

**SENSORIMOTOR BEHAVIOUR IN RATS AFTER LESIONS OF DORSAL
SPINAL PATHWAYS**

**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 Behavioural Neuroscience**

by

SRIKANTH G. KANAGAL

© Copyright Srikanth G. Kanagal, August, 2008

All rights reserved

PERMISSION TO USE

In agreement with the outlines set out by the College of Graduate Studies and Research at the University of Saskatchewan, I allow the University of Saskatchewan Libraries to make this thesis available to all interested parties. Also in accordance with the College of Graduate Studies and Research, I allow this thesis to be copied “in any manner, in whole or in part, for scholarly purposes”. This thesis may not, however, be reproduced or used in any manner for financial gain with my written consent. Any scholarly use of this thesis, in part or in whole, must acknowledge both myself and the University of Saskatchewan.

Any requests for copying or using this thesis, in any form or capacity, should be made to:

Head of Department of Veterinary Biomedical Sciences

University of Saskatchewan

Saskatoon, Saskatchewan

S7N 5B4

ABSTRACT

To investigate the roles of different dorsal spinal pathways in controlling movements in rats, I performed lesions of specific spinal pathways and measured the behaviour abilities of rats using different sensorimotor behavioural tests. The first experiment was designed to understand the contribution of sensory pathways traveling in the dorsal funiculus during locomotion and skilled movements using sensitive behavioural tests. I demonstrated that ascending sensory fibers play an important role during overground locomotion and contribute to skilled forelimb movements. The second experiment compared the differences in sensorimotor abilities caused by dorsal funicular lesions performed at two different levels of rat spinal cord. My results showed that the pathways present in the cervical and thoracic dorsal funiculus exert different functional effects over control of limb movement during locomotion. The third experiment investigated the compensatory potential of dorsal funicular pathways after dorsolateral funicular injuries in rats. My results showed that dorsal funicular pathways do not compensate for loss of dorsolateral pathways during the execution of locomotor tasks, though there is indirect evidence that rats with dorsolateral funicular lesions might rely more on ascending sensory pathways in the dorsolateral funiculus during skilled forelimb movements. Finally, the fourth experiment was designed to investigate the compensation from dorsolateral funicular pathways after injuries to pyramidal tract in rats. I demonstrated that pathways running in the spinal dorsolateral funiculus do provide compensatory input to spinal circuitry to maintain skilled reaching abilities after lesions of the pyramidal tract but these same pathways do not appear to compensate during either overground

locomotion or skilled locomotion. Thus, this compensatory response is task-specific. These results highlight the fact that behavioural context determines the nature of compensation from spared pathways after spinal cord injuries.

ACKNOWLEDGEMENTS

I wish to express gratitude to my supervisor, Dr. Gillian Muir for her unwavering encouragement and unstinted support during my entire PhD program. It is on the account of scientific training that she has imparted to me, that I wish and, I can hope to continue and contribute to science. Her patience and active guidance alone has brought me this far. Being part of her lab was an absolute joy where she set an inimitable example of how to balance family with research, and made me realize what education truly means, and this to me, goes far more than what ever I have learnt about science. My many thanks to her.

I thank the members of my PhD advisory committee (Drs. Baljit Singh, Ron Doucette, David Janz, Gillian Muir and Linda Hiebert) for their guidance, constructive criticisms and encouragement. Special thanks to Teresa Chu, for her inspiration and support during my initial few years. My thanks to K.S.V. Gowri, Carrol Dowdswell, Margeret Dykes and Andrea Chennettee for their technical assistance and help during my research. Thank you to Melanie Vanderloop, Sean Bennett, Cathy Dick, Laura Taylor and Nadine Poulton for their help and company. Thanks to Drs. Don Hamilton and Ron Chaplin for exposing me to wonderful joy of teaching.

Special thanks to my friends for life Seena and Prabha for being, and having been, more than my family through thick and thin and through my many mood-swings. Thanks to my close friends Muraly, Kashi and Prasad for their patient hearing and support. Thank you to Raj and Janardhan for helping me to understand and navigate through life and science and also for exposing me to scientific obsession, I am enjoying it. Thank you to

Chandrashekhar, my friend-in-need. Thanks to Tara, Lakshminarayana Reddy, Kiran, Naveen, Manju, Keshav, Shankar, Nag, Viji and Raghu for your words of encouragement, laughs, time and company.

Thanks to my master's supervisor Dr. K. Narayana for showing me the path of research and for his persistent encouragement and support.

Thanks to Diane Matovich, Joane Payne, Sandra Rose, Sheila Carron for their secretarial support. Thanks to Cathy, Darr and Jim for providing humor and making my stay a memorable one.

Lastly and most importantly, I would like to thank my family- **Anna, Amma, Sharath Attha, Mama, Raghu and Lakshmi** for their patience, unconditional love, support and encouragement. **Anna** and **Amma**, I am here because of your sacrifices, I love you very much. I also like to thank Nagesh mama for all the moral support and encouragement despite the inconveniences I put him through. I could not have done this without these people.

I dedicate this work to my wife Rama and daughter

Adithi.

It was their sacrifices, forced by my whims and fancies, which made the completion of this thesis possible. Thank you Rama, for supporting and encouraging me during my dark days, you filled me with enough courage and purpose that helped me to reach this goal. Thank you Adithi, for showing me that there is a life besides research, which is beyond everything that I had known.

I love you both, thank you.

TABLE OF CONTENTS

PERMISSION TO USE	i
ABSTRACT	ii
ACKNOWLEDGEMENTS	iv
DEDICATION	vi
List of abbreviations	xi
List of figures	xii
List of tables	xv
Chapter 1. LITERATURE REVIEW-----	1
 1.1 Introduction	1
 1.2 Neuroanatomy of spinal cord	2
 1.2.1 Introduction	2
 1.2.2 Dorsal funiculus	6
 1.2.2.1 Dorsal column-medial lemniscal pathway	7
 1.2.2.2 Post-synaptic dorsal column pathway	7
 1.2.2.3 Corticospinal tract	9
 1.2.3 Dorsolateral funiculus	12
 1.2.3.1 Spinocerebellar tract	13
 1.2.3.2 Spinohypothalamic tract	14
 1.2.3.3 Spinomesencephalic tract	15
 1.2.3.4 Rubrospinal tract	15
 1.2.3.5 Reticulospinal tract	20
 1.2.4. Pathways running in ventrolateral and ventral funiculus	21
 1.2.4.1 Coeruleospinal tract	21
 1.2.4.2 Spinomesencephalic tract	22
 1.2.4.3 Spinoreticular tracts	22
 1.2.4.4 Spinothalamic tract	23
 1.2.4.5 Vestibulospinal tracts	23
 1.3 Neural control of locomotion	24
 1.4 Neural control of skilled locomotion	27
 1.5 Neural control of skilled reaching	30
 1.6 Models commonly used to study spinal cord injury	33
 1.6.1 Complete spinal cord injury models	34
 1.6.2 Incomplete spinal cord injury models	34
 1.6.2.1 Contusion injury	35
 1.6.2.2 Compression injury	35
 1.6.2.3 Laceration injury	36
 1.6.2.4 Ischemic injury	36
 1.6.2.5 Chemical-mediated injuries	37
 1.7 Methods to assess functional recovery after partial spinal lesions in rats	38
 1.7.1 End point measures	39
 1.7.2 Qualitative measures	40
 1.7.3 Quantitative measures	41
 1.7.3.1 Quantitative kinematic analysis	41
 1.7.3.2 Kinetic analysis	42

Chapter 2. OBJECTIVES AND HYPOTHESES-----	44
2.1 Rationale	44
2.2 Objectives and hypotheses of the studies	46
 Chapter 3. GENERAL METHODS AND PROCEDURES	48
3.1 Subjects	48
3.2 Training	48
3.3 Surgery	49
3.3.1 Surgical preparation and pre-medication	49
3.3.2 Surgical procedure	49
3.4 Behavioural assessment	51
3.4.1 Training	51
3.4.2 Single pellet reaching- test for skilled forelimb usage	51
3.4.3 Ladder crossing- test for skilled locomotor abilities	54
3.4.4 Kinetic measurements- overground locomotion	54
3.4.4.1 Measurement of ground reaction forces	54
3.4.4.2 Measurement of stride parameters	58
3.5 Histology- microscopic evaluation of the lesion site	58
3.6 Statistical analyses	58
 Chapter 4. BILATERAL DORSAL FUNICULAR LESIONS ALTER SENSORIMOTOR BEHAVIOUR IN RATS-----	60
4.1 Abstract	60
4.2 Introduction	61
4.3 Materials and Methods	63
4.3.1 Animals	63
4.3.2 Surgery	63
4.3.3 Behavioural assessment	63
4.3.3.1 Training	63
4.3.3.2 Skilled reaching- single pellet reaching	64
4.3.3.3 Skilled locomotion -Horizontal ladder	64
4.3.3.4 Overground locomotion	64
4.3.3.4.1 Kinematic measurement of ground reaction forces	64
4.3.3.4.2 Measurement of stride parameters	65
4.3.4 Histology	65
4.3.5 Statistical analyses	65
4.4 Results	65
4.4.1 Histology	65
4.4.2 Behavioural assessment	67
4.4.2.1 Skilled reaching- single pellet reaching	67
4.4.2.2 Skilled locomotion: horizontal ladder	70
4.4.2.3 Overground locomotion	70
4.4.2.3.1 Analysis of ground reaction forces	70
4.4.2.3.1 Step lengths and stride parameters	72
4.5 Discussion	75
4.6 Conclusions	82

Chapter 5. THE DIFFERENTIAL EFFECTS OF CERVICAL AND THORACIC DORSAL FUNICULUS LESIONS IN RATS-----	84
5.1 Abstract	84
5.2 Introduction	85
5.3 Materials and Methods	87
5.3.1 Animals	87
5.3.2 Training	88
5.3.3 Surgery	88
5.3.4 Behavioural assessment	88
5.3.4.1 Overground locomotion	88
5.3.4.1.1 Measurement of ground reaction forces	88
5.3.4.1.2 Measurement of stride parameters	89
5.3.4.2 Skilled locomotion -Horizontal ladder	89
5.3.5 Histology	89
5.4 Results	89
5.4.1 Histology	89
5.4.2 Behavioural assessment	91
5.4.2.1 Overground locomotion	91
5.4.2.1.1 Analysis of ground reaction forces (GRF)	91
5.4.2.1.2 Step lengths and stride parameters	93
5.4.2.2 Skilled locomotion: horizontal ladder	97
5.5 Discussion	97
5.6 Conclusions	103
 Chapter 6. EFFECTS OF COMBINED DORSOLATERAL AND DORSAL FUNICULAR LESIONS ON SENSORIMOTOR BEHAVIOUR IN RATS-----	104
6.1 Abstract	104
6.2 Introduction	105
6.3 Materials and Methods	107
6.3.1 Animals	107
6.3.2 Training	108
6.3.3 Lesion groups	108
6.3.4 Surgery	108
6.3.5 Behavioural assessment	109
6.3.5.1 Overground locomotion	109
6.3.5.1.1 Kinetics and kinematics	109
6.3.5.1.2 Measurement of stride parameters	109
6.3.5.2 Skilled locomotion -Horizontal ladder	109
6.3.5.3 Skilled reaching- single pellet reaching	110
6.3.6 Histology	110
6.3.7 Statistical analysis	110
6.4 Results	110
6.4.1 Histology	110
6.4.2 Behavioural assessment	113
6.4.2.1 Overground locomotion	113
6.4.2.1.1 Analysis of ground reaction forces (GRF)	113

6.4.2.1.2 Step lengths and stride parameters	117
6.4.2.2 Skilled locomotion- horizontal ladder	119
6.4.2.3 Skilled fore-paw usage- single pellet reaching	121
6.5 Discussion	124
6.6 Conclusions	134
Chapter 7. TASK-DEPENDENT COMPENSATION AFTER PYRAMIDAL TRACT AND DORSOLATERAL SPINAL LESIONS IN RATS-----	135
7.1 Abstract	135
7.2 Introduction	136
7.3 Materials and Methods	139
7.3.1 Subjects	139
7.3.2 Behavioural training	139
7.3.3 Experimental plan	139
7.3.4 Surgeries	140
7.3.5 Behavioural measurements	140
7.3.5.1 Overground locomotion	141
7.3.5.1.1 Kinetics and kinematics	141
7.3.5.1.2 Measurement of stride parameters	141
7.3.5.2 Skilled locomotion -Horizontal ladder	141
7.3.5.3 Skilled reaching- single pellet reaching	141
7.3.6 Histology	141
7.3.7 Statistical analysis	142
7.4 Results	142
7.4.1 Histology	142
7.4.2 Experimental groups	145
7.4.3 Behavioural assessment	146
7.4.3.1 Overground locomotion	146
7.4.3.1.1 Analysis of ground reaction forces (GRF)	146
7.4.3.1.1 Step lengths and stride parameters	154
7.4.3.2 Skilled locomotion- Horizontal ladder	158
7.4.3.3 Skilled fore-paw usage- single pellet reaching	162
7.5 Discussion	167
7.6 Conclusions	178
Chapter 8. GENERAL DISCUSSION AND CONCLUSIONS-----	179
8.1 Corticospinal system	179
8.2 Rubrospinal system	181
8.3 Ascending sensory pathways	184
8.4 Conclusions	186
List of publication	189
Reference list	190
Appendix	215

List of abbreviations

ASP	Ascending sensory pathways	Main CST	Main corticospinal tract
C2-3	Cervical spinal level 2,3	MedSTT	Medial spinothalamic tract
CNS	Central nervous system	MLR	Mesencephalic locomotor region
CPG	Central pattern generator	PSDCP	Poly-synaptic dorsal column pathway
CS	Coeruleospinal tract	PT	Pyramidal tract
CST	Corticospinal tract	ReticST	Reticulospinal tract
DC	Dorsal columns	RF	Reticular formation
DCML	Dorsal column medial leminiscal pathway	RSCT	Rostral spinocerebellar tract
DF	Dorsal funiculus	RST	Rubrospinal tract
DLF	Dorsolateral funiculus	SC	Spinal cord
DSCT	Dorsal spinocerebellar tract	SCI	Spinal cord injury
FC	Fasciculus cuneatus	SHT	Spinohypothalamic tract
FG	Fasciculus gracilis	SMT	Spinomesencephalic tract
GRF	Ground reaction forces	T8	Thoracic spinal level 8
LatCST	Lateral corticospinal tract	VCST	Ventral corticospinal tract
LatSTT	Lateral spinothalamic tract	VSCT	Ventral spinocerebellar tract
LED	Light emitting diode	VST	Vestibulospinal tract

List of Figures

Fig 1.1	Schematic representation of spinal cord showing dorsal, lateral and ventral funiculi	3
Fig 1.2	Schematic representation of cat spinal cord showing location of different Rexed's laminae	4
Fig. 1.3	Diagrammatic representation showing approximate locations of different ascending and descending pathways in rat spinal cord	8
Fig 3.1	Picture of a rat in reaching box	52
Fig 3.2	Picture of a rat walking over horizontal ladder	55
Fig 3.3	Picture of a rat trotting across force platform	57
Fig 4.1	Schematic drawing representing the lesion epicenter in ASP lesioned and ASP+CST lesioned rats	66
Fig 4.2	Percentage successful reaches in ASP and ASP+CST rats.	68
Fig 4.3	Qualitative reaching scores in ASP and ASP+CST rats.	69
Fig 4.4	Percentage correct steps of the forelimbs while crossing a horizontal ladder by ASP and ASP+CST rats	71
Fig 4.5	Fore-aft forces generated by pre-surgical, ASP lesioned and ASP+CST lesioned rats.	73
Fig 4.6	Vertical forces produced by the forelimbs and hindlimbs for presurgical, ASP lesioned and ASP+CST lesioned rats.	74
Fig 4.7	Forelimb and hindlimb contact timing for pre-surgical, ASP lesioned and ASP+CST lesioned rats.	76
Fig 5.1	Representative photomicrographs of spinal cords from sham, cervical DF and thoracic DF lesioned groups.	90
Fig 5.2	Vertical forces produced by the forelimbs and the hindlimbs for pre-surgical, cervical DF lesioned and thoracic DF lesioned rats.	92
Fig 5.3	Fore-aft forces generated in for pre-surgical, cervical DF lesioned and thoracic DF lesioned rats.	94

Fig 5.4	Forelimb and hindlimb contact timing for pre-surgical, cervical DF lesioned and thoracic DF lesioned rats.	95
Fig 5.5	Graphs representing percentage of forelimb and hindlimb correct steps while crossing horizontal ladder by cervical DF lesioned and thoracic DF lesioned rats.	98
Fig 6.1	Representative photomicrographs of spinal cords from DLF followed by ASP and DLF followed by DF lesioned rats.	111
Fig 6.2	Vertical forces produced by the forelimbs and the hindlimbs for pre-surgical, DLF lesioned, DLF+ASP lesioned and DLF+DF lesioned rats.	115
Fig 6.3	Fore-aft forces generated in pre-surgical, DLF lesioned, DLF+ASP lesioned and DLF+DF lesioned rats.	116
Fig 6.4	Forelimb and hindlimb contact timing for pre-surgical, DLF lesioned, DLF+ASP lesioned and DLF+DF lesioned rats.	118
Fig 6.5	Forelimb and hindlimb correct steps during ladder walking as percentage of pre-surgical performance for DLF+ASP lesioned and DLF+DF lesioned rats.	120
Fig 6.6	Reaching ability as a percentage of pre-surgical performance in rats with DLF+ASP and DLF+DF lesions.	122
Fig 6.7	Qualitative reaching success in DLF lesioned, DLF+ASP lesioned and DLF+DF lesioned rats.	123
Fig 7.1	Schematic drawings representing the epicenter of pyramidal tract and spinal lesions in PT followed by DLF lesioned, simultaneous PT+DLF lesioned, DLF lesioned and PT followed by DLF+DF lesioned rats.	143
Fig 7.2	Vertical forces produced by the forelimbs fore PT followed by DLF lesioned, DLF lesioned and simultaneous PT-DLF lesioned rats.	147
Fig 7.3	Fore-aft forces produced by the forelimbs fore PT followed by DLF lesioned, DLF lesioned and simultaneous PT-DLF lesioned rats.	152
Fig 7.4	Forelimb and hindlimb contract timing in PT followed by DLF lesioned, DLF lesioned and simultaneous PT-DLF lesioned rats.	155
Fig 7.5	Forelimb and hindlimb correct steps as percentage of pre-surgical ability during ladder walking in PT followed by DLF lesioned, DLF lesioned and simultaneous PT-DLF lesioned rats.	159

Fig 7.6	Individual ladder walking data expressed as percentage pre-surgical performance from V11, KS16 and KS20 rats.	161
Fig 7.7	Reaching ability as a percentage of pre-surgical performance in PT followed by DLF lesioned, DLF lesioned and simultaneous PT-DLF lesioned rats.	163
Fig 7.8	Individual pellet reaching data expressed as percentage pre-surgical performance from V11, KS16 and KS20 rats.	166
Fig 7.9	Qualitative reaching scores in PT followed by DLF lesioned, DLF lesioned and simultaneous PT-DLF lesioned rats.	168

List of tables

Table 4.1	Stance duration of the forelimbs expressed as proportion of stride duration in cervical DF lesioned rats.	96
Table 6.1	Stance duration of limbs expressed as proportion of stride duration and mean trotting speeds in DLF+ASP lesioned and DLF+DF lesioned rats.	114
Table 6.2	Summary of behavioural outcomes associated with dorsal spinal lesions.	125
Table 7.1	Stance duration of limbs expressed as proportion of stride duration and mean trotting speeds in PT followed by DLF lesioned, DLF lesioned and simultaneous PT-DLF lesioned rats.	150

Chapter 1. LITERATURE REVIEW

1.1 Introduction

Spinal cord injuries often cause non-specific damage to spinal pathways, which are not confined to any particular pathway. The end result is a combination of deficits due to loss of all the pathways that are damaged. As a first step, it is pertinent to understand how each of the spinal pathways contributes during normal/trained behavioural tasks. In this dissertation, I have approached this issue by performing selective and specific lesions of pathways in different areas of spinal cord and studying their effects during various sensorimotor tasks.

