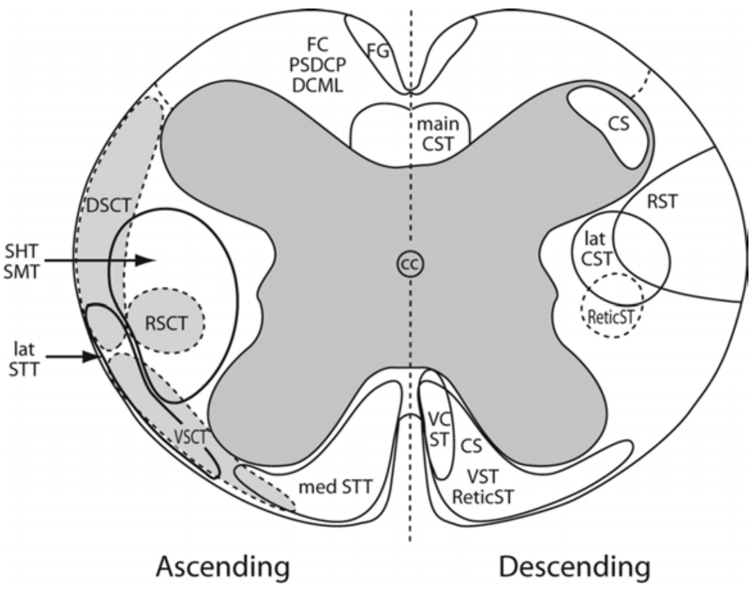


Fig. 9. 2 Approximate areas of ascending and descending pathways within the rat spinal cord. Ascending pathways: **DSCT=dorsal spinal cerebellar tract (Yamada et al. 1991); VSCT=ventral spinocerebellar tract (Yamada et al. 1991; Xu and Grant 1994; Terman et al. 1998); RSCT=rostral spinocerebellar tract (Xu and Grant 1994; Terman et al. 1998); SHT=spinohypothalamic tract (Katter et al. 1996a; Kostarczyk et al. 1997); SMT=spinomesencephalic tract (Zemlan et al. 1978); LatSTT=lateral spinothalmic tract (Giesler, Jr. et al. 1981; Dado et al. 1994c); MedSTT=medial spinothalamic tract (Giesler, Jr. et al. 1981; Dado et al. 1994c); FC=fasciculus cuneatus; FG=fasciculus gracilis; PSDCP=post-synaptic dorsal column pathway (Giesler, Jr. et al. 1984); DCML=dorsal column medial lemniscal pathway (Willis and Coggeshall 1978).** Descending pathways: **Main CST=main crossed corticospinal tract (Brown, Jr. 1971; Terashima 1995; Brosamle and Schwab 1997); LatCST=lateral crossed corticospinal tract; VCST=ventral uncrossed corticospinal tract (Terashima 1995; Brosamle and Schwab 1997); CS=coeruleospinal tract (Clark and Proudfit 1992); VST=vestibulospinal tract (Houle and Jin 2001; Matesz et al. 2002); ReticST=reticulospinal tract (Fox 1970; Houle and Jin 2001); RST=rubrospinal tract (Brown 1974; Antal et al. 1992).**

9.4.3 Ladder Crossing

Statistical analysis of the percent incidence of footfalls indicated that there was a significant difference over time (Chi-square=23.4, 6 df, $P<0.001$). Post-hoc analysis indicated that animals had more footfalls at 2 days and 2 ½ weeks following surgery compared to before surgery ($P<0.05$) (Fig. 9.4).

9.4.4 Forelimb support while rearing

Statistical analysis of forelimb usage while rearing indicated significant differences over time for left ($F_{6,42}=4.32$, $P=0.002$) and both ($F_{6,42}=6.4$, $P<0.001$) forelimbs. Ventrolateral funiculus lesioned animals used their left forelimb (ipsilateral to injury) less frequently than before surgery at 2 days and 1.5 weeks following injury ($P<0.05$)(Fig. 9.5A). Rats used both limbs together more often at 2 days, 1 ½ , and 2 ½ weeks compared to before surgery ($P<0.05$) (Figure 9.5B).

9.4.5 Ground reaction forces

Uninjured rats typically trot symmetrically (Fig. 9.6). Statistical evaluation of Individual Limb Peak Vertical Force Left to Right Symmetry for fore and hind limbs over time indicated that animals had greater asymmetry between their forelimbs 2 days following spinal cord injury compared to presurgery ($F_{6,41}=11.06$, $P<0.001$; post-hoc $P<0.05$, mean left to right peak vertical ratio = 0.832 +/-0.05 SEM) (Fig. 9.6). This difference was found because animals

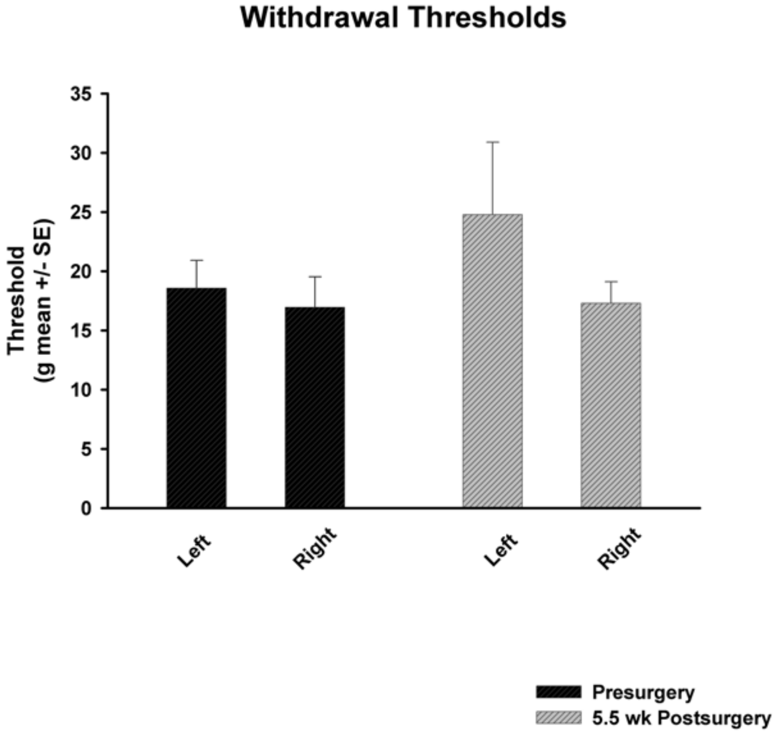


Fig. 9. 3 Withdrawal thresholds for ULVL injured animals. The trend indicates that the hind limb ipsilateral to the injury may have higher von Frey thresholds compared to before surgery, although this difference was not statistically significant.

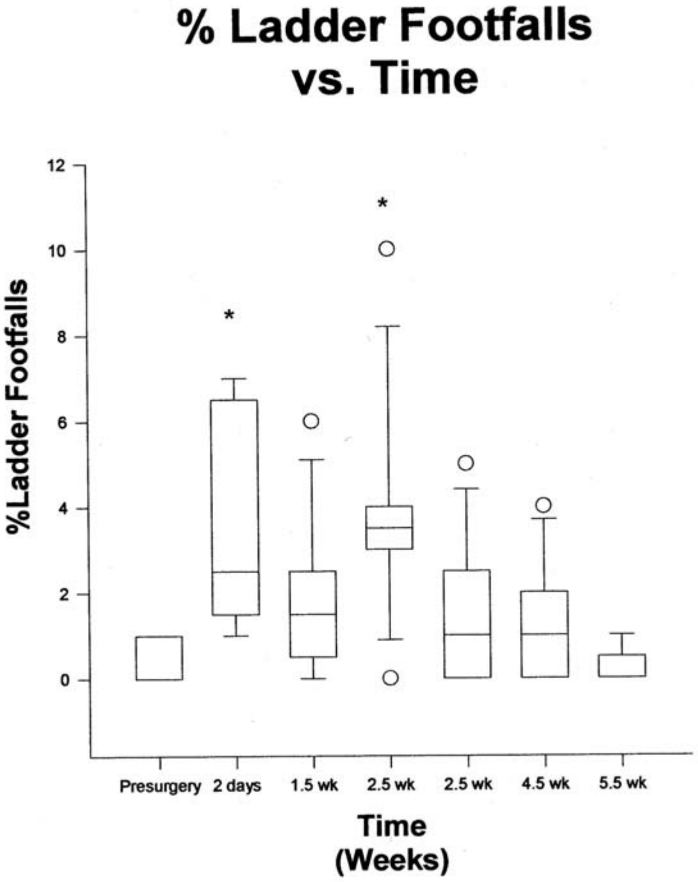


Fig. 9. 4 Percentage incidence of footfalls while ladder walking for ventrolateral spinal cord injured animals. Box plot representing median, 5th, 25th, 75th, and 95th percentiles of % incidence of footfalls while completing the ladder task. Circles represent data points outside of 5th and 95th percentiles. Animals had more footfalls immediately following and at 2.5 weeks after ULVL injury (P<0.05). Animals regained complete skilled locomotor ability by 5.5 weeks following spinal injury (*=significantly different from before surgery).

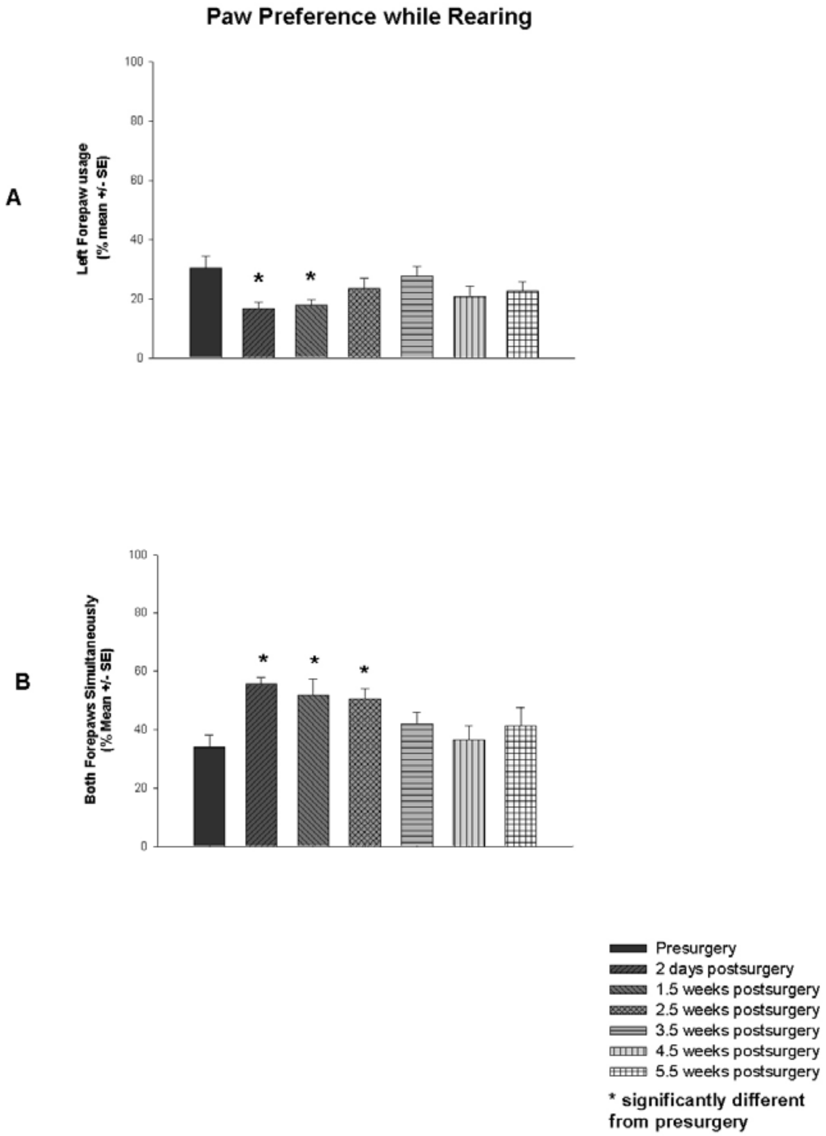


Fig. 9. 5 Paw usage during rearing within a vertical cylinder. (A) Animals used the ipsilateral paw less at 2 days and 1.5 weeks after ULVL spinal injury compared to uninjured animals. (B) Animals tended to use both paws for stabilization more while rearing up to 2.5 weeks following ULVL injury (significance= P<0.05).