The main focus of the work presented in this dissertation pertains to understanding the roles of dorsal spinal pathways in execution of sensorimotor behaviours. The dorsal half of the spinal cord contains both ascending sensory and descending supraspinal pathways. These include 1) pathways travelling in the dorsal funiculus, namely fasciculus gracilis, fasciculus cuneatus and corticospinal tract and 2) those travelling in the dorsolateral funiculus, namely dorsal spinocerebellar tract and rubrospinal tract.

Chapter 1 reviews the information about 1) the neuroanatomy of rat spinal cord, 2) basis for neural control of locomotion, 3) basis for neural control of skilled locomotion, 4) basis for neural control of skilled limb movements 5) spinal cord injury models available for research, and 6) methods to assess functional recovery after partial spinal cord lesions in rats.

1.2 Neuroanatomy of spinal cord

1.2.1 Introduction

In this section, a brief review of the spinal cord anatomy will be presented. Later in the section, different spinal pathways and their roles during various behaviours will be discussed.

The spinal cord is the caudal extension of brain and is part of CNS. It acts as a conduit for pathways travelling up to or from the brain. Histologically, spinal cord cross sections reveal two distinct areas- a central “H” shaped dark region called the gray matter and a surrounding rim of white matter (Fig.1.1).

Gray matter of the spinal cord

The gray matter forms the central core of the spinal cord. It is comprised of cell bodies. Based on the morphology and arrangement of Nissl-stained cell bodies in the cross-sections of cat spinal cord, Rexed in 1952 presented a cytoarchitectonic scheme for the gray matter. According to this classification, gray matter is divided into 10 cytoarchitectonic regions, laminae I- IX and an area around the central canal (lamina X). This classification is followed even in rats with some alterations (Fig.1.2).

White matter pathways in the rat

The white matter is composed of bundles of axons traversing all along the length of the spinal cord. In quadrupedal species, the white matter, based on location from most dorsal to ventral, is classified as the dorsal funiculus, dorsolateral funiculus, ventrolateral

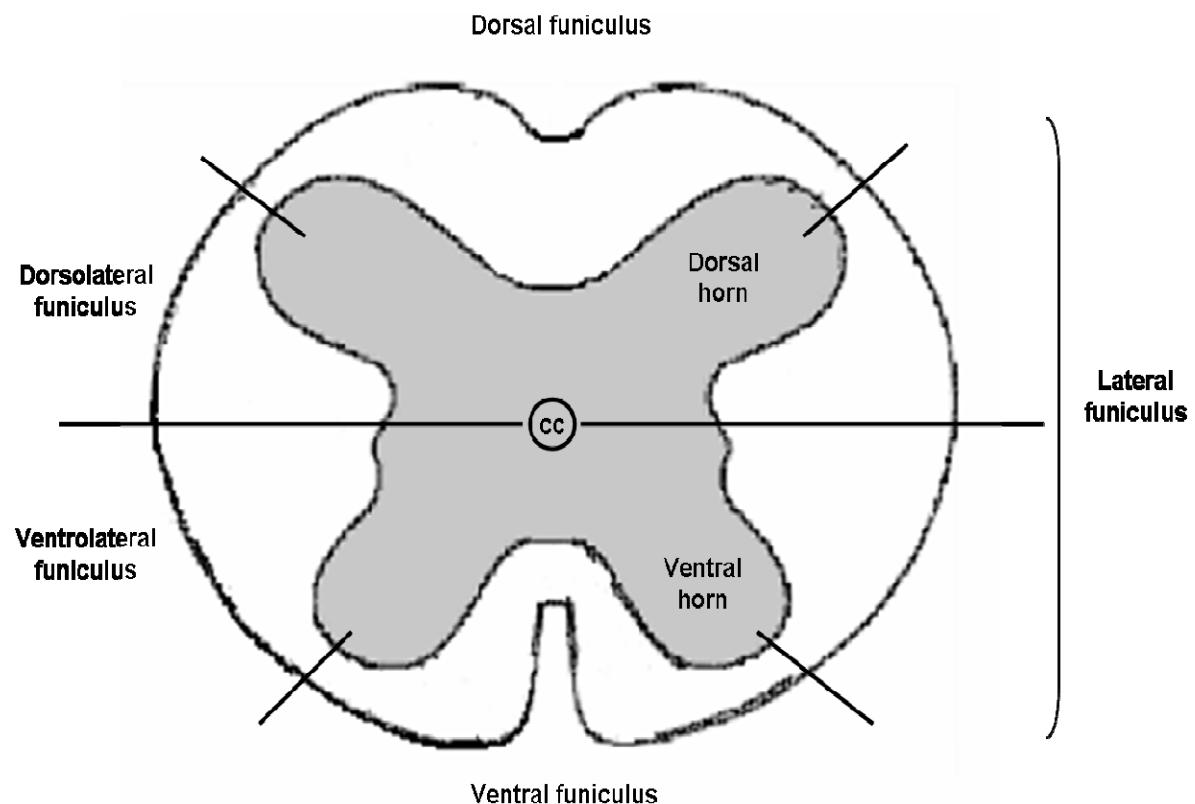


Fig.1.1. Schematic representation of spinal cord showing location of dorsal, dorsolateral, ventrolateral and ventral funiculi of spinal white matter. The gray matter is divided into the dorsal and ventral horns. (cc- central canal) (modified from Webb, 2003)

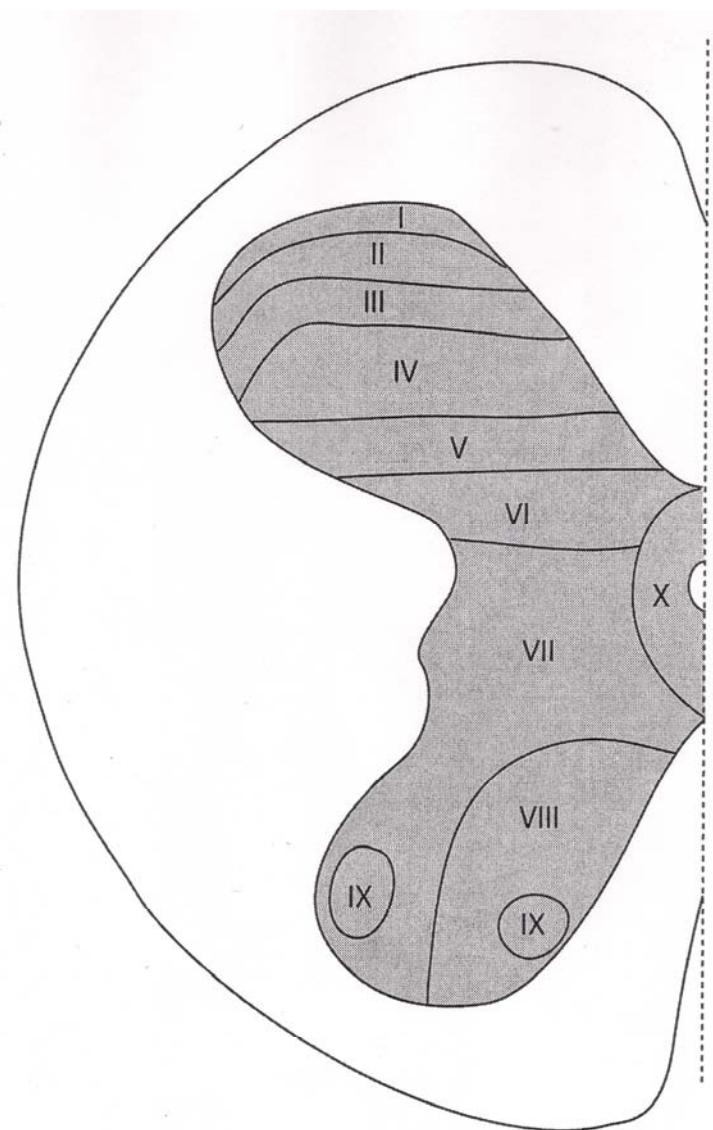


Fig.1.2. Schematic representation of cat cervical spinal cord showing location of different Rexed's laminae. Based on the Nissl stained cytoarchitectural features, Rexed divided the gray matter into ten laminae (I- X). (Adopted from Webb, 2003).

funiculus and ventral funiculus (Fig.1.1). Each quadrant has an admixture of different pathways, which though anatomically indistinguishable from each other, are functionally categorized as either ascending or descending pathways.

The pathways which carry information from sensory receptors and interneurons to the brain are collectively called ascending pathways and their cell bodies are present either in the dorsal root ganglion or in the spinal cord. The pathways which transmit motor commands from brain are called descending or supraspinal pathways and their cell bodies are present in the brain. In addition to relaying motor information, descending pathways are also involved in modulating the transmission of sensory information from the spinal cord to supraspinal levels. All ascending and descending pathways in the white matter of the spinal cord are bilaterally represented. Apart from these pathways, the white matter is also composed of propriospinal neurons, which connect one segment of the spinal cord with another. Propriospinal axons constitute approximately 33% of the axons in the sacral white matter (Chung and Coggeshall, 1983, Chung et al., 1987). The cell bodies of propriospinal axons are located in all laminae except lamina IX and project to the ipsilateral dorsal and ventral horns and lamina X, as well as to the contralateral cord (Menetrey et al., 1985; Matsushita, 1998).

Though neuroanatomy of the spinal pathways is homologous across the species, there still exist inter-species differences in terms of location and function of spinal pathways. To illustrate with an example, the corticospinal tract, which is a direct pathway from cerebral cortex to the spinal cord, is located in the dorsolateral funiculus of spinal cord in

cats, dogs and humans (Brown, 1971; Verhaart, 1962b; Webster et al., 1990; Lemon and Griffiths, 2005). This pathway has terminations on motor neurons. These direct motoneuronal connections suggest an important role in motor control in humans and primates. In rats and opossums, the corticospinal tract is located in the dorsal columns and it does not have any direct motoneuronal connections in rats, implying an indirect role in motor control (Lemon and Griffiths, 2005; Lemon, 2008).

In the recent past, due to the use of efficient tracing and labeling techniques, our knowledge of the organization of spinal pathways in rodents has improved. Still, much of our understanding about the role of pathways during locomotion and motor control comes from studies involving cats.

This chapter proposes to highlight the neuroanatomy of the rat spinal cord with emphasis on the dorsal spinal pathways, which is the focus of this thesis. The reader is referred to Fig.1.3 for diagrammatic representation of dorsal spinal pathways.

1.2.2 Dorsal funiculus

The pathways travelling in the dorsal funiculus are also referred to as the dorsal columns. In humans, these are composed of mostly ascending fibers. In the rat, the dorsal funiculus contains both ascending and descending pathways. Ascending pathways are composed of two groups of ascending fibers 1) the direct dorsal column pathway, composed of ascending collaterals of primary afferents and 2) the post-synaptic dorsal column pathway, which is made up of axons from spinal neurons projecting to other segments of

the spinal cord and to supraspinal centers (dorsal column-medial lemniscal pathway and ascending post-synaptic dorsal column pathway).

The corticospinal tract is the only descending pathway in the dorsal funiculus and that travels in the ventral-most part of the dorsal funiculus. The organization of these pathways has important implications for several of the studies described in this thesis (refer to Chapter 4 and Chapter 6).

1.2.2.1 Dorsal column-medial lemniscal pathway

These are the ascending collaterals of sensory neurons with cell bodies in dorsal root ganglia. These axons are somatotopically arranged rostro-caudally, such that fibers from the lumbar spinal cord occupy the medial position and are named the fasciculus gracilis (Fig.1.3). Fibers from the cervical and thoracic spinal cord are progressively added to the lateral border of the columns and are named the fasciculus cuneatus (Fig.1.3) (Giesler et al., 1984). Fibers running in the fasciculus gracilis and fasciculus cuneatus terminate in the gracile and cuneate nuclei, respectively within the medulla. The sensory information from these tracts is then relayed to the contralateral nuclei in the ventrobasal thalamus and the somatosensory cortex. The dorsal column-medial lemniscus system transmits information about mechanosensation and kinesthesia (Angaut-Petit, 1975).

1.2.2.2 Post-synaptic dorsal column pathway

The vast majority of primary afferent fibers do not project directly to the dorsal column nuclei, but terminate on spinal neurons whose axons then project to the gracile and cuneate nuclei (Al-Chaer et al., 1996, Willis et al., 1999). These axons of post-synaptic

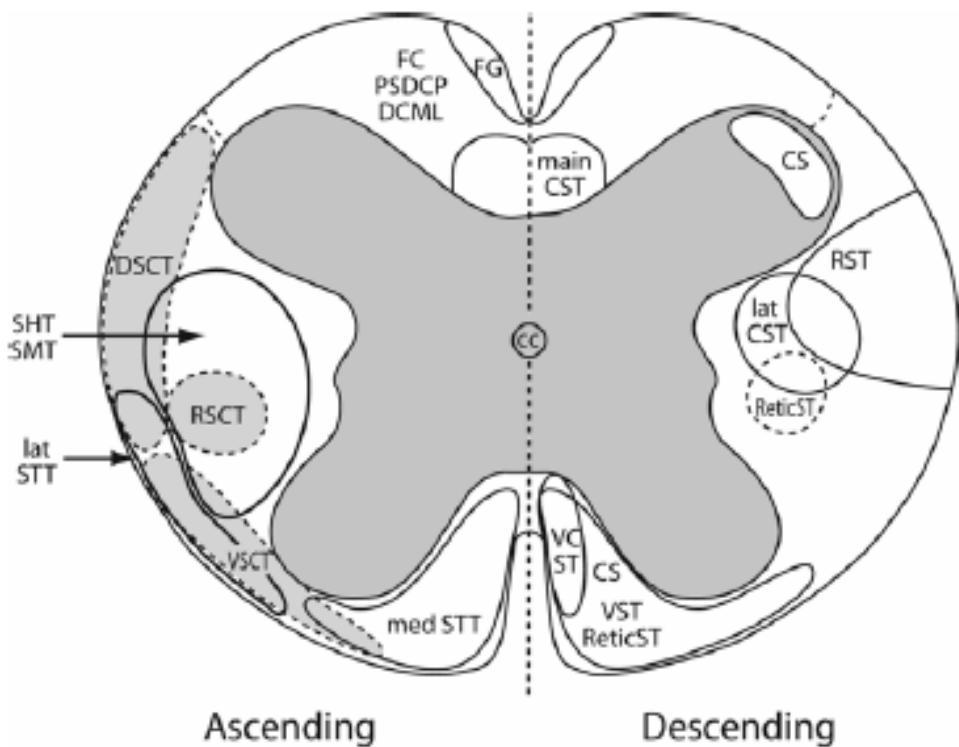


Fig.1.3. Diagrammatic representation showing approximate locations of different ascending (left half of the diagram) and descending (right half of the diagram) pathways in rat spinal cord. (Diagram adopted from Webb, 2003).

Ascending pathways- **1)** DSCT (dorsal spinal cerebellar tract; Yamada et al., 1991), **2)** VSCT (ventral spinal cerebellar tract; Yamada et al., 1991; Xu and Grant, 1994; Terman et al., 1998), **3)** RSCT (rostral spinocerebellar tract; Xu and Grant, 1994; Terman et al., 1998), **4)** SHT (spinohypothalamic tract; Katter et al., 1996a; Kostarczyk et al., 1997), **5)** SMT (spinothalamic tract; Zemlan et al., 1978); **6)** LatSTT (lateral spinothalamic tract; Giesler Jr. et al., 1981; Dado et al., 1994c), **7)** MedSTT (medial spinothalamic tract; Giesler Jr. et al., 1981; Dado et al., 1994c), **8)** FC (fasciculus cuneatus; Giesler, Jr. et al., 1984), **9)** FG (fasciculus gracilis; Giesler, Jr. et al., 1984), **10)** PSDCP (poly synaptic dorsal column pathway; Giesler, Jr. et al., 1984) and **11)** DCML (dorsal column medial lemniscal pathway (Willis and Coggenshall, 1978).

Descending pathways- **1)** Main CST (main crossed corticospinal tract; Brown, Jr. 1971), **2)** Lat CST (lateral crossed corticospinal tract; Brosamle and Schwab, 1997), **3)** VCST (ventral uncrossed corticospinal tract; Terashima, 1995; Brosamle and Schwab, 1997), **4)** CS (coeruleospinal tract; Clark and Proudfoot, 1992), **5)** VST (vestibulospinal tract; Houle and Jin, 2001; Matesz et al., 2002), **6)** ReticST (reticulospinal tract; Fox, 1970; Houle and Jin, 2001) and **7)** RST (rubrospinal tract; Brown, 1974; Antal et al., 1992).

dorsal column pathways are located primarily in lamina 4 or 10. These axons are thought to convey information pertaining to visceral (Al-Chaer et al., 1996, Willis et al., 1999), mechanical, thermal nociception (Angaut-Petit, 1975) and also mechanosensation (Angaut-Petit, 1975).

1.2.2.3 Corticospinal tract

The corticospinal tract is present only in mammals. There is a huge difference in corticospinal system across the species. In primates, dogs, cats and rodents it is well developed, while in domestic farm animals like sheep and horses it is less well developed, its function being replaced by the corticotegmental tract.

In most animals, fibers of corticospinal tract originate from the neurons in lamina V (called the Betz cells) of the sensory-motor cortex. In rats,, they also arise from other cortical areas including frontal and prefrontal cortex (Li et al., 1990; Miller, 1987). The corticospinal axons descend through the internal capsule into the cerebral peduncles and extend caudally at the ventral surface of the medulla oblongata (at this level they are called the pyramidal tract). At the level of medulla and spinal cord, the corticospinal axons decussate incompletely and continue caudally to different segments of the spinal cord. In domestic farm animals like sheep and goats, the axons extend only to the upper cervical levels (Verhaart, 1962), while in rats, axons descend as far as lumbosacral levels (Brown jr, 1971; Terashima, 1995a). The corticospinal tract is divided into crossed and uncrossed components. In rodents and opossums, the main crossed corticospinal tract (dorsal corticospinal tract) which constitutes about 90-95% of the total descending fibers descends and continues in the dorso-ventral portion of the dorsal funiculus (Fig.1.3;

Brown jr, 1971; Weidner et al., 2001). It is at this position, in the dorsal funiculus, that the CST is damaged in many injury paradigms. It is important to note, however, that any injury aimed at transection of the CST in the dorsal funiculus also necessitates damage to overlying ascending sensory fibres, a point that has, until recently, been overlooked in many studies of CST function. I address this issue in Chapter 4. In other animals, including carnivores and primates, the main corticospinal tract fibers descend in the dorsolateral funiculus (Brown, Jr., 1971). In rodents, some portion of the crossed corticospinal fibers also travel in the dorsolateral funiculus (called the lateral corticospinal tract), which constitutes about 1-2% of the total fibers (Fig.1.3; Steward et al., 2004). Apart from this, a small number of uncrossed fibers also travel in the ventromedial funiculus of the spinal cord (called the ventral corticospinal tract). This constitutes about 1-3% of total corticospinal fibers (Fig.3; Brosamle and Schwab, 1997). In rats, the corticospinal neurons also send collaterals to the midbrain, trigeminal nuclei (Catsman and Kuypers, 1981), pontine nuclei and red nucleus (Akintunde and Buxton, 1992).