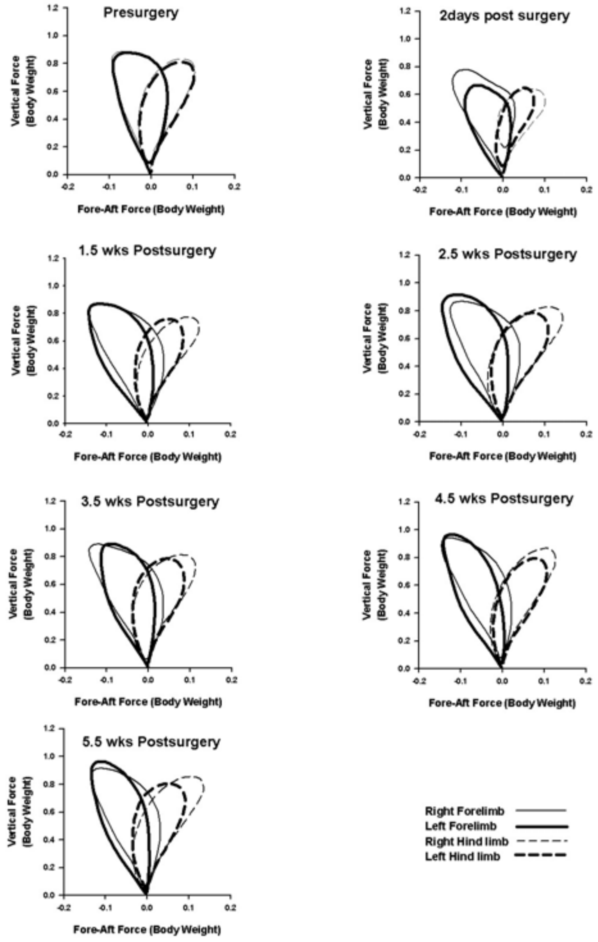


Fig. 9. 6 Vector dynamograms (mean vertical vs. foreaft forces) illustrating symmetry between right and left, fore and hind limbs during freely behaving overground locomotion. *Vertical forces*: Animals have nearly perfect left right symmetry before spinal cord injury. ULVL animals bore less weight on their left forelimb (ipsilateral to spinal injury) 2 days following spinal cord injury (P<0.05) compared to the right forelimb. Animals bore significantly less weight on the left hind limb at 2 days and 1.5 weeks after injury compared to before surgery (P<0.05), while right hind limbs bore more weight compared to the left hind limb at 4.5 (P<0.05) and 5.5 (P<0.05) weeks after spinal cord injury. *Braking and propulsive forces*: ULVL spinal injured animals used both forelimbs more for braking while using the left forelimb less for propulsion during most of the recovery period compared to before surgery. These same animals used the right hind limb more for propulsion than their left hind limb throughout most of the recovery period.

bore less weight on their left forelimb compared to the right forelimb at this time point ($P<0.05$). Analysis of peak vertical forces for the hind limbs indicated that animals bore less weight on their left hind limb at 2 days and 1.5 weeks after surgery compared to before surgery (Chi-square=18.2, 6 df, $P=0.006$; $P<0.05$ post-hoc). In addition, animals bore more weight on their right hind limbs compared to their left hind limb at 4.5 and 5.5 weeks after ventrolateral spinal cord injury ($P<0.05$).

Uninjured rats use their forelimbs mostly for braking while using their hind limbs mostly for propulsion (Fig. 9.6). This is indicated by Individual Limb Peak Braking to Peak Propulsion Forces for the fore and hind limbs. Ratios greater than 1 indicate greater braking forces while ratios less than 1 indicate greater propulsive forces, while values equal to 1 indicate equivalent braking and propulsive forces. Uninjured animals had median peak braking to propulsive force ratios equal to 2.41 and 2.48 for the left and right forelimbs respectively, and 0.29 and 0.27 for the left and right hind limbs respectively. Analysis of braking to propulsive force indices over time revealed no significant differences for right fore and hind limbs (right fore and hind respectively: Chi-square = 7.35, 6 df, $P=0.29$; Chi-square = 9.06, 6 df, $P=0.17$) and left hind limbs (Chi-square = 11.45, 6 df, $P=0.08$). Statistical difference was detected for the left (ipsilateral to injury) forelimb (Chi-square = 16.96, 6 df, $P<0.01$). Animals had larger Peak Braking to Peak Propulsive Force for the left forelimb at 5.5 weeks after spinal cord injury (median=16.43) than before surgery (median=2.41). This indicated that animals were either using their left forelimb less for propulsion or more for braking 5.5 weeks following spinal cord injury. Investigation of peak braking and propulsive forces for each of the limbs over time indicated that 1) animals used their left forelimb more for braking at 1.5, 2.5, 4.5, and 5.5 weeks compared to before surgery while using their right forelimb more for braking at 1.5, 3.5, 4.5 and 5.5 weeks compared to before surgery (left forelimb F6,41=4.5, P=0.001; right forelimb F6,41=3.6, P=0.006); 2) animals used the left forelimb (ipsilateral to injury) less for propulsion at all time points compared to before surgery (Chi-square=19.8, 6 df, P=0.003); and 3) animals used the right hind limb more for propulsion at 2.5 weeks following injury compared to before surgery. Analysis of peak braking and propulsion forces at each time point indicated that animals used their left forelimb less for propulsion compared to the right forelimb at 2.5 and 5.5 weeks following injury (P<0.001). Injured animals used their right hind limb more for propulsion than the left hind limb at 1.5, 4.5 and 5.5 weeks following injury.

No significant differences were detected for Summed Peak Vertical Force Symmetry over time (Fig. 9.7.1-9.7.3) (Chi-square = 11.63, 6 df, P=0.07). Further inspection of differences within each time period, however, revealed that animals generated higher peak vertical force with their left forelimb/right hind limb compared to their left hind limb/right forelimb at 5.5 weeks following injury (P<0.05).