There are differences in the termination pattern of corticospinal tract in the spinal cord across the species. In rodents, they terminate most densely in laminae III-VI of the dorsal horn with sparse terminations in ventral horn (Antal et al., 1984; Casale et al., 1988; Liang et al., 1991; Brosamle and Schwab, 2000). In contrast to rodents, corticospinal fibers in primates and humans have dense terminations in the ventral horns (Porter and Lemon, 1993).

The role of the corticospinal tract has been studied extensively, but yet, the exact role of corticospinal tract in rodents is still unknown. Recent evidence seems to suggest that in rodents, corticospinal tract is more important in modulation of sensory information than in the direct motor control (Lemon and Griffiths, 2005). Nevertheless, corticospinal tract is important for the control of fine hand and finger movements in the humans, primates, cats and rats (Lemon and Griffiths, 2005;Lemon, 2008;Porter and Lemon, 1993;Alstermark et al.,1981;Alstermark et al., 1987;Pettersson et al., 2007;Pettersson et al., 2000; Piecharka et al., 2005;Whishaw et al., 1993;Whishaw et al., 1998;Whishaw and Metz, 2002). Evidence for this has come mostly from lesion studies.

Damage to the corticospinal tract at the medullary pyramids in rats impairs food handling with the forepaws, reaching for food with a forelimb (skilled reaching) including the rotary movements of both the proximal and distal forelimb segments during reaching. (Whishaw et al., 1993;Whishaw et al., 1998) These lesions also impair the ability of rats to walk over challenging terrain such as grids, or horizontal ladders, implying that the corticospinal tract is involved in voluntary modification of gait (Klapka et al., 2005;Piantino et al., 2006;Vavrek et al., 2006) similar to as seen in cats and primates (Whishaw et al., 1986;Lawrence and Kuypers, 1968a;Alstermark et al., 1989). Remarkably, sparing of even a small amount of the pyramidal tract is enough to produce recovery (Terashima, 1995;Piecharka et al, 2005). Spinal CST lesions cause similar changes but are not as severe as pyramidal lesions (Anderson et al., 2005; Li et al., 1997). Damage to the dorsal corticospinal tract in the cervical spinal cord reduced the ability of rats to reach for food pellets, but the animals recovered reaching ability by four weeks

post-lesions (Weidner et al., 2001). However, this functional recovery was lost if the ventral uncrossed pathway are additionally injured (Weidner et al., 2001). Thus it seems that the ventral component, though comprising a mere 1-3% of total corticospinal fibers (compared to 90-95% of dorsal component), has a huge capacity to compensate for the loss of dorsal corticospinal tract. To add to the literature about the effects of CST lesion at different levels during its descent, in the studies presented in thesis, I have performed CST lesions at the level of the caudal medulla (Chapter 7), cervical level (Chapter 4) and mid-thoracic level (Chapter 5)

In addition to control of reaching and grasp, the functions of the corticospinal tract include tactile placing (Hicks and D'amato, 1975;Donatelle, 1977), descending control of afferent inputs including nociception (Cheema et al., 1985;Wall and Lidierth, 1997), selection, gating and gain control of spinal reflexes (Chen and Wolpaw, 2002; Lemon, 2008), excitation and inhibition of motoneurons (Lemon, 2008; Maier et al., 1998) and long term plasticity of spinal cord circuits (Wolpaw, 1997).

1.2.3 Dorsolateral funiculus

In the dorsolateral funiculus, ascending pathways such as the dorsal spinal cerebellar tract, spinohypothalamic tract, spinomesencephalic tracts course along with the descending tracts such as rubrospinal and reticulospinal tracts

1.2.3.1 Spinocerebellar tract

The spinocerebellar tract has 3 divisions in the spinal cord, namely the 1) dorsal, 2) ventral and 3) rostral spinocerebellar tracts (Fig.1.3) based on the site of origin and sensory information they transmit. The ventral spinocerebellar tract which arises from Clarke's nucleus and large cells in laminae VII and IX (spinal border cells) of the lumbar spinal cord transmit sensory information from the hindlimbs. The dorsal spinocerebellar tract arises from the Clarke's nucleus in the thoracic spinal cord and transmits sensory information from the tail, trunk and a portion of hindlimbs. The rostral spinocerebellar tract arises from the nucleus centrobasalis (within the cervical enlargement) and central cervical nucleus (in the upper cervical segments) and transmits sensory information from the forelimbs (Yamada et al., 1991).

Spinocerebellar fibers enter and continue in the lateral funiculus of the spinal cord. The axons of both the dorsal and ventral spinocerebellar tracts overlap during their ascent to the cerebellum via the rostral cerebellar peduncle (Yamada et al., 1991). In rats, some of the ventral spinocerebellar fibers also enter the cerebellum via the caudal cerebellar peduncle (Yamada et al., 1991). Spinocerebellar fibers entering the cerebellum are called mossy fibers and terminate on the granule cells of the cerebellar cortex and on interposed and fastigial cerebellar nuclei (Bloedel and Courville, 1981). While the axons of rostral and dorsal spinocerebellar tracts ascend predominantly to the ipsilateral cerebellum (Snyder et al., 1978; Tracey et al., 1988; Xu and Grant, 1994), the ventral spinocerebellar tract decussates twice before terminating in the cerebellum, ipsilateral to the cells of their origin (Bloedel and Courville, 1981, Tracey et al., 1988).

Besides these divisions, other spinocerebellar axons, originating from Stilling's nucleus and the ventrolateral nucleus (Terman et al., 1998) have been described for the sacral and coccygeal regions of spinal cord. Though they are not considered as part of the dorsal spinocerebellar tract, they ascend in the DLF before terminating in the cerebellum.

Though the spinocerebellar system transmits both proprioceptive and exteroceptive information to the cerebellum (Bloedel and Courville, 1981), very little is known about the role of spinocerebellar system during locomotion. Bilateral lesions involving dorsal spinocerebellar tract in cats do not affect temporal gait patterns (English, 1985) but they might affect the inter-limb coordination (Poppele et al., 2003).

1.2.3.2 Spinothalamic tract

The neuron cell bodies of the spinothalamic are located within lamina I (lateral reticular area) and lamina X (Giesler, Jr. et al., 1994). The axons of these cells cross over to the contralateral spinal cord and ascend deep within the dorsolateral funiculus (Katter et al., 1996a; Kostarczyk et al., 1997). The fibers travel through the brainstem and thalamus and terminate ipsilaterally in several regions in the hypothalamus, including the lateral, caudal and dorsal areas (Giesler Jr et al, 1994; Cliffer et al., 1991; Wang et al., 1999). The spinothalamic tract is known to transmit information about noxious stimuli (Burstein et al., 1991; Katter et al., 1996b) and visceral pain (Zhang et al., 2002). Because of its direct connection with the hypothalamus, it is also suspected that it is involved in mediating autonomic and endocrine responses to noxious stimuli such as

elevations in blood pressure, increased blood circulation to heart and skeletal muscles and decreased blood flow to viscera and skin, and elevations in cortisol (Burstein et al., 1996, Giesler, Jr. et al., 1994).

1.2.3.3 Spinomesencephalic tract

The spinomesencephalic tract neurons originate in laminae I, IV, V and VI in the dorsal horn of the spinal cord and are mainly located in the cervical cord (Yezierski and Mendez, 1991). The fibers ascend in the lateral funiculus to terminate in areas of midbrain like intercollicular nucleus, deep layers of superior colliculus, the external nucleus of the inferior colliculus, the central gray and the cuneiform nucleus. These areas of the midbrain receive nociceptive inputs and form part of the neural circuitry involved in localization or descending control of pain (Willis and Westlund, 1997). There are also terminations in the anterior and posterior pretectal nuclei, the red nucleus, the Edinger-Westphal nucleus and the interstitial nucleus of Cajal (Menetrey et al., 1982; Willis and Westlund, 1997, Yezierski, 1988). The spinomesencephalic tract is important in transmitting information regarding pain and thought to be involved in integrating somatic sensation with visual and auditory information (Menetrey et al., 1982; Willis and Westlund, 1997)

1.2.3.4 Rubrospinal tract

The rubrospinal tract arises in the midbrain (mesencephalic tegmentum) from paired structures called the red nuclei. The red nucleus is present on both sides, at the level of the rostral colliculus, ventrolateral to the oculomotor nucleus and dorsal to substantia

nigra (Boseila et al., 1975). The red nucleus sends projecting fibers to the contralateral spinal cord and also receives afferent input from the contralateral cerebellum and cerebral cortex. Phylogenetically, the red nucleus and the rubrospinal tract have an interesting feature in that they are present in lower and higher vertebrates including amphibians and reptiles, as well as birds and mammals (Ten Donkelaar, 1988) but are markedly reduced in apes and humans in comparison with quadrupedal mammals (Massion, 1988). It has been suggested that during evolution, the red nucleus and the rubrospinal tract appeared with the appearance of extremities, where the rubrospinal tract is important for the independent use of the limbs. In contrast the development of the neocerebellum and the corticospinal tract in apes and humans resulted in a new level of reorganization of motor control, making the rubrospinal system redundant (Massion, 1988). In rats and cats, both the corticospinal tract and the rubrospinal tract are well developed and share many common characteristics including spinal innervation (Gorska and Sybirska, 1980; Martin and Ghez, 1988; Sybirska and Gorska, 1980; Kennedy, 1990).

The red nucleus has two divisions: 1) the magnocellular (large cell) and 2) the parvocellular (small cell) components (Jerath, 1964). Across species, there are huge variations in the development of these two divisions. In primates, magnocellular portion is very small but it is very well developed in opossums, while the parvocellular portion is well developed in primates. In cats and rats there are no distinct magnocellular or parvocellular divisions (Adogwa and Lakshminarsimhan, 1982).

The magnocellular portion of the red nucleus in monkeys receives input from the cerebral cortex and interposed nucleus of the cerebellum, and sends afferents to the spinal cord (Oka and Jinnai, 1978; Flumerfelt, 1978). The parvocellular portion of the red nucleus receives afferent information from the parietal association cortex, and from the dentate nucleus of the cerebellum in cats, and sends efferents to the ipsilateral inferior olfactory nucleus in rats, cats and monkeys (Kennedy et al., 1986; Oka and Jinnai, 1978; Flumerfelt, 1978). Interestingly, the inferior olfactory nucleus (which sends afferents to the dentate nucleus of the cerebellum) is better developed in mammals having primarily the parvocellular red nucleus. This has led to the suggestion that the parvocellular portion is important in the control of highly coordinated movements or postures.

In the rat, the rubrospinal tract arises from both the magnocellular and parvocellular portions of the red nuclei, with a somatotopic arrangement of cells (Shieh et al., 1983). The neurons projecting to the cervical spinal cord are found in the dorsal and dorsomedial regions and those projecting to lumbosacral segments are located in ventral and ventrolateral regions (Shieh et al., 1983). The rubrospinal axons decussate at the level of the mesencephalic tegmentum to extend contralaterally in the dorsolateral funiculus of the spinal cord (Fig.3.3) (Brown, 1974; Ten Donkelaar, 1988).

It has been shown that the rubrospinal axons run throughout the entire length of the spinal cord in opossum, yet only a few fibers extend to the uppermost cervical segments in humans (Martin and Dom, 1970; Nathan and Smith, 1982). In rats, the rubrospinal axons extend as far as the lumbar spinal cord (Antal et al., 1992). In the spinal cord, rubrospinal

axons make connections to the neurons in laminae V, VI and VII throughout the length of the spinal cord. Axons originating from the dorsomedial portion of the red nucleus project to forelimb motor neurons and those originating from the ventrolateral portions of the red nucleus project to hindlimb motor neurons (Huisman et al., 1981). It has been established that rubrospinal axons terminate on distal and intermediate muscle motor neurons but not proximal muscle motor neurons (Kuchler et al., 2002).

There are many views about the role of rubrospinal system during locomotion. It has been proposed that the red nucleus is important in movement execution (through cerebello-rubrospinal connections) but not during movement initiation (Massion, 1988) and that it is important in the braking phase and postural fixation of movement (in concert with cerebral cortex through corticorubral connections) (Tsukahara et al., 1968). The rubrospinal tract has polysynaptic excitatory effects on flexor motor neurons and inhibitory effects on extensor motor neurons (Hongo et al., 1969a; Orlovsky, 1972a). However, electrically stimulating the red nucleus elicits an inhibitory effect on flexors and excitatory effect on the extensors (Hongo et al., 1969a). Stimulating the red nucleus also causes activation of contralateral limb flexors (Gassel et al., 1965). In adult cats, the destruction of the red nucleus had no marked effects in locomotion (Shik and Orlovsky, 1976). In another study, de-cerebellation in thalamic cats (in which corticorubral connection is damaged) abolished cyclic modulation and decreased the mean frequency of discharge of rubrospinal spinal neurons during locomotion (Orlovsky, 1972a). In the same study, rubrospinal neurons were found to be most active during swing and at the end phases of locomotion (when flexors are most active). In rats, unilateral lesions of the

red nucleus (Muir and Whishaw, 2000) and rubrospinal tract (Webb and Muir, 2003) have been shown to cause alterations in the generation of ground reaction forces and in interlimb coordination during locomotion. In addition, the rubrospinal system has also been shown to be important during skilled walking, voluntary modifications of gait and during skilled movements of the forelimbs both in cats and rats (Alstermark et al., 1981; Alstermark et al., 1987; Pettersson et al., 2000; Pettersson et al., 1997; Whishaw et al., 1990; Whishaw and Gorny, 1996; Whishaw et al., 1998; Whishaw et al., 1992; Webb and Muir, 2003; Schrimsher and Reier, 1993; Hermer-Vazquez et al., 2004). In cats, required to step over obstacles while walking, the rubrospinal neurons were found to exhibit multiple periods of activity during both swing and stance for both flexors and extensor muscles (Lavoie and Drew, 2002), implying that the rubrospinal system provides necessary input to both flexors and extensors during more skilled locomotor activity. Damage to the red nucleus or rubrospinal tract compromises the ability to step correctly in tasks requiring skilled limb placements such as locomotion over a horizontal ladder or a rope (Webb and Muir, 2003; Hendriks et al., 2005; Soblosky et al., 2001). During skilled movements of the forelimbs, the rubrospinal system is thought to cooperate with corticospinal systems (Whishaw and Gorny, 1996) to provide a tonic framework against which forelimb movement occurs. Interestingly, it is also thought that corticospinal and rubrospinal systems can substitute for each other's role during the execution of a skilled locomotor task such as walking on a rotating bar (Kennedy, 1990). The switch between the two systems may involve intermediate circuits, which re-route descending motor commands from the injured system to the uninjured system (Kennedy 1990; Fanardjian et al., 2000a,b,c). The collaborative and compensatory nature of the

corticospinal and the rubrospinal systems has been addressed in Chapters 6 and 7 in this thesis.

1.2.3.5 Reticulospinal tract

The reticulospinal fibers originate from the reticular nuclei in the midbrain, pons and medulla (Satoh, 1979). These fibres are present in many species starting from fishes through to mammals. In rats, approximately 13 medullary reticular nuclei, and 13 pontine and mesencephalic reticular nuclei have been described (Newman, 1985). Many of these nuclei project to both cerebral cortex and spinal cord (Newman and Liu, 1987). Reticular fibers originating from the midbrain descend in the ventromedial funiculus and extend only to the level of mid-thoracic spinal cord in rats (Waldron and Gwyn, 1969). Reticular fibers originating from the medullary reticular formation descend into spinal cord and course bilaterally in the ventral and lateral funiculi and reach the lower lumbar segments (Martin et al., 1985; Fox, 1970; Shapavolov and Gurevitch, 1970). In the gray matter, these fibers terminate in all laminae including I and II (Martin et al., 1985). The reticulospinal system is the main source of serotonergic input into the spinal cord (Satoh, 1979), which is important for locomotion (Rossignol et al., 1998).

The reticulospinal system has been shown to be involved in lordosis (Robbins et al., 1992; Sasaki, 1999; Zemlan et al., 1983), the startle reflex (Yeomans et al., 2002), pain modulation (Villanueva et al., 1996), modulation of blood pressure (Aicher et al., 2000). Besides all this, importantly, medullary reticular nuclei are important for initiating locomotion (Noga et al., 1991). In rats, it is thought that those reticulospinal fibers

descending in the ventral and ventrolateral spinal funiculi are most important for locomotion (Loy et al., 2002a).

1.2.4. Pathways running in ventrolateral and ventral funiculus

Among the studies included in the thesis, pathways running in ventrolateral and ventral funiculus were not lesioned directly. As we shall be referring to them in some of our studies, a brief description regarding the ascending and descending pathways which travel in the ventral half of the rat spinal cord is presented here.

1.2.4.1 Coeruleospinal tract

The majority of these axons originate from the locus coeruleus within the pons, with some of them also arising from other structures such as the ventrolateral brain stem, and subcoeruleus nucleus (Commissiong, 1981; Kwiat and Basbaum, 1992). The coeruleospinal axons, which are considered to be an important source of noradrenergic input to the spinal cord, descend bilaterally and decussate within the spinal cord (Commissiong, 1981). The course and termination patterns of these axons differs between different substrains of rats (Sluka and Westlund, 1992; West et al., 1993). In Sprague-Dawley rats supplied by Harlan, most of the locus coeruleus axons travel within laminae I-II and the dorsolateral funiculus and project to the dorsal horn. In contrast, in Sprague-Dawley rats supplied by Sasco, most locus coeruleus axons travel in the ventral funiculus and project to the ventral horns (Clark and Proudfit, 1992; Proudfit and Clark, 1992; Sluka and Westlund, 1992). The differences in termination patterns of coeruleospinal axons also have functional implications. For example, antinociceptive

properties in the animals with dorsal terminations are not present in animals with ventral horn terminations (West et al., 1993). Generally, coeruleospinal tract have been implicated in the control of autonomic functions, modulating the perception of pain and modifying motor behaviour such as locomotion (Jones, 1991).

1.2.4.2 Spinomesencephalic tract

See section **1.2.3.3**

1.2.4.3 Spinoreticular tracts

The spinoreticular tract neurons are located in the lateral part of the neck of the dorsal horn, laminae VII, VIII and some portions of X (Chaouch et al., 1983). There are three main groups of spinoreticular neurons: (1) those projecting to the lateral reticular nucleus; (2) a group projecting to the medial nuclei of the pontomedullary reticular formation, including the gigantocellular reticular nucleus, the paragigantocellular nucleus and the caudal part of the pontine reticular nucleus; and (3) neurons that innervate the dorsal reticular nucleus of the medulla (Wang et al., 1999). The axons ascend mainly in the ventrolateral funiculus (Zelman et al., 1978; Bing et al., 1990). The spinoreticular axons are thought to carry information about noxious stimuli and non-noxious cutaneous stimuli (Menetrey et al., 1980). In addition, spinoreticular axons projecting to lateral reticular nucleus are thought to transmit sensory information from joints, muscles and tendons (Menetrey et al., 1984) and may be involved in motor control (Magnuson et al., 1998).

1.2.4.4 Spinothalamic tract

The spinothalamic tract has lateral and medial components based on the cell bodies of origin. The lateral spinothalamic axons arise from cells which are located in laminae I, III-V and VII and those which give rise to the medial spinothalamic tract are located more ventrally in laminae VI-VII (Giesler, Jr et al., 1981; Granum, 1985). In the rat, at least 50% of spinothalamic neurons are located in the first four cervical segments, and about 90% of the axons terminate contralateral to their site of origin (Granum, 1986; Kemplay and Webster, 1986). All the axons travel in the ventral or ventrolateral funiculus (Giesler et al., 1981). The lateral spinothalamic tract terminates in the lateral thalamus and the medial spinothalamic tract terminates in the medial thalamus (Giesler, 1981). The spinothalamic tract is considered to be the main pathway for information from receptors signaling pain and temperature (Willis and Westlund, 1997).

1.2.4.5 Vestibulospinal tracts

The vestibulospinal tract arises from the vestibular nuclear complex. This system is phylogenetically old and is present in fishes (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). In mammals the vestibular system has four divisions; lateral, medial, spinal and superior vestibular nuclei. In rats, the vestibulospinal tract originates from all four divisions (Leong et al., 1984; Masson et al., 1991) and descends as far as the lumbosacral segments. This tract courses ipsilaterally in the ventral and ventromedial funiculus (Houle and Jin, 2001; Matesz et al., 2002). The course of vestibulospinal axons differs in cats, where the lateral and medial

vestibulospinal tracts remain ipsilateral but the caudal branch descends bilaterally within the ventral and dorsolateral funiculi (Peterson et al., 1978). In rats, vestibulospinal axons terminate mainly in lamina VIII, but also in laminae II-VII (Matesz et al., 2002).

Along with the reticulospinal tract, the vestibulospinal tract forms the medial system in the spinal cord and is important in maintaining posture and balance. It is also essential for maintaining normal locomotion. Vestibulospinal axons have excitatory effects on extensor motor neurons and inhibitory effects on flexor motor neurons (Hongo et al., 1971; Hongo et al., 1975). In cats, these axons have been shown to have important roles in maintaining and increasing extensor tone bilaterally during locomotion (Matsuyama and Drew, 2000a, Matsuyama and Drew, 2000b)

1.3 Neural control of locomotion

Movement of an organism from one point to another is termed locomotion. Depending on the environment in which organisms live, there are various modes of locomotion including swimming, flying, running, walking, galloping, hopping or even crawling. All these movements are initiated and executed by neuronal networks. It is now established that the building blocks responsible for basic alternating pattern of limb movements exists entirely within the spinal cord, with sensory information and supraspinal command centers modifying the output of intrinsic spinal networks, to suit the context and environment.

The concept of spinal locomotor centers was introduced based on the experiments of Brown (Brown and Sherrington, 1912), in which cats with a transected spinal cord and with cut dorsal roots still produced rhythmic alternating contractions in ankle flexors and extensors. Based on these observations, Brown proposed that the generation of alternating activity might be due to the existence of two centers within the spinal cord, one each for flexors and extensors, with mutual reciprocal inhibition. This scheme is referred to as the half-center hypothesis (Brown and Sherrington, 1912). This work formed the basis of our understanding about networks in the spinal cord, which are intrinsically capable of producing alternating rhythmic activity.

The network of neurons in spinal cord which generate “self-sustained” patterns of rhythmic activity and shapes the pattern of bursts of motor neurons is collectively called the central pattern generator (CPG) (Grillner, 1981, 1985). In mammals, it is assumed that there is at least one such CPG for each limb (Forssberg et al., 1980; Viala and Vidal, 1978), with interconnections between them. The CPGs are made up of many neurons and are spread across many spinal segments. In neonatal rats, the neurons constituting hindlimb CPGs are located in the thoraco-lumbar spinal cord (extending from last thoracic spinal segment thorough the caudal lumbar spinal segment) (Kjaerulff and Kiehn, 1996; Kremer and Lev-Tov, 1997) and the forelimb CPGs are located with in the last two cervical and first thoracic spinal cord segments in neonatal rats (Ballion et al., 2001). In the lumbar spinal cord, the CPG neurons are located medially at the level of ventral commissure (Kjaerulff and Kiehn, 1996). The forelimb centers are connected with one another (Ballion et al., 2001), as are hindlimb centers (Kjaerulff and Kiehn, 1996;

Stokke et al., 2002). The long projecting propriospinal neurons couple the cervical and lumbar enlargements, probably underlying the coordination of limb movements (Nathan et al., 1996).

Although CPGs are self-sufficient to generate typical alternating pattern, they can be influenced by afferent (sensory) and supraspinal input (Brooks, 1979; Pearson, 1995, Bouyer and Rossignol, 1998). Afferent information influences the CPG pattern and the CPG selects the appropriate afferent information according to the external requirements (McCrea, 2001). However, both the CPG and the reflexes that mediate afferent input to the spinal cord are under the control of the brainstem (Jankowska and Lundberg, 1981). To oversimplify, behaviourally, in order to compensate for unexpected postural disturbances and changes in terrain, the eventual locomotor output utilizes both sensory feedback and dynamic descending input (Hultborn et al., 1998).

Locomotion can be initiated either by voluntary (supraspinal control) or involuntary (spinal) mechanisms. The cerebral cortex, through its interactions with other structures like basal ganglia, the medial or lateral hypothalamus, regions in midbrain and medullary reticular formation is involved with the voluntary initiation of locomotion (Jordan, 1998). Voluntary commands interact with the CPG to change the gait characteristics (Bosco and Poppele, 2001). Specifically, stimulation of an area in the midbrain called the mesencephalic locomotor region (MLR) results in quadrupedal locomotion in a decerebrate animal. Interestingly, increasing the strength of stimulus changes the animal's gait from walk to gallop (Shik et al., 1969).

Recently, Sinnamon (Sinnamon, 1993, reviewed in Jordan, 1998) has suggested 3 different subsets of neural circuits initiating locomotion based on the behavioural contexts, namely: 1) exploratory system, 2) primary appetitive system and 3) primary defensive system. It is thought that locomotion is initiated upon activation of these centers based on the different behavioural needs of the animal. Primary appetitive systems functions to bring the organism in contact with incentive and consummative stimuli, the primary defensive system functions to increase the distance between the organism and painful stimuli and in the exploratory system, locomotion is directed to stimuli that comprise the features of an environment (Jordan, 1998).

1.4 Neural control of skilled locomotion

Skilled locomotion tests the ability of an animal to make anticipatory gait modifications. The neural substrates that are responsible for the skilled limb movements are not clearly elucidated, but it is thought that skilled locomotion is an elaboration of the basic locomotor pattern and rhythm with adaptive regulation from the supraspinal control centers (Armstrong, 1988; Drew et al., 1996). During ladder walking, an animal needs to guide its limbs to specified points in the environment and accurately place its limbs on a rung. This sequence of skilled limb movements involves sensorimotor integration. Most of the work concerning neural control of skilled locomotion has been done in cats. Among the brain structures involved, role of motor cortex during skilled locomotion has received the most attention. Trendelenberg in 1911 showed that after temporarily cooling the motor cortex in dogs and cats, the animals could not walk across the grids (as

reviewed in Armstrong, 1988). Similar findings have been demonstrated after cortical lesions (Adkins et al., 1971; Jiang and Drew, 1996), pyramidal tract lesions (Jiang and Drew, 1996; Eidelberg and Yu, 1981) and corticospinal tract lesions (Gorska and Zmyslowski et al, 1993). Interestingly, while the lesions of the motor cortex and its efferent pathway, the pyramidal tract, cause modest deficits during overground locomotion (Muir and Whishaw, 1999a; Metz et al., 1998), they cause severe problems during skilled locomotion (Metz and Whishaw, 2002). This suggests that motor cortex plays a very important role during skilled placements of limbs (Drew et al., 1996; Kalaska and Drew, 1993). This finding has been confirmed even electrophysiologically. During ladder walking, neuronal recordings from motor cortex including pyramidal neurons show higher levels of activity than during overground walking, and that this increase is greatest in late swing-early stance in the contralateral forelimb (Beloozerova and Sirota, 1993; Drew, 1988). These observations have led to the suggestions that the function of motor cortex during skilled locomotion is to control the accuracy of limb movements (Armstrong, 1986; Beloozerova and Sirota, 1993) and to regulate stance duration (Drew et al., 1996).

Along with the motor cortex, the cerebellum contributes to modifications of the base locomotor activity required to control paw placement and limb trajectory (Drew et al., 1996). The cerebellum is also thought to prime the other directly involved motor regions of CNS during visually controlled locomotion (Armstrong and Marple-Horvat, 1996). In addition the afferent inputs from the forelimbs and the hindlimbs converging on the

cerebellum also play important roles during limb placements and adaptations during skilled walking (Marple-Horvat and Armstrong, 1999).

Apart from the motor cortex and the cerebellum, the red nucleus and its efferent pathway the rubrospinal tract are directly involved in placement of limbs during skilled walking. Recordings of neuronal activity from the red nucleus suggests that, along with the motor cortex, rubral neurons contribute to modifications of the pattern of EMG activity that are required to produce changes in limb trajectory, and are also important in regulating intra- and inter-limb coordination (Lavoie and Drew, 2002).

Though the neural control of skilled locomotion in rats has not been investigated in detail as in cats, some of the information can be gathered from lesion studies. In rat, ladder walking ability is affected by lesions of the pyramidal tract (Metz and Whishaw, 2002), motor cortex (Soblosky et al., 1997; Metz and Whishaw, 2002), nigrostriatal fibers (Metz and Whishaw, 2002), dorsal column (Webb and Muir, 2003) and rubrospinal tract (Webb and Muir 2003).

Thus there is a growing acceptance that skilled walking uses the same neuronal networks as overground locomotion, including the spinal centers and supraspinal centers but requires an ongoing adaptation from supraspinal centers, particularly the motor cortex.

1.5 Neural control of skilled reaching

Reaching is a skilled movement, which refers to movements of the limbs, paws and digits for catching, holding and manipulating objects (Whishaw, 2003). These movements involve complex interactions between motor and sensory systems. Normally, rats use skilled forelimb movements for variety of purposes, including eating and grooming (Ivancic et al., 1996). Reaching in rats was first described by Peterson (1932). He described the importance of the motor cortex for hand preference. In recent years the most detailed descriptions of rat reaching are studied in the single pellet reaching task, in which rats are trained to obtain a food pellet located in a small depression on a shelf (Whishaw, 1993). Most of the information about the neural structures involved in skilled forelimb movements comes from lesion studies.

In primates, the corticospinal and rubrospinal systems play very important roles in skilled forepaw movements (Lawrence and Kuypers, 1968a, 1968b). After damage to corticospinal tract in monkeys, reaching ability is severely reduced but the rubrospinal system can mediate the residual ability. However, combined damage to both the systems abolishes reaching ability in primates (Lawrence and Kuypers, 1968b). Similarly in cats, reaching ability is mediated by corticospinal and rubrospinal system (Petterson et al., 2000). In absence of both these systems, the residual ability is mediated by either ventrally located reticulospinal tracts or propriospinal neurons (Petterson et al., 2007).

Similar to primates and cats, in rats the corticospinal and rubrospinal systems play an important role during skilled forelimb reaching (Whishaw and Metz, 2002; Whishaw et

al., 1986, 1990, 1991, 1992a, 1992b, 1993, 1996, 1998; Metz and Whishaw, 2002; Piecharka et al., 2005; Schrimsher and Reier, 1993; Hermer-Vazquez, 2004). Neurons in the forelimb area of motor cortex and red nucleus fire during specific phases of reaching (Hermer-Vazquez et al., 2004) indicating their involvement during skilled reaching. In addition, electrical stimulation of motor cortex by microelectrodes elicits movements of the limb and paw in rats (Donoghue and Wise, 1982; Neafsey et al., 1986). Accordingly, damage to motor cortex and or red nucleus along with their spinal projection, i.e. corticospinal and rubrospinal tracts respectively, have the greatest effect on reaching ability in rats.

Corticospinal damage reduces the pellet retrieval more severely than the damage to the rubrospinal tract (Whishaw et al., 1998). Damage to the red nucleus or rubrospinal tract has a moderate effect on pellet retrieval but causes permanent impairments in the reach movements (Whishaw and Gorny, 1996; Whishaw et al., 1992). Interestingly, damage to red nucleus exacerbates the reaching deficits after motor cortex lesions in rats (Whishaw et al., 1990). Recently, it was shown that combined damage to corticospinal and red nucleus cause additive effects both in pellet retrieval and reach movement (Whishaw et al., 1998), similar to those seen in monkeys after pyramidal tract and rubrospinal tract lesions (Lawrence and Kuypers, 1968a, 1968b). Hence it is thought that both these systems act concertedly to bring about skilled movement of forepaws in rats (Whishaw and Gorny, 1996). In Chapters 6 and 7 I have looked at the collaborative and compensatory roles of corticospinal and rubrospinal systems during skilled reaching.

In addition to the corticospinal and rubrospinal systems, skilled reaching is also affected by damage to the lateral striatum (Pisa, 1988; Pisa and Cyr, 1990), cerebellum and inferior olfactory nucleus (Whishaw et al., 1993), all of which act along with the motor cortex in bringing about movement of the limbs.

Sensory feedback also plays an important role both for haptic (sense of active touch) and postural coordination. The sensory pathways in the dorsal columns carry information about haptic, although damage to the dorsal columns does not reduce pellet retrieval ability. Instead, these lesions affect the components of reach movement permanently (McKenna and Whishaw, 1999) and rats lose their tactile discrimination ability (Ballermann et al., 2001). Dorsal rhizotomy, which removes the sensory feedback from forelimb, affects postural coordination and impairs aiming of the forepaw and grasping of food pellet (Saling et al., 1992). In Chapter 4, I have looked at the effects of bilateral damage to ascending fibers during skilled reaching.

Though skilled movements in rats and primates are homologous and similar, an important difference between them is the sensory control of skilled movements (Whishaw et al., 1992). Primates use vision to locate objects which they reach and they also shape their hand to object size using visual cues. In contrast, rats use olfaction to locate the food object (Whishaw and Tomie, 1990) and use haptic information from forelimbs via dorsal column pathways for digit shaping (Ballermann et al, 2001).

1.6 Models commonly used to study spinal cord injury

Laboratory animal models have been used to recreate spinal cord injuries to study various facets of injury, including regeneration and repair. Based on the histology and imaging of injured human spinal cord, Bunge and colleagues (Bunge et al, 1993) have classified spinal cord injuries as 1) contusion injury 2) compression and 3) laceration. To recreate each of these conditions, appropriate animal models have been developed. Animal models have been in use since the time Allen (1911) described the use of weight drop techniques in dogs.

Rats are the most widely used species to study traumatic spinal cord injuries, mainly because the morphological, biochemical and functional changes that occur after spinal cord injury are similar to those seen in humans (reviewed in Onifer et al., 2007) and also because they are readily available. There are as many injury paradigms as there are types of spinal cord injury. Generally, experimental models are classified as complete or incomplete, and incomplete injuries can be further categorized as unilateral or bilateral. Based on the type of traumatic insult, the models could be categorized as contusion, compression, laceration, ischemic and chemical-mediated. Injury can be inflicted at different levels of spinal cord. Basically, choice of a particular model over others depends on the aspect of SCI being studied, as no particular model completely addresses all aspects of traumatic SCI. For detailed literature, the reader is referred to the review by Onifer and colleagues (Onifer et al., 2007). The present section briefly lists the SCI paradigms commonly used in the research, along with their advantages and disadvantages.

1.6.1 Complete spinal cord injury models

This model involves transection of the spinal cord transversely, disrupting the continuity of cranio-caudal connections. The site of injury is usually in the lower thoracic spinal cord. The complete spinal cord injury model is useful in studies looking at regenerative potential of different axons. After complete spinal transection as all the spinal axons are severed, axons that grow past the transected site into the distal end of the injured spinal cord can be unequivocally confirmed as regenerated axons. These regenerated axons can be identified using anterograde or retrograde axonal labeling. This model is also used in studies of central pattern generator in the lumbar spinal cord (Barbeau et al., 1987; Barbeau and Rossignol, 1991). In addition to studying the effects of depriving supraspinal influences (Hiebert et al., 1994), the role of afferent input in driving the pattern generators could also be studied (Barbeau and Rossignol, 1987). It is also a convenient model to study the roles of various pharmacological agents controlling locomotion (Barbeau et al., 1987; Barbeau and Rossignol, 1991). A major limitation of this model is the intensive post-operative care, as the animals will be paraplegic.

1.6.2 Incomplete spinal cord injury models

These models are used in evaluating potential treatments for spinal repair, and in understanding the roles of different pathways during different behaviours. One major advantage of these models is less intensive post-operative care. The lesions could be unilateral or bilateral and depending on the lesion type, injury could be contusion, compression, laceration, ischemic or chemical-mediated.

1.6.2.1 Contusion injury

This is probably the oldest and most relevant model of human spinal cord injury in terms of pathology (Allen, 1911; Metz et al, 2000a). The injury site undergoes hemorrhagic necrosis, with subsequent development of cavitation surrounded by glial scarring. This injury is caused either by dropping a weight from a height or by electromechanically displacing the spinal cord for a specific amount of time. In the weight drop technique, depending on the weight and the height, different amounts of force is applied to the cord resulting in varying degrees of reproducible spinal cord injury. The most commonly used weight drop model is New York University (NYU) impactor (Gruner, 1992). The most commonly used electromechanical impactor is Ohio State University (OSU) device (Noyes, 1987). This device injures the spinal cord by means of a solenoid-controlled air cylinder mounted on a rigid frame with a tip that impacts the exposed dorsal spinal cord. Using both these techniques, reliable and graded forms of spinal cord injury can be reproduced when factors like animal strain, age and anesthetic agent are controlled (Kwon et al., 2002).

1.6.2.2 Compression injury

This paradigm was developed to resemble the ventral compression of spinal cord that is commonly seen in the human spinal cord injury. This involves exposing the rat spinal cord and compressing it with an aneurysm clip or modified forceps (Rivlin and Tator, 1977; Khan and Griebel, 1983). The clip is calibrated for a known compression force to obtain various intensities of lesion. This type of injury can also be inflicted by using inflating balloons (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). This model is helpful to determine the behavioural outcomes after various durations of spinal cord compression.

1.6.2.3 Laceration injury

Though laceration injuries are not clinically typical in human spinal cord injuries, this is frequently used in experimental models. Techniques used include transection, incision, hemisection, resection and aspiration. Injury is inflicted by using scalpel blade (Ballermann et al., 2001), modified tapered needle (Webb and Muir, 2002, 2003, 2004) or with scissors. Laceration injuries sever spinal axons, and therefore could be used to lesion specific pathways and study the behavioural implications (Muir and Whishaw, 1999a; Webb and Muir, 2002, 2003, 2004). It can also be used to study the plasticity and regenerative potential of specific pathways through various cellular transplants (Kwon et al., 2007; Coumans et al., 2001; Ramon-Cueto et al., 2000; Bareyre et al, 2004).

1.6.2.4 Ischemic injury

Clinically, primary ischemic disturbance causing spinal cord injury is rare, though ischemia is one sequelae of any type of spinal cord injury. In rats, ischemic injury is inflicted by inserting a Fogerty catheter through the femoral artery to the level of the subclavian arteries. The Fogerty catheter tip is then filled with saline to occlude the thoracic aorta (Taira and Marsala, 1996). Following the occlusion, gray and white matter of the spinal cord undergoes apoptosis and necrosis (Follis et al., 1993; Kato et al., 1997). The severity of the injury is dependent on the duration of occlusion. Another method

used to cause ischemic damage of spinal cord is by injection of photosensitive dyes like Rose Bengal (Watson et al., 1986) or erythrosin B (Cameron et al., 1990; Hao et al., 1991) intravenously and irradiating the exposed spinal cord to produce vascular thrombosis.

1.6.2.5 Chemical-mediated injuries

This is used exclusively in experimental models to study various facets of secondary spinal cord pathology, seen after primary injury. Administering excitatory amino acids like glutamate, aspartate, or kainate causes destruction of oligodendrocytes and neurons in the gray matter of the spinal cord, mimicking excitotoxicity after traumatic spinal cord injury. Administering the herbicide paraquat mimics damage to lipids and proteins by free-radical, peroxynitrate and calpain (Hall, 2001). Injecting zymosan, yeast particulate activates microglia and macrophages to cause inflammatory damage similar to that seen after traumatic spinal cord damage (Popovich et al., 2002). Injecting ethidium bromide, lysolecithin or L-alpha lysophosphatidyl choline alone or with irradiation causes demyelination and oligodendrocyte death similar to that seen after traumatic spinal cord injury (Blakemore and Patterson, 1978; Graca and Blakemore, 1986; Yajima and Suzuki, 1979).