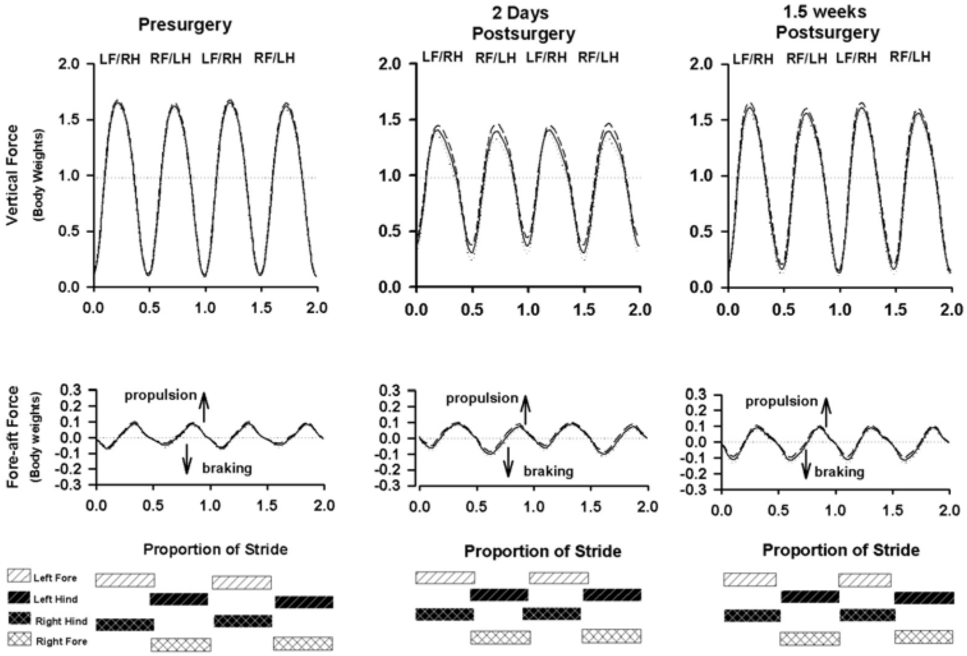


Fig. 9.7. 1 Summed ground reaction forces with footfall patterns. Before surgery, animals locomoted symmetrically using a trotting gait (alternation between diagonal limb pairs). ULVL spinal injured animals adopted an altered gait characterized by placing their right hind limb (contralateral to spinal injury) earlier than the left forelimb 2 days following spinal cord injury. This abnormal gait subsided by 1.5 weeks following spinal cord injury and remained normal throughout the recovery period. Dashed lines = mean + SEM; dotted lines = mean - SEM; solid lines = mean.

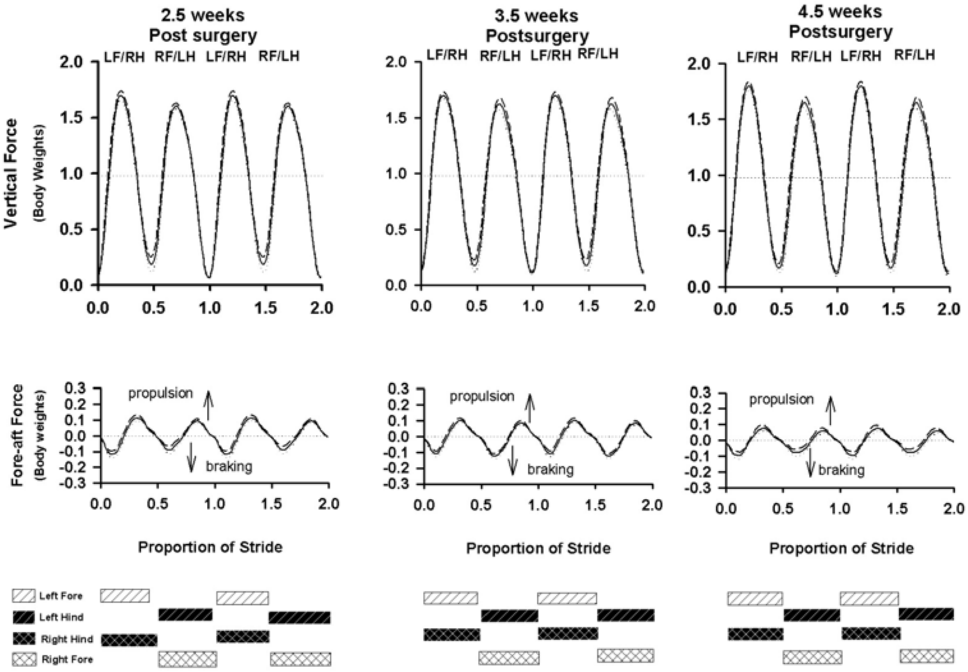


Fig. 9.7. 2 Summed ground reaction forces with footfall patterns. Animals continued to locomote symmetrically from 2.5 through to 4.5 weeks following injury. Dashed lines = mean + SEM; dotted lines = mean - SEM; solid lines = mean.

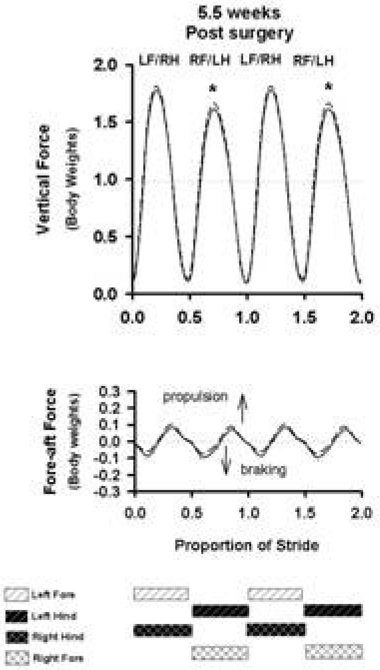


Fig. 9.7. 3 Summed ground reaction forces with footfall patterns. Although no differences were detected for diagonal limb pairs for peak vertical force symmetry, animals bore more weight on their left forelimb/right hind limb than the left hind limb/right forelimb at 5.5 weeks after injury (*=signficantly different (P<0.05) from left forelimb/right hind limb). Dashed lines = mean + SEM; dotted lines = mean - SEM; solid lines = mean.

Upon inspection of individual footfall patterns, animals tended to have a substantial amount of limb overlap following ventrolateral spinal cord injury at 2 days following surgery (Fig. 9.7.1). During normal trotting an animal places its diagonal limbs on and off the ground at approximately the same time (Fig. 9.7.1). The alteration in temporal footfall pattern did return to normal by 1 ½ weeks following spinal cord injury (Fig. 9.7.1) and remained normal throughout the remainder of the recovery period (Fig. 9.7.1-9.7.3).