To summarize, animal models, particularly rat models, of spinal cord injury have advanced our understanding of the pathophysiology following spinal cord injury. Though no particular model addresses all the aspects of human spinal cord injury, the choice of a particular model is dependent on the questions to be answered. As more and more

treatments are being explored for human spinal cord injury repair, rat models enable testing of these therapies and aid for comparisons between different studies.

1.7 Methods to assess functional recovery after partial spinal lesions in rats

Behaviour in animals is the integration of multiple sensorimotor processes to produce a meaningful movement such as locomotion or skilled limb action. Behavioural testing serves to evaluate and describe the ability of animals while performing a task. The aim of behavioural testing is to understand how the processes which subserve movements are organized and also to understand how damage to these structures modifies or breaks down the ability of the animals to perform the behaviour. In spinal cord injury research, behavioural testing could help us (1) in correlating the degree of functional deficits with lesion severity, location and duration, (2) in assessment and comparison of functional recovery after various interventions, and (3) in investigating the compensatory potential of structures that are not damaged.

The criteria for selection of a behavioural test to assess functional recovery following SCI has been described (Goldberger et al., 1990). Based on the recommendations, a behavioural test should be (1) sensitive and quantitative, (2) a trained behaviour (so that confounding issues of motivation is removed) and (3) appropriate for the species being used in experiments.

There are several behavioural tests available for testing rat models of spinal cord injury and they can be classified into (1) end point measures (2) qualitative measures (3)

quantitative measures (kinetics and kinematics). In this section, a brief introduction will be given about the advantages and disadvantages in each of these different behavioural testing approaches. For detailed descriptions, readers are referred to reviews by Webb and Muir (2002, 2005).

1.7.1 End point measures

In general, end point measures evaluate the ability of the animals to accomplish a behavioural task. There is a huge variation as to what different end point tests measure. Some tests assess behaviour that involves the use of the whole body, including body orientation and balance, in addition to assessing limb action. These tests include measuring the time required to cross a length of beam (Kunkel et al., 1993) or to climb a rope (Kunkel et al., 1993), scoring the number of times a paw slips between the metallic rungs while the animal walks over it (Soblosky et al., 1997), or measuring the angle at which the animal can no longer maintain a fixed position on a tilted surface (inclined plane; Rivlin and Tator, 1977).

There are end point measures that focus more on the function of individual limbs. These include measuring time for the animals to retrieve a fixed number of pellets or the number of food pellets animals retrieve successfully (pellet reaching; Whishaw and Metz, 2002; Whishaw et al., 1986, 1990, 1991, 1992a, 1992b, 1993, 1996, 1998; Metz and Whishaw, 2002; Piecharka et al., 2005) or the time required to remove a sticker placed on a paw (sticker removal test; Diener and Bregman, 1998b).

There are also end point measures that assess reflexes. These include testing the ability of the animal to place its foot on the surface (reflex placing; Gale et al., 1985;

Kunkel et al., 1993), testing the degree to which animal withdraws its limb following toe pinch (reflex withdrawal; Gale, 1985), or time taken for the animal to move from supine to prone position (righting reflex; Kerasidis et al., 1987; Kunkel-Bagden et al., 1992; Diener and Bregman, 1998).

Advantages of end point measures-

- a) objective
- b) relatively simple to score
- c) quick to perform once the animals are trained
- d) inexpensive equipment required to perform tests
- e) easy to score and analyze the data obtained

Disadvantages of end point measures-

- a) Does not elaborate on deficits
- b) Does not take into consideration the compensatory ability of animals

1.7.2 Qualitative measures

These measurements describe movements qualitatively based on presence, absence or modification. Qualitative kinematics such as Eshkol-Wachman Movement Notation (EWMN) (Whishaw et al., 1991) describes the movement of limbs in space relative to the body and has been used to describe the detailed movements of rat forelimbs during pellet retrieval. Qualitative measures are also used to describe locomotor abilities in animals.

One of the most common open field locomotor scoring systems, the Basso Beattie Bresnahan scale (BBB scoring) (Basso et al., 1995) and its earlier version, the Tarlov scale, are used to describe the recovery of hindlimb function after thoracic spinal cord

injury. In all these tests, the movement of one limb segment relative to another or the body is scored based on either presence or absence of each movement.

Advantages of qualitative measures-

- a) less time consuming and easy scoring
- b) can be used as a complement to end-point measures to reveal how a task might be completed/accomplished

Disadvantages of qualitative measures-

- a) can be subjective
- b) to score the behaviour consistently, personnel should be experienced

1.7.3 Quantitative measures

This approach attempts to quantify behavioural abilities in animals. Quantitative measures can further be classified into kinematics and kinetics.

1.7.3.1 Quantitative kinematic analysis

This analysis quantifies how the body moves in space; it takes into consideration variables such as timing, distance, velocities and angular variables of a movement (Clayton, 1996). In this method, commonly, a video recording of the animal performing the behaviour is made. Limb segments are measured and markers which have been applied to certain points of the limbs (i.e. joint markers) are followed by digital tracking.

Advantages of quantitative kinematic measures-

- a) provides an objective, quantitative and detailed assessment of movements

- b) describes and quantifies the changes during each phase of movements

Disadvantages of quantitative kinematic measures-

- a) requires expensive equipment
- b) time consuming, both in setting up the markers and during analysis

1.7.3.2 Kinetic analysis

Kinetic analysis measures the forces involved in the production and modification of motion (Muir and Whishaw, 1999a, 1999b, 2000; Webb and Muir, 2002, 2003, 2004). Kinetic analysis provides information about forces generated on the ground as the animal moves overground and reveals how limbs are being used during locomotion. Kinetic measurements are very sensitive in measuring even the most subtle changes during locomotion. As used in the present thesis, kinetic analysis requires animals to locomote over force platforms which are capable of measuring forces in 3 orthogonal directions.

Advantages of quantitative kinetic measures-

- a) provides a quantifiable and sensitive measure of locomotor ability in animals
- b) provides a standardized measure which could be used to compare studies between different laboratories

Disadvantages of quantitative kinetic measures -

- a) requires expensive equipment,
- b) requires extensive personnel training,
- c) can not be used in paraplegic animals

The behavioural testing employed should: (1) be relevant to the model of spinal cord injury, (2) best represent the behavioural ability of animals after spinal cord injuries, (3) be sensitive enough to delineate the deficits, and 4) be reproducible, so that it enables comparison between different institutions/laboratories. If the spinal cord injury models permit, a combination of behavioural tests should be performed rather than any one particular test.

In the studies presented in the thesis, a laceration/transection injury paradigm was used to lesion specific pathways in the brain (Chapter 7) and spinal cord (Chapters 4, 5 and 6) and to study its behavioural implications. The behavioural abilities of lesioned rats were tested during overground locomotion, skilled locomotion and also during skilled reaching. The sensorimotor assessment included combination of kinetic, kinematic and endpoint measurements. Kinetic parameters consisted of measurement of ground reaction forces during overground locomotion. Kinetic parameters such as stance duration and limb timing were also measured during overground locomotion. Endpoint measurements were used during skilled locomotion and skilled reaching (see detailed description refer to Chapter 3).

Chapter 2. OBJECTIVES AND HYPOTHESES

The general objective of the studies presented in the thesis was to understand the contribution of dorsal spinal pathways during locomotion and other sensorimotor behaviours.

2.1 Rationale

Rats are the most commonly used species for spinal cord injury research and the focus of many rat spinal cord injury studies involve pathways which are functionally relevant to human medicine. Nevertheless, due to the inter-species variation in regards to anatomical location and functional contribution of different spinal pathways, results from one species can not necessarily be extrapolated to another species. Not much is known about how different spinal pathways contribute to different sensorimotor behaviours in rats. Better understanding of the importance of spinal pathways in rats may help in developing therapies for spinal cord injury.

The CST in humans is a very important motor pathway and for this reason, many laboratory spinal injury models involve corticospinal damage. Furthermore, in rats the CST is readily accessible in the dorsal funiculus (see Section 1.2.2.3). Despite this advantage, the CST cannot be lesioned without collateral damage to overlying ascending sensory fibers. Thus the functional deficits after a spinal CST lesion is actually a combination of deficits due to the loss of both the CST and ascending sensory fibers. The deficits due to loss of ascending sensory fibers have, with few exceptions, been ignored in the literature. To investigate the deficits due to damage to ascending sensory fibers, we

designed experiments outlined in Chapter 4. One of the findings from Chapter 4 was that cervical DF lesions affect only forelimb function but not hindlimb function. We reasoned that it was possibly because of cervical level of the spinal injury. To investigate if thoracic DF lesions cause hindlimb deficits, we designed experiments outlined in Chapter 5.

After partial spinal cord injuries, the behavioural capabilities of rats are a combination of 1) deficits due to loss of spinal pathways and 2) compensation from the intact pathways. In order to effectively interpret behavioural abilities, the roles played by the spared spinal pathways needs to be understood. Earlier studies have shown that the rubrospinal system is capable of compensating the loss of corticospinal system and vice-versa (Kennedy, 1990; Fanardjian et al., 2000a; 2000b; 2000c). As the corticospinal and the rubrospinal tracts travel in different funiculi in the rat (see Section 1.2.3.4), this offers a unique model to study the compensatory nature of one system after damage to another. To investigate the nature of behavioural compensation from the corticospinal system after damage to rubrospinal tract along with other DLF pathways, we designed the experiments outlined in Chapter 6. We then investigated the compensatory potential of the rubrospinal pathways in the DLF after damage to the corticospinal system through experiments outlined in Chapter 7. The objectives and hypotheses of the respective chapters are listed below.

2.2 Objectives and hypotheses of the studies

1. To compare the sensorimotor abilities of rats with ascending sensory fiber pathways lesions in the dorsal funiculus to those of rats with complete dorsal funicular lesions. Specifically, I hypothesized that bilateral lesions of the ascending sensory pathways in the dorsal funiculus would produce persistent changes during overground locomotion and that these changes would be independent of concurrent damage to the CST (Chapter 4).
2. To compare the locomotor abilities of rats with cervical dorsal spinal funicular lesions to those of rats with mid-thoracic dorsal spinal funicular lesions. Specifically, I hypothesized that rats with bilateral lesions of the cervical dorsal funiculus would display deficits involving all four limbs, while the rats with bilateral lesion of thoracic dorsal funiculus would display deficits involving only the hindlimbs (Chapter 5).
3. To investigate the compensatory role of undamaged dorsal funicular pathways after lesions to dorsal lateral funiculus in rat spinal cord. Specifically, I hypothesized that rats with staggered lesions to dorsolateral and dorsal spinal funiculi would show more severe sensorimotor deficits than the rats with simultaneous lesions to dorsolateral and dorsal spinal funiculi (Chapter 6).
4. To determine whether the pathways in the dorsal part of the lateral funiculus can compensate for loss of corticospinal input to the spinal cord. Specifically, I

hypothesized that rats with dorsolateral funiculus lesions which were performed six weeks after pyramidal tract lesions would exhibit more deficits on several behavioural tasks compared to animals which received pyramidal tract and dorsolateral funiculus lesions simultaneously (Chapter 7).

Chapter 3. GENERAL METHODS AND PROCEDURES

3.1 Subjects

Long Evans female rats (225-250g) used in the experiments were obtained from Charles River Laboratories (Quebec). They were housed as pairs and were maintained in 12h dark/12h light rooms at the laboratory animal care facility at the Department of Veterinary Biomedical Sciences, Western College of Veterinary Medicine at the University of Saskatchewan. Upon their arrival, rats were rested for a period of one week after which training was initiated, but were handled every day to get acclimatized to the conditions. Rats were fed rodent chows but were food-restricted so that their body weights were maintained between 250-300g. All animals were examined daily by a Veterinarian and their body weights recorded. All animals were cared for according to the guidelines prescribed by the Canadian Council on Animal Care.

3.2 Training

After one week of their arrival, all rats were trained daily to trot on a runway (180 X 20 cm) for food reward. After this, the rats were trained to walk over a horizontal ladder. At the same time, rats were also trained to retrieve sugar pellets in a pellet reaching task. The training was deemed complete when the rats could trot on the runway and walk over the ladder without stopping in between and when they could successfully retrieve 70% of the food pellets in their first attempt.

3.3 Surgery

3.3.1 Surgical preparation and pre-medication

Prior to surgery all rats were weighed. Pre-anesthetic medication included subcutaneous injections of an anti-cholinergic agent, glycopyrrolate (0.03mg/kg; Sabex Inc., Boucherville, QC) to reduce the salivary, tracheo-bronchial and pharyngeal secretion; an analgesic, buprenorphine (0.05mg/kg; Buprnex, Reckitt Benckiser Pharmaceuticals Inc., Richmond, VA); and an antibiotic, trimethoprim and sulfadoxine (30mg/kg ; Trivetin, Schering Canada Inc., QC). Fifteen minutes after pre-anesthetic medications, rats were anesthetized by a intraperitoneal injection consisting of a mixture of medetomidine hydrochloride (sedative, 0.3mg/kg ; Sabex Inc., Boucherville, QC), ketamine (dissociative anesthetic, 0.3mg/kg ; Vetalar, Bioniche Animal Health, ON) and fentanyl (analgesic, 50 μ g/kg ; Sabex Inc., Boucherville, QC). Following the induction of a surgical plane of anesthesia, skin over the surgical site was shaved, scrubbed using chlorhexidine soap and rinsed with 70% isopropyl alcohol. Ocular lubricant was applied to both eyes to keep them moist. Through out surgery and recovery period, animals were administered 100% oxygen nasally. Animals were kept warm on a recirculating warm water blanket. Heart rates and pedal withdrawal responses were assessed periodically throughout the surgical procedure to ensure that a surgical plane of anesthesia was maintained.

3.3.2 Surgical procedure

Rats were routinely surgically prepared and draped. For spinal surgeries, rats underwent either cervical or thoracic dorsal laminectomies at either vertebra C2/C3 or T7/T8

respectively. Briefly, using an operating microscope, a sagittal incision was made either at the nape of the neck or at the mid-thoracic level, and underlying epaxial muscles were bluntly dissected to reveal the dorsal spinous process of C2 vertebra or T7/T8 vertebrae respectively. The dorsal spinous process was removed using a microrongeurs and a durotomy was then made using a pair of microscissors and sharp forceps to reveal the dorsal surface of the spinal cord. Appropriate bilateral spinal cord lesions to either C2 or T8 were performed using a modified 25 gauge hypodermic needle. 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. Overlying muscles were closed using 3-0 braided polyglycolic acid (Dexon II, Sherwood Davis and Geek, St. Louis, MO) in a simple continuous pattern. Skin was closed using 3-0 monofilament polybutester (Novofil, United States Surgical, CT) in a simple interrupted pattern.

For pyramidal tract lesions, rats were placed in dorsal recumbency with their heads immobilized using a Styrofoam restraint and their fore limbs taped to their thorax. Using a magnifying surgical microscope, a ventral midline incision was made on the neck followed by a blunt incision of sternocleidomastoid muscle to expose the trachea. The sternohyoid and sternothyroid muscles were retracted and trachea and oesophagus were laterally displaced to one side using blunt retractors. A deep blunt dissection exposed the ventral occipital bone. Using a dental drill a hole was made on the midline of the ventral occipital bone, which was extended to both sides using a sharp microrongeurs (Fine Science Tools, Inc, BC). A durotomy was made using fine forceps. Under higher magnification pyramidal tract fibers were transected using a fine forceps, carefully so that there was no damage either to the centrally located basilar artery or the underlying

inferior olfactory nucleus. Underlying muscles and skin was sutured as before. When ever the sham animals were used, they were treated similarly except that no damage was done to the spinal cord.

After surgery, the rats were given analgesic (buprenorphine for 2 days; 0.05mg/kg; Buprnex, Reckitt Benckiser Pharmaceuticals Inc., Richmond, VA) and antibiotic (trimethoprim and sulfadoxine for 5 days; 30mg/kg ; Trivetin, Schering Canada Inc., QC) medication.

3.4 Behavioural assessment

3.4.1 Training

Prior to surgery, all rats were trained daily to cross a runway for food reward. Usually, rats took about 3 weeks to achieve the optimal performance on the runway. After this, the rats were trained for a week on the horizontal ladder. Rats also underwent training in a skilled pellet reaching task for a period of 3-4 weeks. They were deemed trained if they could successfully reach for at least 70% of the pellets.

3.4.2 Single pellet reaching- test for skilled forelimb usage

Skilled reaching is a task involving complicated movement pattern and has been used to assess the sensorimotor abilities in rats after lesions of the brain (Whishaw et al., 1986, Whishaw et al., 1991) and spinal cord (Whishaw et al., 1993, Whishaw et al., 1998, Ballermann et al., 2001; McKenna et al., 1999, Anderson et al., 2005).

All rats were trained to reach for food pellets (Rodent chow pellets, Bioserve Inc.) in a single pellet reaching box (Fig. 3.1). The reaching box, made of clear Plexiglas, had a



Fig. 3.1. Picture of a rat in reaching box.

Pellet reaching is a test for skilled forelimb usage. In this task, rats are introduced inside the reaching box and are trained to reach for a food pellet kept on the shelf, by reaching through a vertical slit.

vertical slit in the middle, and horizontal platform on the outside. A small indentation on the horizontal platform, served as a well to hold the food pellet (Whishaw et al., 2002). The set up prevented the rats from retrieving pellets with their tongues and forced them to use their preferred paw. To reach for the pellet, rats had to extend their forelimb through the slit, grasp the pellet in their paws, retrieve the pellet and eat it. When the rats did all this in a single attempt, it was considered a successful reach. After each successful or unsuccessful attempt to reach for a pellet, rats were trained to retrieve a food reward at the back of the reaching box and then approach the reaching shelf at the front of the box anew for the next attempt. This allowed each reaching attempt to be relatively independent of the previous one. At each time point, the rats were initially allowed to reach for 10 pellets to become acclimatized to the set up, and then were videotaped as they reached for a further 20 pellets. For quantitative data, video-recordings were later analyzed and the number of successful reaches counted and expressed as percentage successful reach. In addition, the first 5 successful reaches were scored for the qualitative aspects of a reach using the ten component rating scale developed by Whishaw and colleagues (McKenna et al., 1999; Whishaw et al., 1993; Whishaw et al., 1998). In this, each reach was analyzed for ten sequential components: limb lift, digits close, aim, advance, digits open, pronate, grasp, supination 1, supination 2 and food release. In addition, the arpeggio movement of the paw was analyzed. Each component was rated on a 3 point scale: 0=normal movement, 1=abnormal movement but present, 2=absent.

3.4.3 Ladder crossing- test for skilled locomotor abilities

A horizontal ladder was used to test the skilled locomotor abilities of rats. Ladder walking has been used commonly to evaluate sensorimotor abilities of both forelimbs and hindlimbs in rats after experimental brain and spinal cord injuries (Chan et al., 2005; Klapka et al., 2005; Piantino et al., 2006; Soblosky et al., 1997; Soblosky et al., 2001; Valverde, 1966; Vavrek et al., 2006). The ladder was comprised of 2 mm diameter dowels placed 10 mm apart from each other (Fig.3.2. non-variable ladder setup). The ladder was placed equidistant from the ends of a clear plexiglass runway. At each data collection, rats were allowed to cross the ladder 10 times, to acclimatize to the set up before 15 runs were recorded from each rat. Animals were videotaped using a super VHS camera. Videotape was analyzed field by field (60 fields/s) and the total number of footfalls by each limb was recorded for 15 runs and expressed as a percentage correct steps for each limb/rat.