9.5 Discussion

Few studies have evaluated behavioural recovery following unilateral spinal cord injury in adult rats (McKenna and Whishaw 1999; Schallert et al. 2000; Ballermann et al. 2001; Soblosky et al. 2001; Mills et al. 2001; Webb and Muir 2002a). Moreover, very few studies have evaluated behavioural recovery following cervical spinal cord injury in rats (McKenna and Whishaw 1999; Schallert et al. 2000; Ballermann et al. 2001; Soblosky et al. 2001; Webb and Muir 2002a; Webb and Muir 2002b). In part, this has resulted from the lack of measurements sensitive enough to detect subtle behavioural changes in a small animal. Consequently, little is known about the importance of particular spinal cord pathways in contributing to locomotor behaviour in this species. The present study demonstrated that adult, freely behaving, female Long-Evans rats typically recover a substantial amount of their sensorimotor, including locomotor, abilities following unilateral ventrolateral (ULVL) spinal cord injury. Overall, these findings are in agreement with previously published work that showed functional redundancy of pathways within the thoracic ventral and ventrolateral spinal funiculus (Loy et al. 2002a). Animals with ULVL injury to the cervical spinal cord do not develop enhanced sensitivity to pedal mechanical stimulation at 5.5 weeks following injury. ULVL injured animals fall between the rungs of the ladder more often immediately after spinal cord injury compared to before surgery, but they quickly recover. During exploratory behaviour, these animals use the forelimb ipsilateral to the lesion less and use both forelimbs together more than do uninjured animals. During locomotion, injured animals tend to bear less weight on the forelimb ipsilateral to the spinal cord injury immediately post-operative. All behaviours tended to return to near normal values, although, a trend indicated that animals tended to bear less weight on the hind limb ipsilateral to the spinal cord injury throughout the recovery period. In addition, animals used the forelimb ipsilateral to the spinal cord injury less for propulsion compared to the contralateral limb. Ground reaction force determination supports the hypothesis that unilateral injury to the ventrolateral spinal funiculus results in mild and long lasting locomotor changes. Rats with unilateral ventrolateral funiculus injury are much less affected than unilateral rubrospinal and/or dorsal column injured animals. In addition, because ULVL injured animals regained a substantial amount of sensorimotor recovery over the entire recovery period, these results support the idea that pathways running in the ventral and ventrolateral funiculus show a substantial amount of redundancy with respect to sensorimotor, behaviour including locomotion.

Central pain is a common sequela to spinal cord injury in humans (Christensen and Hulsebosch 1997). Recently, behaviours indicative of central pain have been observed in unilateral and bilateral thoracic spinal cord injured rats, including increased responsiveness to peripheral sensory stimulation (Mills et al. 2001). The sensory abilities of ULVL rats were assessed by determining von Frey threshold responses. Presurgery values were similar to those reported for uninjured adult female Long-Evans rats (Webb et al. 2003). Statistically significant differences for hind limb von Frey threshold values 5.5 weeks following cervical spinal cord injury were not found, although, animals tended to have higher thresholds for the hind limb ipsilateral to the spinal cord injury. Von Frey threshold testing is often used for determining sensory abilities of laboratory rats; however, it is also indicative of an animal's motor abilities because animals are required to withdraw the limb in response to stimulation. If these animals had central pain resulting from spinal cord injury, one would expect a lower threshold. Consequently, the lack of statistical difference for this test, and the possibility of a higher threshold response for the left hind limbs, indicates that central pain did not develop in ULVL injured animals. In fact, ULVL animals may have higher thresholds for the left hind limb because of impaired motor abilities. Although, it is possible that impaired sensory abilities may also contribute to higher thresholds of ULVL injured animals. Indeed, the present study was not designed to evaluate whether the resulting von Frey thresholds were due to sensory or motor impairments. Regardless, further investigation is warranted to determine the importance of pathways traveling in the ventrolateral funiculus for withdrawal responses.

The ladder task is a test used to assess the skilled locomotor abilities of laboratory rats following various central nervous system disturbances. The results indicate that animals with ULVL spinal cord injuries have impaired skilled locomotor abilities immediately after and again at 2.5 weeks after injury but these impairments are not permanent. There are several explanations for this result. First, there may be damage to tracts in the ULVL which are normally used but are not required for skilled locomotion. Second, the spinal injury may have caused inflammation but not permanent damge to tracts located near the injury which are normally used for skilled locomotion. The vestibulospinal tract may be one tract that is involved in either, or both of these situations. The vestibulospinal tract is known to descend predominantly ipsilaterally within the ventral and ventrolateral funiculus in rats (Houle and Jin 2001; Matesz et al. 2002) and is thought to be important for extensor muscle activation and flexor muscle inhibition and is most important during stance (Grillner et al. 1970; Orlovsky 1972b; Orlovsky 1972c; Matsuyama and Drew 2000a). If extensor muscle activation is impaired during stance, animals would not be able to adequately extend their knee (stifle) forward in anticipation of “hitting” the next rung, would not extend their limb backward fully (end of stance) and may not be able to make appropriate postural adjustments during this form of skilled locomotion. These deficits could result in increased incidence of footfalls by altering the animals posture and interlimb coordination. Interestingly, animals had a significantly higher incidence of footfalls at 2.5 weeks following injury but not at 1.5 weeks following injury. It has been shown that monocytic inflammatory responses within the spinal cord are maximal at approximately 1 week following partial transection of the spinal cord (Dusart and Schwab 1994). Immunohistochemical reactivity for microglia/macrophages can be found up to three months following injury however (Dusart and Schwab 1994). At 2-3 weeks following partial spinal cord transection, however, glial scarring is present and cavitation begins (Dusart and Schwab 1994). Considering that these animals were administered a high dosage of glucocorticoid at the time of surgery, the inflammatory process would have been impaired and may have resulted in a delay of the normal inflammatory responses outlined above. The behavioural changes observed at 2.5 weeks could be due to extension of the primary injury site due to secondary injury mechanisms (especially more chronic microglial/macrophage infiltration) but without histological evidence at these time points one can only speculate. Nevertheless, these results demonstrate that pathways running in the ventrolateral funiculus are not necessary for skilled locomotion when injured unilaterally.