3.4.4 Kinetic measurements- overground locomotion

3.4.4.1 Measurement of ground reaction forces

As a quantitative measure of locomotor abilities in rats, ground reaction forces were recorded during overground locomotion (Webb et al., 2002; Webb et al., 2003a; Webb et al., 2003b; Webb et al., 2004). As mentioned previously, rats were trained to trot across the runway (Fig.3.3). Three force plates each measuring 10.5 X 11 cm located adjacent to each other in the middle of the runway, were used to measure forces in three orthogonal directions- vertical, fore-aft and medio-lateral. Body weights of the rats were recorded prior to collecting data. Collection of ground force data was triggered by the rat breaking

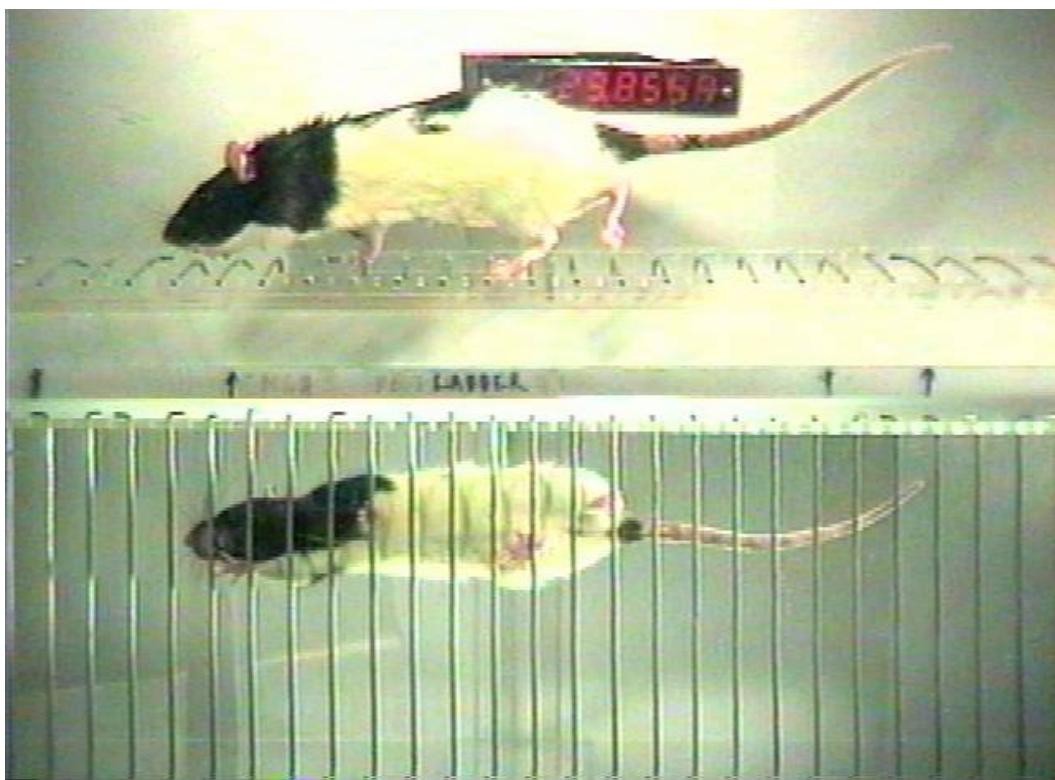


Fig. 3.2. Picture of a rat walking over horizontal ladder.

Ladder walking is a test of skilled locomotor ability. In this test, rats are trained to walk over series of metallic bars spaced 1cm from each other. Video-recording of the performance is used to assess the ability of the rats to step correctly on the bars.

an infrared beam (also triggering an LED timer and digital camera, recording the run). Force platform data was amplified, analogue to digitally converted (RC Electronics, San Raphael CA) at a sampling frequency of 1,000 Hz and collected on a personal computer (Midas 2.0 Xcitex Inc, Boston). Digital video was simultaneously recorded and collected using a high-speed (125Hz) digital camera (Motionscope 1050, Redlake, MASD, Inc). The data and video recording was stopped when the rats broke a second infrared beam of light placed behind the last force plate. The video and ground reaction force data was stored in a personal computer for later analysis.

Runs from the rats were selected based on the 2 inclusion criteria (1) that the rat had to be traveling at a constant velocity and (2) its paws needed to be clearly placed on the plates. The force data from the selected runs was then filtered in the forward and reverse direction using a modified low pass recursive filter. The data was expressed in proportion of body weight and normalized over time as a proportion of stride using custom written software (Microsoft Visual Basic, Microsoft Corp., San Francisco, CA) (Poulton et al., 2005; Webb et al., 2002; Webb et al., 2003a; Webb et al., 2003b; Webb et al., 2004). Data for each limb was averaged at each data collection time point for each rat, (minimum of 10 runs per trial) using custom written software (Microsoft Visual Basic, Microsoft Corp., San Francisco, CA). Group averages were obtained by averaging individual limb averaged forces with others in the same group. Variables of ground reaction forces analyzed included peak vertical, propulsive and braking forces and impulses in vertical and fore-aft directions for each limb.



Fig. 3.3. Picture of a rat trotting across force platform.

Rats are trained to trot on a runway which consists of 3 force platforms located adjacent to each other. Approaching the first force plate rats break a LED beam and trigger data acquisition and video recording, and as they step off the last force plate they break 2nd LED beam, which shuts off the recording system. Data from the force plates are used to measure the forces exerted on the ground during trotting (ground reaction forces) and to calculate step lengths and stride parameters.

3.4.4.2 Measurement of stride parameters

For each of the accepted runs, using the onset and offset timing of individual limbs on all 3 force plates, the stride variables including stance duration, stride length, step lengths and overlaps of limbs were measured. The distance measurements were normalized to stride length and timing measurements were normalized to stride duration using custom written software (Microsoft Visual Basic, Microsoft Corp., San Francisco, CA).

3.5 Histology- microscopic evaluation of the lesion site

On the day of the last experimental time point, after all the behavioural data was collected, rats were deeply anaesthetized with 90-100 mg/kg sodium pentobarbital (injected intra peritoneally). Rats were transcardially perfused with approximately 200 ml of heparinized physiological saline followed by an equal volume of 4% paraformaldehyde dissolved in 0.1M PBS. Spinal cords were collected and routinely processed and embedded in paraffin. Serial sections of the spinal cord were cut at 7-10 µm throughout the injury site. Sections were mounted on poly-L-lysine coated slides, and stained with Luxol fast blue and counterstained with Cresyl violet. The spinal sections were examined under the microscope to determine the extent of spinal cord damage at the lesion epicenter.

3.6 Statistical analyses

Data from reaching, ladder walking and ground reaction forces were expressed as mean ± S.E. values. Group means were compared using repeated measures ANOVA and post-hoc Bonferroni analysis. Qualitative reaching scores were analyzed using Friedman repeated

measures analysis on ranks and post-hoc Dunn's analysis. A *p*-value of <0.05 (ANOVA) was considered as the level of significance (Sigmastat, SYSTAT, San Jose, CA).

Chapter 4. BILATERAL DORSAL FUNICULAR LESIONS ALTER SENSORIMOTOR BEHAVIOUR IN RATS*

(*manuscript published: Kanagal and Muir, 2007. Experimental Neurology, 205; 513-524)

4.1 Abstract

Spinal cord injury models often involve damage to the corticospinal tract (CST) because of the functional importance of this pathway in humans. In rats, the main component of the CST travels in the dorsal funiculus and cannot be damaged without concurrent damage to overlying sensory fibers. To distinguish deficits due to the loss of CST from those due to sensory fiber damage, we bilaterally axotomized ascending sensory fibers in dorsal columns without CST damage in one group of rats (ascending sensory pathways, ASP) and compared the results to a group with damage to ascending sensory fibers with CST damage (ASP+CST). We assessed the ability of rats to perform a skilled reaching task and to walk over a horizontal ladder. We also measured the forces exerted on the ground (ground reaction forces, GRF) and limb contact patterns produced during overground locomotion. After ASP lesions alone, endpoint measurements of reaching success and footslip errors on the ladder showed transitory impairments, although detailed analysis revealed persistent deficits in skilled forelimb movements. ASP+CST lesions caused persistent deficits in reaching success and ladder footslips throughout the 8 week post-surgical period. Measurement of GRF's and limb timing during overground locomotion revealed differences in both groups at 8 weeks post-surgery compared to pre-surgical values, but no differences between ASP and ASP+CST groups. These results emphasize the normal contribution of both ascending sensory axons and CST axons during skilled

limb movements and support a role for ascending sensory information, but not descending CST input, during overground locomotion. These results also illustrate the value of using sensitive methods to reveal detailed behavioural changes after spinal injury.

4.2 Introduction

In humans, the CST is an important descending tract involved in many functions, including fine digit control (as reviewed in Lemon, 2005). Spinal cord injury studies in rodent models often involve damage to the CST, owing to its functional relevance in humans. The CST is easily accessible in the rodent spinal cord because the majority of CST axons descend in the dorsal funiculus, ventral to ascending primary sensory afferent fibres (Valverde, 1966). Because of this location, any injury model involving damage to CST at the spinal level involves collateral damage to ascending sensory fibers overlying the CST. These sensory fibers transmit information about hapsis (sense of active touch) and proprioception (sense of the position of the body parts,) to the brainstem and sensorimotor cortex in primates and in cats (Davidoff, 1989). In rats, ascending sensory pathways (ASP) in the dorsal funiculus mediate the skilled movement of the forelimbs (McKenna et al., 1999) and are also used for hapsis (Ballermann et al., 2001), and damage to these pathways cause sensorimotor deficits (Onifer et al., 2005; Schrimsher et al., 1993; Soblosky et al., 2001). Therefore, the functional deficits observed after complete lesions of the dorsal funiculus would logically be due to the combination of loss of ascending sensory input and loss of descending CST input to the spinal cord.

Combined damage to sensory and CST axons becomes especially important because one of the major functions of the CST, in humans and rodents, is the modulation of ascending sensory information (Lemon et al., 2005). In rodents, it has been argued that this is the primary function of the CST. CST fibres terminate most densely in the dorsal horn (Brown, Jr., 1971;Gribnau et al., 1989;Valverde, 1966) and make very few, if any, functional connections with spinal motoneurons in rats (Alstermark et al., 2004;Gemma et al.,1987;Kuang et al.,1990;Yang et al., 2003;Liang, 1991;Babalian, 1993). Thus, combined damage to primary sensory axons (loss of ascending sensory information) as well as to the CST (loss of sensory modulation) might be expected to produce functional deficits that are worse than either lesion alone.

We have found that unilateral lesions of the ascending sensory pathways in the dorsal columns, sparing the CST, resulted in persistent changes during the forces produced during overground locomotion (Webb et al.,2003b). Some of these changes were similar to those found after unilateral lesions of other regions of the CNS, which led us to hypothesize that rats might make compensations for unilateral CNS injury that are not necessarily specific to the lesioned pathway (Muir et al., 1999a; Muir et al., 2000b;Webb et al., 2002; Webb et al., 2003b;Webb et al., 2004). Bilateral lesions, such as those used in the present study, should therefore produce changes that are more specifically associated with the lesioned pathway. Furthermore, we and others have shown that unilateral lesions of CST axons at the level of the medullary pyramidal tract do not result in persistent changes during overground locomotion (Metz et al., 1998;Muir et al.,1999a). We therefore hypothesized that bilateral lesions of the ascending sensory pathways in the dorsal funiculus would produce persistent

changes during overground locomotion, and that these changes would be independent of concurrent CST damage. We also examined performance on a skilled reaching and skilled locomotor task after bilateral dorsal column lesions, with and without sparing of the CST.

4.3 Materials and Methods

4.3.1 Animals

Sixteen adult female Long-Evans rats (225-250g), from Charles River Laboratories (Que., Canada) were used for the experiment. The rats were housed in a light controlled room (12h light/ 12h dark) within the Animal Care Facility at the University of Saskatchewan. All rats were fed with rat chow but were slightly food restricted throughout the experiment such that their body weights were maintained below 300g. Their body weights were recorded every week. All rats were examined daily for their health and were cared for according to the regulatory standards set out by the Canadian Council on Animal Care.

4.3.2 Surgery

All animals underwent standard anesthesia, analgesia and cervical cord lesions as described in Sections 3.3.1 and 3.3.2 of this thesis. Lesions were made bilaterally to either ascending sensory fibers (ASP: n=7) or ascending sensory fibers with dorsal corticospinal tract (ASP+CST: n=7).

4.3.3 Behavioural assessment

4.3.3.1 Training

All animals were trained according to the procedure described in Section 3.4.1 in this thesis

Prior to surgery, all rats were assessed behaviourally using endpoint, kinetic and kinematic measurements. Endpoint measurements were used to score reaching success during skilled reaching and to count the number of footslips made while crossing horizontal ladder (skilled locomotion). Kinetic parameters consisted of measurement of ground reaction forces during overground locomotion. Kinematic parameters ie., step lengths, stance durations and overlaps of limbs were also measured during overground locomotion. Data was collected prior to surgery (pre-surgery) and again after surgery every 2 weeks till the end of experiment at 8 weeks.

4.3.3.2 Skilled reaching- single pellet reaching

Skilled reaching is used as a test to assess fine voluntary forelimb movement as described in Section 3.4.2 in this thesis.

4.3.3.3 Skilled locomotion -Horizontal ladder

A horizontal ladder was used to test the skilled locomotor abilities of rats, as described in Section 3.4.3 in this thesis

4.3.3.4 Overground locomotion

4.3.3.4.1 Kinematic measurement of ground reaction forces

Ground reaction forces were recorded and analyzed as described in Section 3.4.4.1 in this thesis.

4.3.3.4.2 Measurement of stride parameters

As described in Section 3.4.4.2 in this thesis.

4.3.4 Histology

As described in Section 3.5

4.3.5 Statistical analysis

As described in Section 3.6

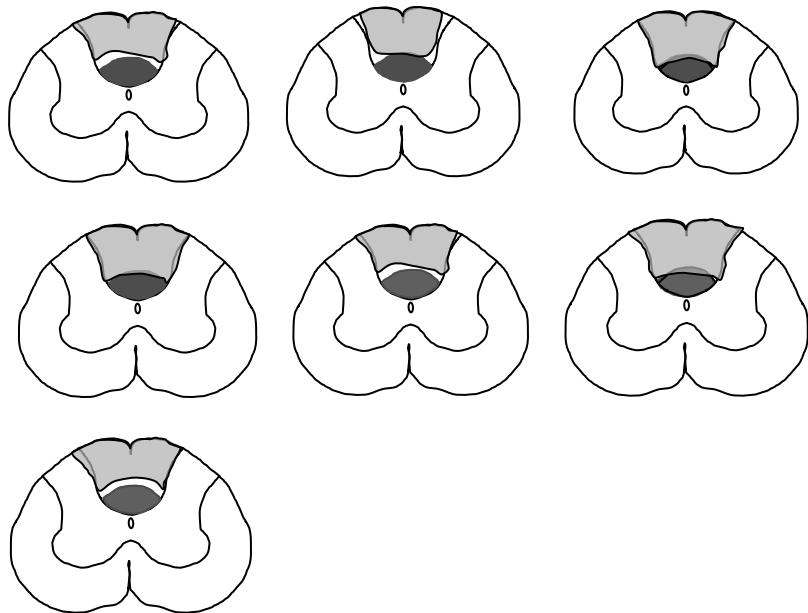
Briefly, all data are expressed as mean \pm SEM. The differences between the groups were analyzed using two-way repeated measures ANOVA (SigmaStat, SYSTAT, San Jose, CA). Bonferroni's test was used for post-hoc analysis. The qualitative reach scoring was analyzed using Friedman repeated measures analysis on ranks and post-hoc Dunn's test. A $p<0.05$ (ANOVA) was considered as the level of significance.

4.4 Results

4.4.1 Histology

Light microscopic evaluation of the histological sections revealed consistent and discrete lesions in the 2 groups. In the ASP group, the ascending sensory fibers were destroyed with complete to near complete sparing of the ventrally located dorsal component of CST in the dorsal funiculus (Fig. 4.1A). In the ASP+CST group, both the ascending sensory and dorsal component of CST were damaged (Fig. 4.1B). We found no damage to the central canal in any of the rats in the ASP+CST group. In both groups, there was occasional evidence of slight damage to the medial aspect of the dorsal horns. One rat in the ASP+CST group was

(A) ASP lesions



(B) ASP+CST lesions

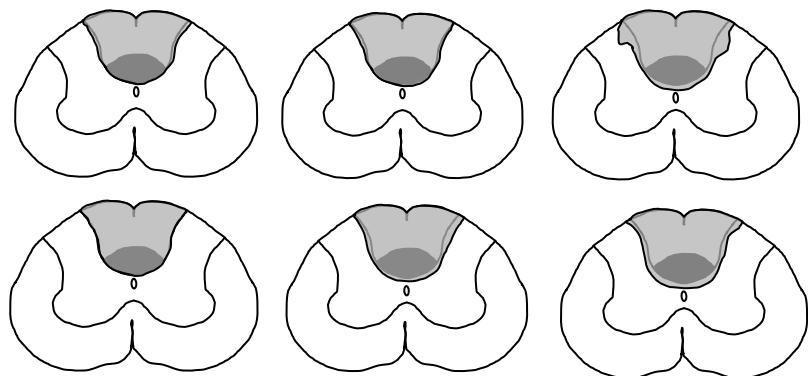


Fig. 4.1: Schematic drawings representing the lesion epicenter (C2) in (A) ASP lesioned and (B) ASP+CST lesioned rats. In ASP rats, the ascending sensory fibers in the dorsal columns were damaged with minimal damage to the CST. In the ASP+CST rats, both the ascending sensory fibers and the CST were damaged. In each drawing, dark grey represents the position of the dorsal component of the CST whereas light grey represents the extent of damaged tissue.

eliminated from the study, as it had sustained damage to the dorsal horns that extended to the lateral aspect of the horns. For the final analyses, there were 7 rats in the ASP group and 6 rats in the ASP+CST group. Sham animals did not show any evidence of damage to the spinal cord.

4.4.2 Behavioural assessment

In all the behavioural tests, at 2 weeks post-surgery sham animals did not differ from the pre-surgical animals, hence they are not discussed further.

4.4.2.1 Skilled reaching- single pellet reaching

Both the ASP and ASP+CST groups showed reduced ability to reach for food pellets after surgery (Fig. 4.2). Statistical analysis revealed significant differences in percentage correct reaches over time for each group ($p<0.05$ for ASP and $p<0.001$ for ASP+CST). Post hoc analysis revealed that animals without CST damage recovered their ability to retrieve pellets by 4 weeks post surgery, whereas animals with CST damage did not recover their reaching abilities by 8 weeks post operative, the latest time point studied (* $p<0.001$ in Fig. 4.2). Animals with CST damage also had reduced reaching success rates at all post-surgical time-points compared to animals without CST damage.

In both ASP and ASP+CST groups, qualitative analysis revealed changes in the individual components of the reach. Though changes were seen in most of the components, the elements consistently affected were aim, pronation, supination 2 and arpeggio ($p<0.05$ for ASP and $p<0.001$ for ASP+CST in Fig. 4.3). Both ASP and ASP+CST lesioned rats could not independently bring the pellet to the mouth (abnormal supination 2); instead they used the opposite paw to aid this movement. Additionally, in ASP+CST group, supination 1 was

Skilled Reaching in rats with ASP and ASP+CST lesions

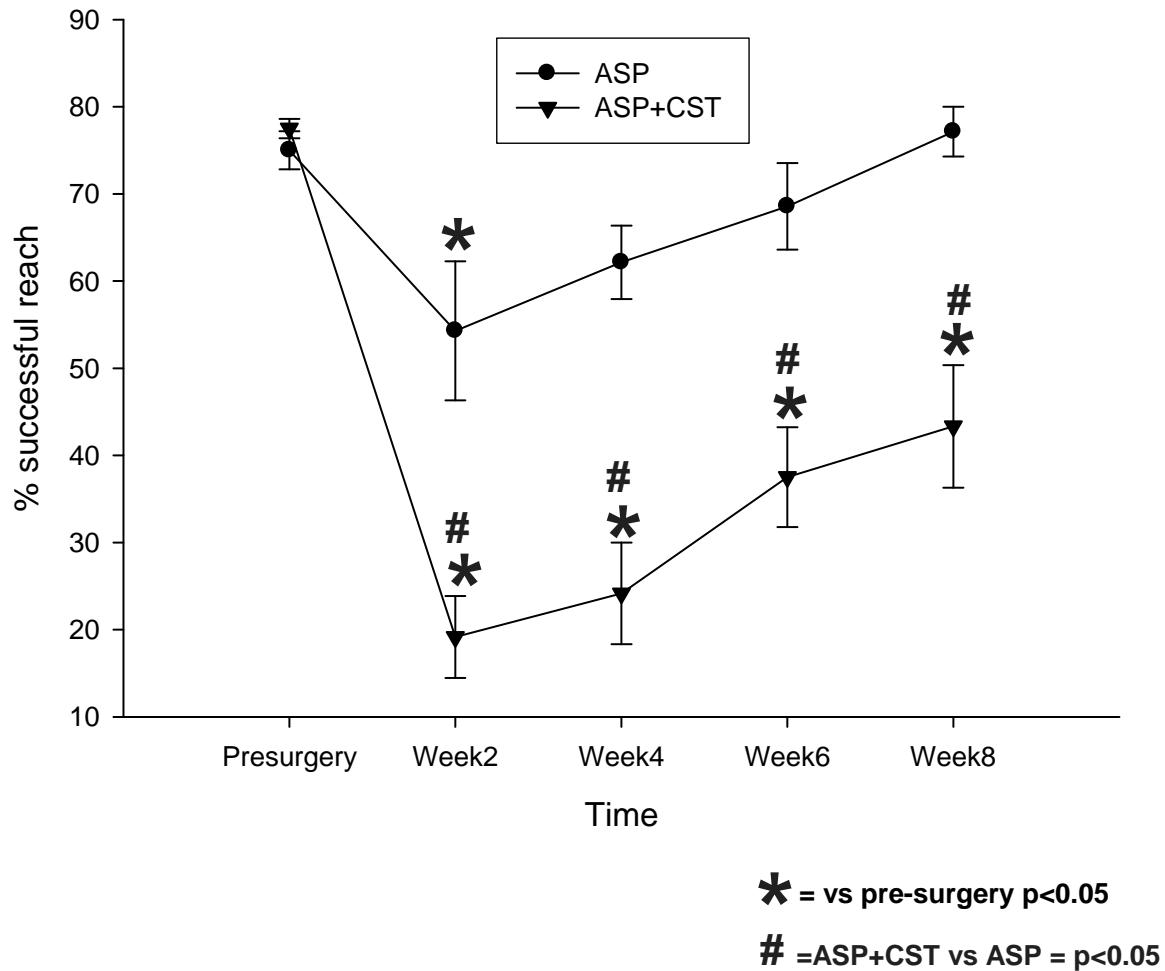


Fig.4.2: Percentage successful reaches in ASP and ASP+CST rats. In the ASP group, the reaching success dropped at week 2 post-surgery ($p < 0.05$) but recovered at later time points. In the ASP+CST group, the reaching success dropped after surgery and remained lower compared to pre-surgical ($p < 0.001$) and ASP group ($p < 0.05$) values at all time points tested.

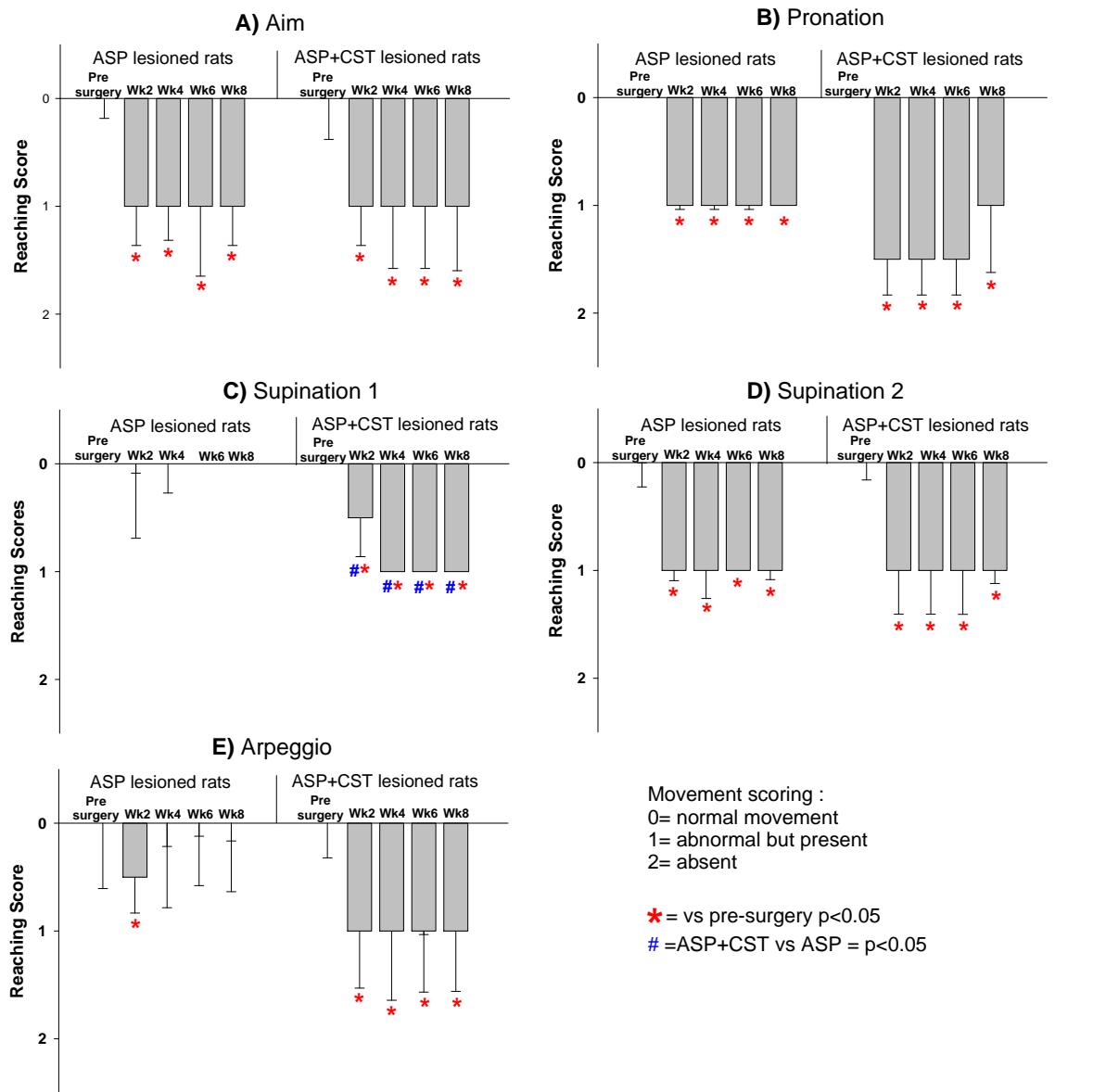


Fig.4.3: Qualitative reaching scores in ASP and ASP+CST rats. Bars represent median values + 95% confidence limits. ASP rats showed abnormal or absent aim, pronation and supination type 2 movements compared to pre-surgery (A, B, D, $p < 0.001$). They also had abnormal arpeggio movements at 2 weeks post-surgery compared to pre-surgery (E, $p < 0.05$). ASP+CST rats showed changes in aim, pronation, supination 1 and supination type 2 movements compared to pre-surgery (A – D, $p < 0.001$), which persisted even at 8 weeks post-surgery. In addition, ASP+CST rats also showed persistent abnormal or absent arpeggio movement of the paws compared to pre-surgery (E, $p < 0.001$). ASP+CST rats showed abnormal supination type 1 movements compared to ASP rats at all time points post-surgery (C, $p < 0.05$).

also affected post-surgery ($p<0.001$; Fig. 4.3c). ASP+CST rats could grasp the pellet, but they rotated their heads to eat the pellet.

4.4.2.2 Skilled locomotion: horizontal ladder

In both ASP and ASP+CST groups, there were no differences in hindlimb errors at any time points before or after surgery and thus the results refer to data for forelimbs only. Animals in both ASP and ASP+ CST groups made fewer correct steps with the forelimbs after surgery compared to pre-surgical performance ($p<0.001$ for both groups) (Fig. 4.4). Post –hoc analysis indicated that ASP animals recovered to pre-surgical performance by 6 weeks post surgery, whereas ASP+CST animals had not recovered by 8 wks post-operative ($p<0.001$; Fig. 4.4). Compared to animals with an intact CST, animals with CST lesions made fewer correct steps at all timepoints after surgery ($p<0.05$; Fig 4.4).

4.4.2.3 Overground locomotion

4.4.2.3.1 Analysis of ground reaction forces

When rats are trotting overground, they alternatively bear the weight on diagonal limb pairs. Both the forelimbs and the hindlimbs produce similar peak vertical forces (Fig. 4.6A). In the fore-aft direction, when the limb touches the ground, it initially produces a braking force (negative fore-aft force) followed by a propulsive force (positive fore-aft force). As the rat is trotting, most of the braking force is generated by the forelimbs, while hindlimbs generate predominantly propulsive forces (Fig. 4.5A). The displacements of limbs in the lateral direction are small and hence a very small medio-lateral force is generated.

Horizontal ladder in rats with ASP and ASP+CST lesions

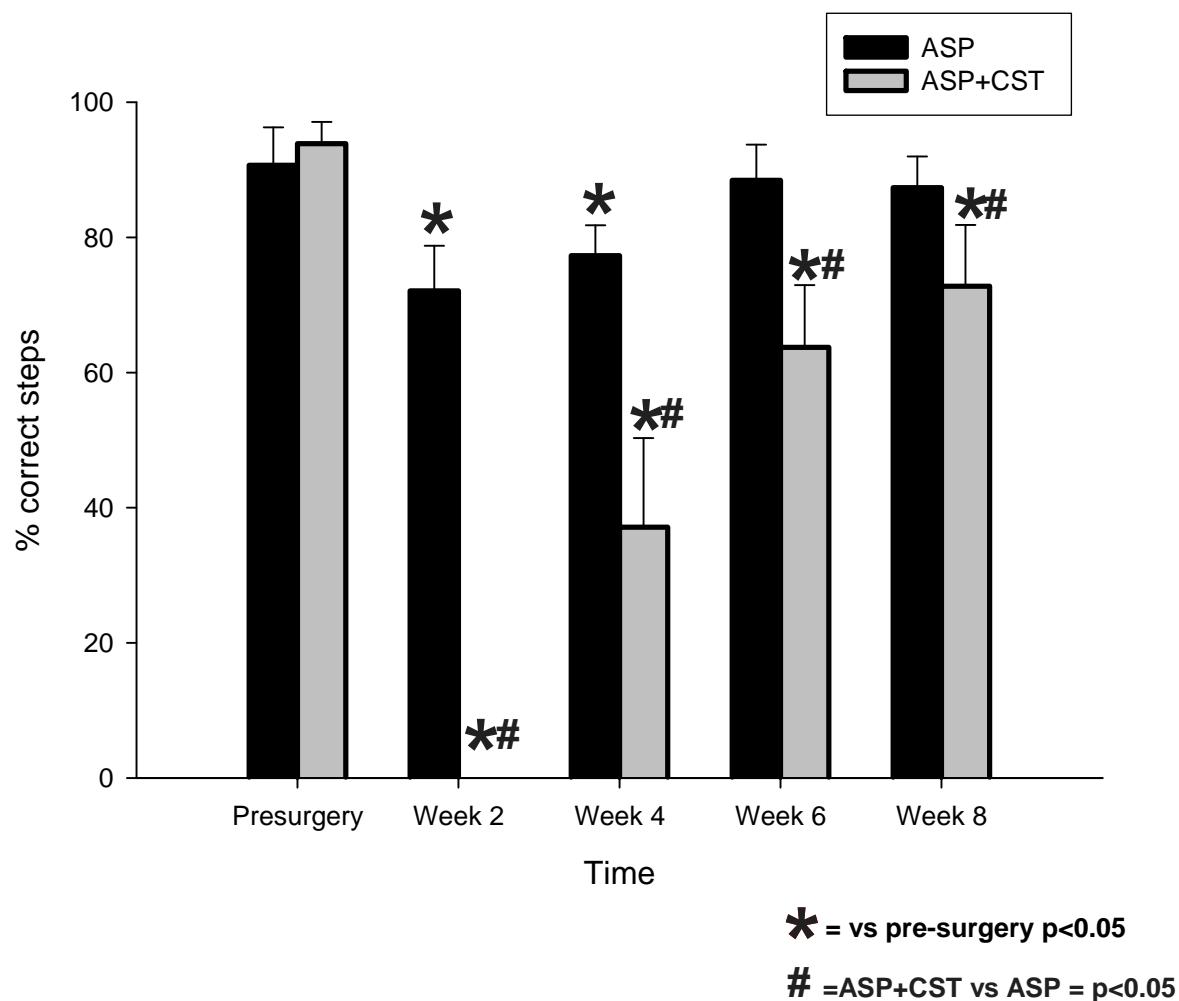


Fig. 4.4: Percentage correct steps of the forelimbs while crossing a horizontal ladder by ASP and ASP+CST rats. ASP rats made errors only at weeks 2 ($p < 0.001$) and 4 ($p < 0.05$) compared to pre-surgery, and recovered to pre-surgical performance by week 6 post-surgery. ASP+CST rats persistently made more errors on the ladder at all the time points (weeks 2, 4, 6: $p < 0.001$ and week 8, $p < 0.05$) compared to pre-surgery and compared to ASP rats.

As we did bilateral lesions, the changes in both of the forelimbs (right and left) and both of the hindlimbs (right and left) were similar. For the most part, alterations in ground reaction forces from pre-surgical values were similar for both ASP and ASP+CST groups and in both groups these changes remained unchanged after surgery for the duration of the study. For simplicity, only the week 8 data is presented here. In both groups, the fore-aft forces generated by the fore- and hindlimbs changed in an identical manner after surgery (Fig. 4.5, $p<0.05$ for both groups). The forelimbs generated more propulsive forces in both groups, whereas the hindlimbs generated more braking forces (Fig. 4.5). Peak vertical forces produced by the hindlimbs were also reduced in both groups compared to pre-surgical values, although this change was only significant for the ASP+CST group (Fig. 4.6, $p<0.05$ for ASP+CST, $p<0.06$ for ASP group). ASP + CST animals also had reduced peak vertical forces in the forelimbs ($p<0.05$). Comparisons between the groups did not reveal any differences in ground reaction forces. Interestingly, all ASP+CST rats, but not ASP rats, adopted a curled-paw posture during rest for the first 2 weeks post-surgery, which was not present at later time points. Nevertheless, the curled posture was not observed whilst rats were trotting on the GRF runway nor when the rats were walking on the horizontal ladder.

4.4.2.3.1 Step lengths and stride parameters

In both the ASP and ASP+CST groups, there were no differences in stride length or stride duration at any time point compared to pre-surgical values, indicating that animals were moving at the same speed both before and after surgery. For both groups, the only change in limb timing parameters was the increase in stance duration of the forelimbs. At all time points after surgery, animals spent proportionately more time with the forelimbs in contact

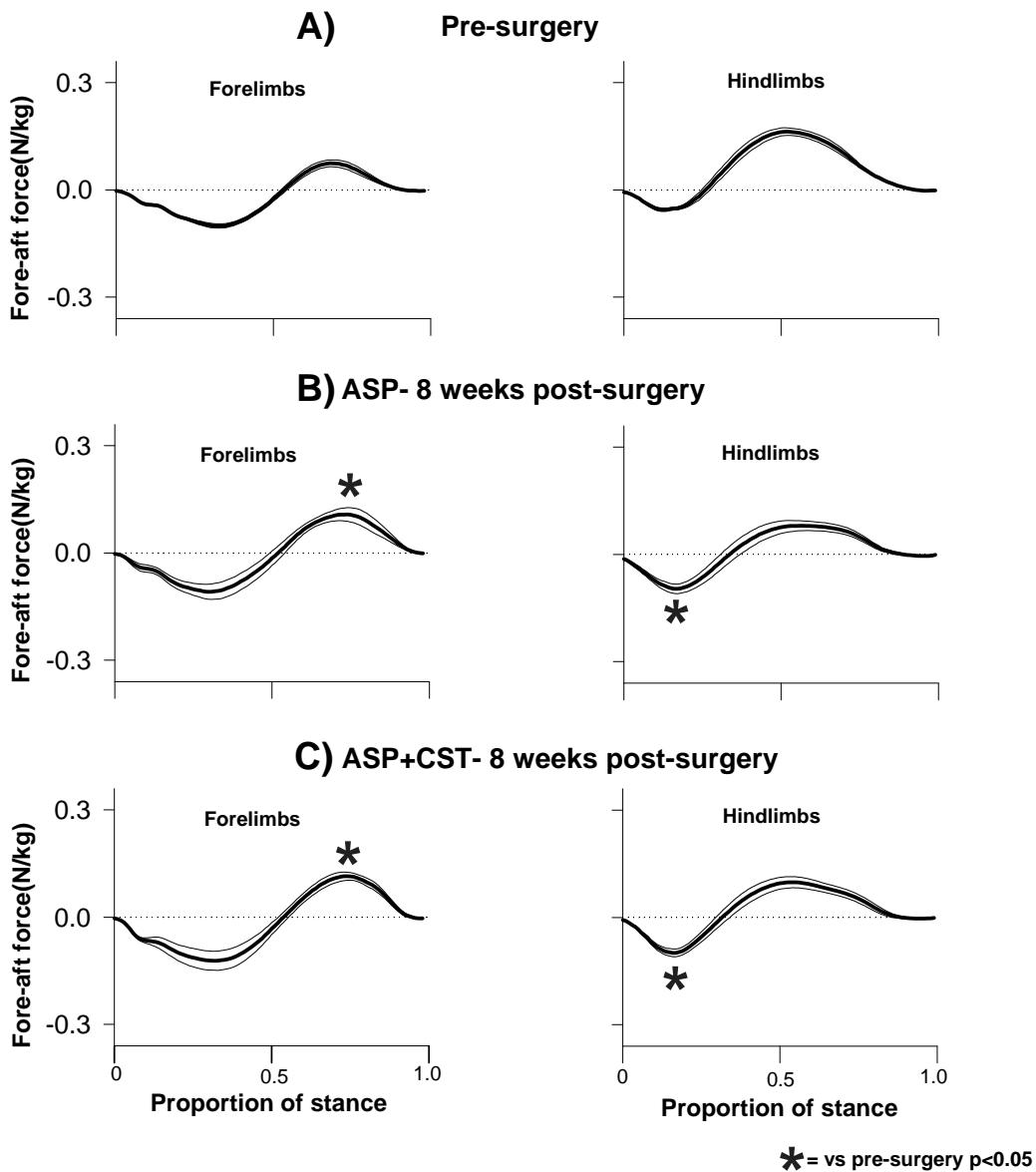


Fig. 4.5: Fore-aft forces generated by pre-surgical (A), ASP lesioned (B) and ASP+CST lesioned (C) rats. Eight weeks post-surgery in both ASP and ASP+CST rats, the forelimbs generated increased peak propulsive forces ($p<0.05$) and the hindlimbs generated increased peak braking forces ($p<0.05$) compared to the pre-surgical rats. Thick lines represent mean data for each group of animals, thin lines represent \pm standard error of the mean.