Uninjured rats typically use their forelimb for support and balance while rearing against a vertical surface. This naturally produced behaviour has been exploited to evaluate the efficacy of various treatments for spinal cord injury and for studying behavioural consequences of various experimentally induced CNS lesions including spinal cord injury (Liu et al. 1999; Schallert et al. 2000;

Ballermann et al. 2001; Webb and Muir 2002a). In the present study, animals used both forelimbs for support more often immediately after spinal cord injury compared with before surgery. In addition, ULVL injured animals initially used their left (ipsilateral to the injury) forelimb less often for support compared to before surgery. These animals did, however, use their limbs similar to before surgery by 5.5 weeks after spinal cord injury. It appears that injury to the ventrolateral funiculus impaired the abilities of animals to use the forelimb ipsilateral to the spinal cord injury early in the course of recovery. Again, one tract possibly involved in these impairments is the vestibulospinal tract. Animals may not have used the forelimb ipsilateral to the spinal cord injury because they were unable to fully extend it forward during rearing. Consequently, animals used both forelimbs together for added support which is most likely a manifestation of behavioural compensation. These results indicate that pathways running in the ventrolateral funiculus are not necessary for forelimb placement when injured alone unilaterally.

Ground reaction force determination revealed that spinal cord injured animals used their left and right limbs differently during locomotion. In addition, animals developed a gait characterized by substantial alterations in temporal footfall patterns two days after spinal cord injury. Two days following injury, animals bore significantly less weight on the forelimb ipsilateral to the spinal cord injury compared to the contralateral forelimb. These animals also bore less weight on the hind limb ipsilateral to the spinal cord injury up to and including 1.5 weeks following surgery. This has also been observed in rats with cervical spinal cord hemisections (Webb and Muir 2002a). Meanwhile, the hind limb contralateral to the injury bore more weight during the later phases of recovery. Animals may have borne less weight on the fore and hind limb ipsilateral to the spinal cord injury because of altered intralimb coordination and/or impaired sensory abilities of these limbs. For example, if flexor and extensor muscles were not activated in a coordinated fashion, normal intralimb coordination may be altered and the limb may not be used as effectively for normal weight bearing. Analysis of peak propulsive forces indicated that animals used the hind limb ipsilateral to the spinal cord injury less for propulsion than the contralateral hind limb. This could be a reflection of impaired activation of hind limb extensor muscles needed to generate propulsion at the end of stance. The asymmetry observed at 5.5 weeks for peak vertical force for diagonal limb pairs is the result of the fact that less weight was being borne on the hind limb ipsilateral to the spinal cord injury. Temporal footfall patterns revealed that animals have a substantial alteration in their gait immediately after spinal cord injury. This gait was characterized by the right (contralateral to spinal injury) hindlimb being placed on the ground before the left forelimb and while the left hindlimb was still on the ground. This gait modification was also seen at 2 days following unilateral pyramidal tract transection (Muir and Whishaw 1999a), and long after unilateral red nucleus ablation (Muir and Whishaw 2000) and cervical spinal cord hemisected adult rats (Webb and Muir 2002a). The animals in the present study regained a typical trotting gait by 1.5 weeks following injury. The abnormal gait observed soon after surgery could have occurred because of 1) impaired extensor muscle activation because of damage to the vestibulospinal tract, 2) asymmetric input from the reticulospinal tract to the central pattern generating network of neurons for the fore and hind limbs, or 3) acute inflammatory reactions involving other spinal tracts. Of course, these potential explanations are not necessarily mutually exclusive.

9.6 Conclusions

In conclusion, tracts running within the ventrolateral funiculus of the cervical spinal cord are not necessary or important for locomotion when injured by themselves. These findings support the idea that there is functional redundancy of pathways running within the ventral and ventrolateral spinal funiculus of the adult rat. These findings also support the idea that it may not be necessary to have complete repair of pathways running in the ventral half of the spinal cord to have meaningful return of sensorimotor function (Loy et al. 2002a).

Chapter 10. GENERAL DISCUSSION, CONCLUSIONS, AND FUTURE STUDIES

10.1 General Discussion

The work contained within this thesis has provided valuable information for those examining sensorimotor behaviour, particularly locomotor behaviour in uninjured rats and in rats with unilateral spinal cord injury. Upon examining the sensorimotor, and especially locomotor abilities, of five different strains of rats it was apparent that not all strains of animals are morphologically similar to one another and not all strains behave similarly to each other. Specifically, the Fischer (F-344) strain was both morphologically and behaviourally most dissimilar from the other strains examined. Although not enough data could be gleaned from either the Fischer or Sprague-Dawley strains following cervical spinal cord hemisection, behavioural data from cervically spinal cord hemisected Long-Evans, Lewis and Wistar rats indicated that these three strains of rats develop similar locomotor adjustments following unilateral spinal cord injury. Experimental results within this dissertation also contribute to the ever expanding volume of knowledge pertaining to the role or importance of particular spinal pathways for overground locomotion in quadrupedal mammals. This work has provided evidence that the rubrospinal tract and ascending dorsal column pathways are important for locomotion and other sensorimotor behaviours. Combined injury to these pathways did not result in enhanced behavioural deficits, thus suggesting that there is redundancy between these two pathways. Injury to the ventrolateral funiculus (ULVL), however, results in relatively minor locomotor adjustments when injured unilaterally. These locomotor adjustments were initially observed for both skilled and unskilled locomotion but returned to near preoperative values 5.5 weeks after spinal cord injury. These results are consistent with the idea that there is a substantial amount of functional redundancy within the ventral and ventrolateral funiculi.

A long thought anecdotal belief in spinal cord injury research is that different strains may locomote differently, especially after spinal cord injury. It has been thought that these differences may account for differences between similar experiments that use different strains of animals. Based on these beliefs, as well as personally observing morphological differences between strains of rats, the experiment in Chapter 5 was executed. Differences between strain morphology (including long bone length and joints angles) may account for some of the differences observed during locomotion in some of the strains. This is important for those designing experiments to evaluate locomotion using a particular strain of rat. For example, experiments designed to examine skilled reaching or locomotion using a strain of rat with small physical stature may require custom made or altered behavioural apparatuses. The results in Chapter 5 also have potentially important implications for those evaluating therapies for experimentally induced spinal cord injury. Unfortunately, enough data could be obtained for only three of the five strains from Chapter 5 following cervical spinal cord hemisection, with a lack of data obtained from both Fischer and Sprague-Dawley rats. Although Lewis, Long-Evans and Wistar rats responded similarly to cervical spinal cord hemisection, future studies evaluating behavioural responses of Fischer rats with cervical spinal cord hemisection may identify a difference for this strain of rat. If, however, ground reaction forces are to be measured for spinal cord injured Fischer rats, the size of the force platform would need to be smaller to account for their shorter stride length following spinal cord injury (one of the reasons why ground reaction force data could not be obtained from this strain in Chapter 6). Of course, strain consideration is not an important factor for scientists comparing different groups of animals within a study providing that only one strain of rat was used. Rather, the effects of strain on sensorimotor behaviour becomes important when comparing different studies within or between laboratories when the strain of animal used is different. Recently it was shown that olfactory ensheathing glia were responsible for remarkable behavioural recovery following spinal cord transection (Ramon-Cueto 2000). Because these results showed almost unbelievable recovery, some research groups are investigating, and in some cases are attempting to reproduce these findings (Keirstead 2001). A study performed recently was unable to demonstrate the therapeutic potential of olfactory ensheathing glia as was shown by Ramon-Cueto (Takami et al. 2002a). This study, however, used the Fischer strain of rat while Ramon-Cueto (Ramon-Cueto 2000) used Wistar rats. Based on the results of uninjured animals in Chapter 5, it is possible that strain of rat could have been at least partially responsible for these conflicting results.

Chapter 7 provides evidence that a battery of behavioural tests are required to fully evaluate sensorimotor function in spinal cord injured animals. These findings are in accordance with those of others (Metz et al. 2000b). If only one particular test is used to examine behavioural recovery following spinal cord injury certain sensorimotor abilities may be overlooked, particularly if the testing procedure has not been used for a particular model of spinal cord injury. For example, the BBB locomotor rating scale is commonly used as the sole method for detecting locomotor recovery in spinal cord injured rats (Mills et al. 2001; Takami et al. 2002a; Noble et al. 2002; Takami et al. 2002b). The present work has shown that the BBB scoring system is inadequate in detecting abnormalities in locomotor abilities of cervical spinal cord injured animals compared to sham-operated controls. If the BBB locomotor rating scale was the only test used to evaluate locomotor abilities of these animals one would incorrectly conclude that cervical spinal cord injured animals had identical locomotor abilities to sham-operated animals. Of course this is an extreme example, however, it amplifies the importance of using a battery of behavioural tests to describe the locomotor adjustments rats make following spinal cord injury. The work presented within Chapter 7 illustrates the need to use sensitive measurements such as ground reaction force determination in unilateral spinal cord injury models, especially those involving the cervical spinal cord.

Aside from illustrating the importance of using a battery of behavioural tests in spinal cord injury research, Chapter 7 also provides evidence that rats develop similar locomotor adjustments following unilateral spinal cord injury regardless of the neuroanatomic level of the spinal cord injury (without damage to motor neurons to the fore or hind limbs). Thoracic spinal cord hemisected animals develop a gait similar to that adopted by cervical spinal cord injured animals. This implies that this "new" gait is the most efficient and/or practical way for unilaterally spinal cord injured animals to locomote. It may be that this gait is adopted in an attempt to provide extra stabilization during locomotion, and by doing so, this would prevent the animal from falling. Alternatively, unilateral spinal cord injury may interrupt pathways important for interlimb coordination, although the experiment in Chapter 7 was not designed to address such a possibility. Nevertheless, the work presented in Chapter 7 expands our understanding and knowledge pertaining to the locomotor adjustments animals make following unilateral spinal cord injury. This knowledge provides us with reasons why multiple testing procedures should be used to evaluate behavioural recovery following spinal cord injury and also how animals adapt their locomotor abilities to assymetrical spinal cord injury.

From the preceding chapters, it is obvious that unilateral cervical spinal cord hemisection causes animals to develop a characteristic gait. Presumably, this gait is adopted by animals that have had unilateral injury to spinal pathways important for overground locomotion. Based on this premise, the experiments described in Chapters 8 and 9 were designed to determine whether the rubrospinal tract, ascending dorsal column pathways or pathways traveling in the ventrolateral funiculus are important for overground locomotion. While the present results cannot lend further insight into the potential importance of these pathways for flexor and extensor muscle activation as has been done in work using cats (Orlovsky 1972a,b,c; Matsuyama and Drew 2000a,b; Lavoie and Drew 2002), the current results provide evidence that the ascending dorsal column pathways and pathways traveling in the dorsolateral funiculus (presumably the rubrospinal tract) are important for locomotion. Animals with injury to these pathways developed a gait reminiscent of rats with unilateral cervical spinal cord hemisection.

Until now, it was thought that animals would compensate, and consequently completely recover (almost immediately) following rubrospinal tract injury by "switching" function of the rubrospinal to the corticospinal system (Kennedy 1990). The results presented within this thesis, however, demonstrate that this switching phenomenon does not occur up to six weeks following spinal cord injury. This present work also supports the premise that the rubrospinal tract is important for locomotion in the unrestrained rat (Muir and Whishaw 2000).

The results of Chapter 8 also demonstrate that the ascending pathways traveling within the dorsal columns play a role in locomotion. Aside from two studies examining the role of the dorsal column in reaching, no study has specifically examined the motor, particularly locomotor, changes that occur following dorsal column sectioning (ascending pathways only) in rats. One study, performed in cats, has demonstrated that bilateral dorsal column injury results in animals adopting a pacing gait (where ipsilateral limbs are in-phase with one another) (English 1980). Rats with unilateral dorsal column injuries, however, develop a gait similar to that adopted by rubrospinal tract injured animals. This gait does not, however, worsen when combined with rubrospinal tract injury. These present results demonstrate that ascending dorsal column pathways are as important in overground locomotion as the rubrospinal tract. Furthermore, these results imply that there is functional redundancy of the dorsal spinal pathways with respect to behavioural recovery. Consequently, there would need to be generalized regeneration of these pathways to increase the likelihood that complete behavioural recovery would occur following spinal cord injury.

It is traditionally thought that pathways traveling in the ventral and ventrolateral funiculi are essential for locomotor initiation in the decerebrate preparation (Steeves and Jordan 1980). Recently it has been shown that severe locomotor disturbances occur in freely behaving rats with bilateral demyelination of these areas of the spinal cord together (Loy et al. 2002a). Yet when these pathways are only partially demyelinated, however, rats regain a substantial amount of their initial locomotor ability. The present work supports that of Loy et al. (2002a). The present work demonstrates that pathways traveling in the ventrolateral funiculus within the cervical spinal cord contribute minimally to locomotion. Injury to pathways traveling in the ventrolateral funiculus of adult rats appears to result in less obvious locomotor disturbances compared to animals with unilateral injury to the rubrospinal tract and/or dorsal column pathways.

Overall, this thesis provides unique information pertaining to the sensorimotor abilities of unilaterally spinal cord injured rats and uninjured rats of various strains. Further experiments are required to describe the molecular changes occurring within the spinal cord of these animals, and to exploit these behavioural changes in work evaluating therapies following spinal cord injury. Some future experiments are described in Section 10.3, although it should be noted that this is not a comprehensive list.

10.2 Conclusions

1. Fischer rats are morphologically distinct and have different sensorimotor and locomotor abilities compared to Lewis, Long-Evans, Sprague-Dawley, and Wistar rats.

2. Adult female Lewis, Long-Evans, and Wistar rats behave similarly to each other following unilateral cervical spinal cord hemisection.

3. Adult, freely behaving, female Long-Evans rats develop a characteristic gait following spinal cord hemisection regardless of whether the injury occurs above the level of the cervical enlargement or between the cervical and thoracolumbar enlargements.

4. Adult, freely behaving, female Long-Evans rats' hind limbs are more severely affected by thoracic spinal cord hemisection compared to cervical spinal cord hemisection.

5. Tactile placing is useful for identifying the neuroanatomic level of the spinal cord injury.

6. Unilateral injury to pathways traveling in the dorsolateral funiculus results in long-lasting sensorimotor behavioural changes in adult freely behaving Long-Evans rats. These changes show similarities to rats with unilateral cervical spinal cord hemisection.

7. Unilateral injury to ascending pathways traveling within the fasiculus cuneatus and gracilis result in long-lasting sensorimotor behavioural changes in adult freely behaving Long-Evans rats. These changes show similarities to rats with unilateral cervical spinal cord hemisection.

8. Unilateral injury to pathways traveling in the ventrolateral spinal funiculus result in mild, short-lasting sensorimotor behavioural changes in adult freely behaving Long-Evans rats. These animals recover to near presurgery values.

10.3 Future Studies

1. The first experimental chapter of this thesis demonstrated that Fischer rats are smaller in physical stature compared to Lewis, Long-Evans, Sprague-Dawley and Wistar rats. Although Fischer rats did not have statistically different locomotor abilities during the ladder task, these animals tended to have more footfalls compared to the other strains examined. It would be useful to design an experiment to determine whether this perceived difference actually exists. If the perceived difference does in fact exist it would be important to determine whether this difference is due to scaling effects of ladder size to strain size. This could be achieved by using the same strains, but instead of using a ladder with one size of rungs/rung spacing evaluate all of the different strains using different rung sizes and spacing. Given that Fischer rats also had abnormal hind limb posture it would also be important to examine exactly how these animals place their limbs on the ladder rungs compared to other strains.

2. Results from this work demonstrate that Fischer rats are morphologically distinct from Lewis, Long-Evans, Sprague-Dawley and Wistar rats. In addition, Fischer rats behave much differently than these strains. Because some of the larger spinal cord injury research laboratories use this strain (Takami et al. 2002a; Takami et al. 2002b) and they have found conflicting results with previously published work from other laboratories (Ramon-Cueto et al. 2000; Bethea et al. 1999) it is important to determine whether Fischer rats behave similarly to spinal cord injury. This could be accomplished by repeating the experiment in Chapter 6 and adding Fischer rats to the experiment. Based on the lack of statistical power, more than 11 animals would be required.

3. Recent studies evaluating neurotransmitters in the spinal cord of completely spinalized cats that were trained to stand or step on a treadmill have shown that untrained animals or animals trained to stand have increases in an enzyme (glutamic acid decarboxylase (GAD-67)) necessary for inhibitory neurotransmitter production within the spinal gray matter (Tillakaratne et al. 2002). Furthermore, this study showed that animals that were trained to stand unilaterally had elevated GAD-67 expression within the lumbar gray matter ipsilateral to the side trained to stand. This same study has demonstrated that animals trained to step have decreased levels of this enzyme. In the case of unilateral spinal cord injury, such as in this thesis, it would be interesting to determine whether

an asymmetry between GAD-67 mRNA exists within the cervical and thoracic enlargements, considering rats with unilateral cervical spinal cord hemisection use the left and right limbs asymmetrically.

4. As with all behavioural studies it is impossible to discern, without doubt, the behaviours that result from deficits and those that result from compensatory responses to those deficits. Recent electrophysiological studies examining neuron activity within the red nucleus (Lavoie and Drew 2002), lateral vestibular nucleus (Matsuyama and Drew 2000a) and reticular nuclei (Matsuyama and Drew 2000a) have correlated activity within these nuclei to different components of the step cycle in cats walking on a treadmill. It would be interesting to use similar methodology in experiments designed to evaluate locomoting unilateral spinal cord injured rats. Instead of evaluating electromyographs, however, it would be useful to examine both limb kinematics and ground reaction forces during locomotion. Using this combination of techniques would provide evidence to substantiate the speculated role of these neural structures in behaviours produced by unilaterally spinal cord injured rats.

5. Unilateral spinal cord injury results in an asymmetry in an animals' gait. Consequently, it has been proposed that identifying the importance of particular pathways for overground locomotion can be more readily determined in unilaterally spinal cord injured animals compared to

bilaterally spinal cord injured animals. Work from this thesis has identified the rubrospinal tract and ascending pathways within the dorsal column as being two of the pathways that result in an asymmetric gait. It would be important to determine how bilateral damage to these pathways affects sensorimotor behaviours, especially locomotion. Presumably, animals with bilateral damage to these pathways would locomote symmetrically and have symmetrical sensorimotor behavioural changes. In addition, it would be important to determine if damage to these pathways unilaterally followed by damaging the contralateral pathway some time later would cause the animals to adopt a symmetric behaviour from a previous asymmetric behaviour. Again, if the rubrospinal tract and ascending dorsal column pathways are important for locomotion, one would expect that subsequent injury to the second pathway would induce a symmetric sensorimotor behaviour.

6. Of course, having now identified the rubrospinal and ascending dorsal column pathways as being important for overground locomotion, it would be interesting to use these models for studies evaluating spinal cord regeneration or sensorimotor training to improve behavioural recovery following spinal cord injury. For example, it would be interesting to design an experiment using asymmetric training to determine whether the asymmetric behaviours outlined in this thesis would recover to near baseline values. For example, one could: 1) encourage impaired limb

usage or 2) design a treadmill training protocol such that a rat would locomote on a tilted (roll direction) treadmill so that the animal is forced to bear more weight on the limbs ipsilateral to the spinal cord injury.

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