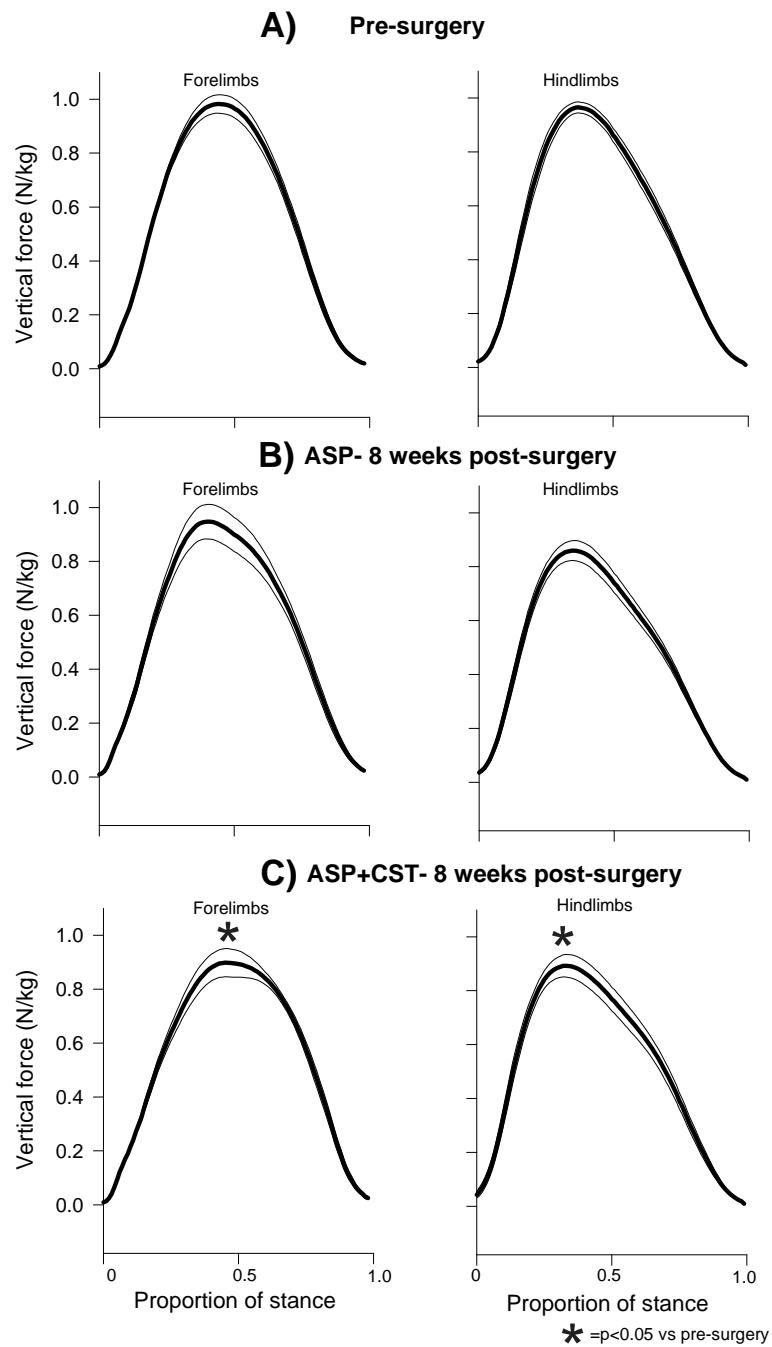


Fig. 4.6: Vertical forces produced by the forelimbs and hindlimbs for pre-surgical (A), ASP lesioned (B) and ASP+CST lesioned (C) rats. Eight weeks post-surgery, ASP rats produced reduced peak vertical forces in the hindlimbs ($p < 0.06$) compared to the pre-surgical rats (B). Eight weeks post-surgery, ASP+CST rats produced smaller peak vertical forces in both the forelimbs and the hindlimbs ($p < 0.05$) compared to pre-surgical rats (C). Thick lines represent mean data for each group of animals, thin lines represent \pm standard error of the mean.

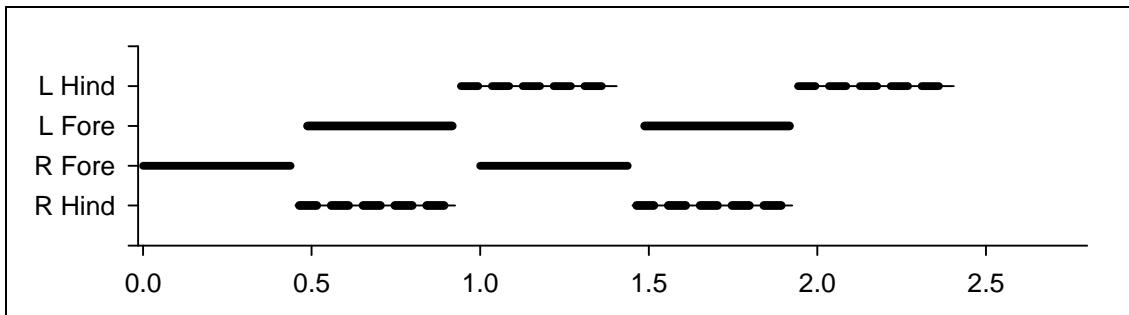
with the ground compared to pre-surgery (Fig. 4.7 ; $p<0.001$ for ASP group, $p<0.05$ for ASP+CST group) .

4.5 Discussion

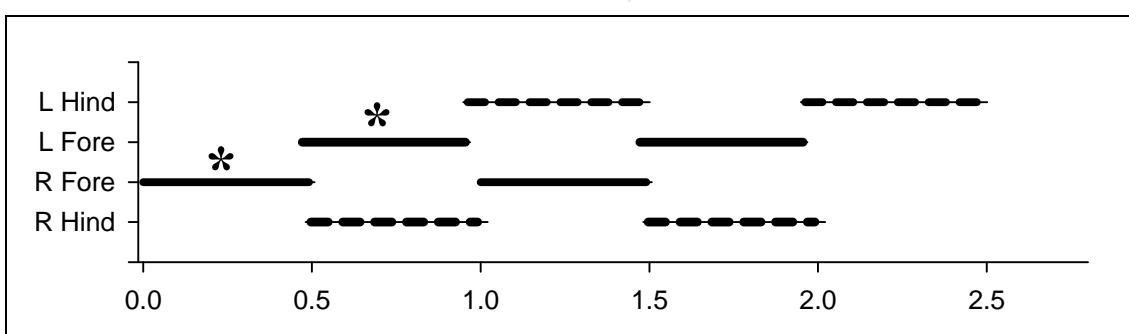
Rats with bilateral damage limited to the ascending sensory fibres in the cervical dorsal spinal funiculus showed transitory deficits on endpoint measures of reaching success and footslip errors during skilled movements. Nevertheless, detailed examination of forelimb movements revealed persistent changes in the actions used to perform the reaching task. Similarly, detailed locomotor measurements demonstrated small but persistent changes in limb timing and forces during overground locomotion. Comparatively, rats with damage to both sensory axons and descending CST axons in the dorsal funiculus displayed more severe and persistent deficits on endpoint measures of reaching ability and ladder locomotion, but no additional changes during overground locomotion. These results emphasize the normal contribution of both ascending sensory and descending CST input during skilled limb movements and also support a role for ascending sensory information, but not CST input, during overground locomotion. These results also illustrate the value of using detailed analysis to reveal behavioural changes after spinal injury.

An important distinction is made in the current study between the functional contributions made by the ASP compared to those of the CST. One of the limitations of ascribing behavioural deficits after discrete spinal lesions is variation in the extent of damage to the intended pathways. On the basis of histological data alone, we cannot discount the possibility that some animals in the ASP group did sustain some damage to the CST. If this

A) Pre-surgery



B) ASP rats- 8 weeks post-surgery



C) ASP+CST rats- 8 weeks post-surgery

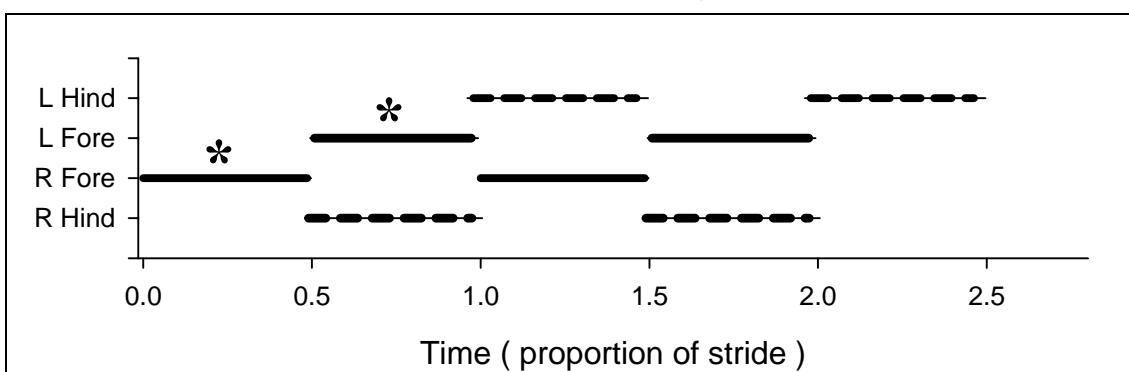


Fig. 4.7: Forelimb (solid lines) and hindlimb (dashed lines) contact timing for pre-surgical rats (A) ASP lesioned rats (B) and ASP+CST rats(C). During trotting, diagonal limb pairs (i.e. right fore and left hind) are in contact with the ground at the same time. Both ASP and ASP+CST rats moved similarly to the pre-surgical rats and the only difference was the increased stance duration of the right and left forelimbs (* = $p < 0.001$) compared to pre-surgery. The lengths and positions of the thick solid and dashed bars represent mean limb contact times for each group of animals, while the thin lines at either end of each bar represent the standard error of the mean.

were the case, some of the behavioural changes seen in ASP animals might therefore be due to CST damage. Upon closer examination of individual animals in the ASP group, however, we found that the behavioural results did not differ between animals which sustained slight (unintentional) damage to the CST and those with a clearly undamaged CST. Similarly, although we cannot confirm that all CST axons were damaged in the ASP+CST group, we could find no relationship between increased severity of lesion and more marked behavioural deficits. Additionally, animals in the ASP-CST group actually showed more severe and persistent deficits compared to those described in the literature (discussed later), suggesting that our CST lesions were at least as complete as those of other studies. We are therefore confident that the behavioural differences between these groups are most strongly related to the differences between the contributions of ASP and those of the CST.

Our reaching results in ASP lesioned animals were comparable to those of other studies involving damage to sensory pathways in the dorsal column. Unilateral dorsal column lesions involving only sensory axons at C2 transiently decreased reaching success, from 50% to 30%, 2 days after surgery (McKenna et al., 1999). Recovery occurred by 1 week post surgery in the latter study, however, whereas the present results using bilateral lesions showed reduced reaching success (80% to 60%) persisting for at least 2 weeks. The higher percent reaching successes overall in our study were probably because of long training periods and our strict inclusion criterion (pre-surgery success needed to be at least 70%). The slower recovery of reaching success in our study is harder to explain but may be due to the bilateral nature of the lesion – both limbs are affected, which may require a different

compensatory mechanism, possibly related to body posture during the reach. Nevertheless, detailed analysis of reaches in ASP lesioned rats showed persistent alterations in aim, pronation and supination movements, similar to the reaching pattern described previously for rats with specific damage to sensory pathways in the dorsal columns (McKenna et al., 1999). These changes in limb action in ASP lesioned rats demonstrate that sensory feedback in the dorsal columns plays an ongoing role during skilled movements (Ballermann et al., 2001;Onifer et al., 2005).

Rats with bilateral damage to both sensory axons and the dorsal CST showed persistent deficits in reaching success up to 8 wks post-lesion in the present study. Comparable studies have shown more rapid recovery of reaching success (Schrimsher et al., 1993;Weidner et al., 2001;Chan et al., 2005). Closer examination of each study, however reveals similarities to the present results. In one study, pre-lesion reaching success was much lower than in the present study (37% compared to 80%) although post-lesion successes were comparable (range of 25 – 30% over 4 weeks, compared to 20 – 40% over 8 weeks in the present study (Schrimsher et al., 1993). In a more recent study, reaching success after bilateral ASP+CST lesions dropped significantly from 70% pre-lesion to approximately 45% at 2 and 3 weeks post lesion and then increased slightly to approximately 50% at 4 wks post lesion, although the latter result was not significantly different from pre-lesion values (Weidner et al., 2001). More rapid recovery has also been documented after dorsal column lesions at C4-C5 which caused a drop in performance from 60% to 30% at 1 week post-injury with recovery to 55% at 2 weeks (Chan et al., 2005). Compared to the latter studies, we saw a more severe drop in performance

immediately post lesion (80% to 20%) but similar or better improvement post-lesion, albeit over a longer time period (e.g.. 20 % - 40% over 8 weeks in the present study compared to 45% - 50% over 4 weeks in the Weidner, et al. study). These differences in ‘recovery’levels might arise from methodological differences, including the use of different rat strains. The Long-Evans rats used in the present study display different reaching characteristics compared to the more commonly used Sprague-Dawley or Wistar rats (VandenBerg et al., 2002;Whishaw et al., 2003).

The qualitative changes in reaching movements in rats with damage to sensory and CST components of the dorsal funiculus are consistent with those seen after CST damage at the level of the medullary pyramids. Complete pyramidal tract lesions impair the ability of the rats to reach for pellets (Whishaw et al., 1998) and incomplete lesions still alter the qualitative aspects of reaching (Piecharka et al., 2005) with reaching success being proportional to sparing of CST (Keyvan-Fouladi et al., 2003;Li et al., 1997). Limb movements in ASP+CST rats showed abnormalities in aim, pronation and supination, similar to rats with medullary pyramidal lesions (Whishaw et al., 1998). Interestingly, the absence of the arpeggio movement seen with ASP+CST lesions here was not seen with pyramidal lesions alone, but may be due to the combined loss of sensory and CST axons (McKenna et al., 1999;Schrimsher et al., 1993). The persistence of these changes in limb movements suggests that at least part of the recovery in reaching success in the present study is due to the development of compensatory limb movements (McKenna et al., 1999). These may or may not be mediated by changes in connectivity of descending and ascending pathways, including the ventral segment of CST as has been suggested (Weidner

et al., 2001). Injured CST tracts have also been shown sprout to contact long propriospinal neurons, which might contribute to gradual improvement in the behavioural tasks (Bareyre et al., 2004). Furthermore, descending input from the red nucleus, which remains intact after dorsal column lesions, has been shown to influence skilled limb movements in rats and may mediate recovery after loss of sensory and CST axons (Kuchler et al., 2002; Whishaw et al., 1998; Muir et al., 2007). The more rapid recovery of limb use in ASP rats compared to ASP+CST rats suggests that alternate sensory pathways compensate relatively quickly for loss of dorsal sensory pathways, including the spinothalamic tract and several spinoreticular pathways, which ascend in the ventrolateral part of the spinal cord (Tracey, 2004; Onifer et al., 2005).

The ability of cervically spinal injured rats to cross a horizontal ladder has been examined in several studies (Soblosky et al., 2001; Metz et al., 2002; Webb et al., 2003b; Webb et al., 2004; Onifer et al., 2005; Chan et al., 2005; Gensel et al., 2006). Previous work from our lab has demonstrated increased ipsilateral fore- and hindlimb footslips after unilateral cervical ASP lesions (Webb et al., 2003b). Rats with unilateral CST lesions at the level of medullary pyramids also made more errors on the contra-lateral forelimb and hindlimb, suggesting that loss of CST in lesions of the spinal dorsal columns might contribute to the increase in footslips (Metz et al., 2002). After unilateral cervical contusion injuries, both forelimb and hindlimb footslips occur, although forelimb errors are more frequent (Gensel et al., 2006; Soblosky et al., 2001). Studies of rats with bilateral lesions involving the entire dorsal half of the spinal cord have also shown increases in both forelimb and hindlimb footslips during ladder locomotion, although again the forelimbs were more severely

affected than the hindlimbs (Onifer et al., 2005). It is a bit surprising in the present study that, for both ASP and ASP+CST groups, we found no hindlimb deficits during ladder locomotion. One possibility is the ladder used in the present study is a less challenging one than those used in the above studies (Metz et al., 2002; Onifer et al., 2005). Our ladder allowed the hindlimbs to frequently span the regular 1 cm distance between rungs, thus making it simpler for the hindpaw to find a foothold. Nevertheless, these results are consistent with findings from a number of studies that hindlimb sensorimotor abilities are less affected by cervical dorsal column injuries compared to the forelimbs. Hindlimb placement on a rung might be influenced more than the forelimbs by direct proprioceptive information arising from the limb and torso, with less reliance on ascending sensory and supraspinal input (Bolton et al., 2006; Pearson, 2000). This latter input would be more vital for the positioning of the forelimbs, and it has been suggested that the forelimbs are normally used in a more exploratory fashion (Clarke, 1995). This could explain why, despite the damage to sensory information arising from both fore- and hindlimbs, the hindlimbs could still adapt to loss of inputs which caused forelimb footslip errors.

Unlike skilled movements, limb action during overground locomotion is relatively stereotyped and much of the limb action is determined spinally (Grillner, 1981). This makes it less likely that damage to ascending and descending supraspinal projections will be reflected in the locomotor pattern compared to movements more reliant on supraspinal control. Nevertheless, small adjustments in the locomotor pattern were detected in both ASP and ASP+CST rats using GRF analysis. Furthermore, these adjustments, particularly the changes in the braking and propulsive forces generated by the limbs, were similar to

those produced in the forelimb ipsilateral to a unilateral cervical ASP lesion, suggesting that ascending sensory information does affect limb action during locomotion (Webb et al., 2003b). Other changes in the locomotor pattern, such as increased forelimb stance durations (Fig. 4.7) seen here were not seen after unilateral lesions, however. Instead, the limb contact pattern seen after unilateral ASP lesions was markedly asymmetric (Webb et al., 2003b). This pattern was common to that of rats with different unilateral CNS lesions and is possibly part of a general compensatory response to unilateral injury (Muir et al., 1999a; Muir et al., 1999b; Muir et al., 2000b; Webb et al., 2002; Webb et al., 2003b; Webb et al., 2004; Poulton et al., 2005). In the present study, the bilateral nature of the lesion resulted in a symmetrical locomotor pattern and a much less marked change in gait, which was nevertheless detectable and persistent throughout the 8 week post surgical period. Interestingly, dorsal funicular lesions at the thoracic level did not produce detectable changes in limb contact patterns (Hendriks et al., 2006) or in BBB scoring (Schucht et al., 2002; Ballermann et al., 2006). Importantly, the similarities of the gait changes in both ASP and ASP+CST groups suggest that the CST plays a minor role, if any, during overground locomotion, consistent with earlier locomotor studies involving pyramidal tract lesions (Metz et al., 1998; Muir et al., 1999a).

4.6 Conclusions

The results of this study highlight the different effects of using endpoint measurements vs detailed kinematic or kinetic measurements of performance (Muir et al., 2000a). Endpoint measures of reaching success and footslip errors revealed differences between ASP and ASP+CST lesioned animals but were not sensitive enough to detect the abnormalities in the

ways in which both groups of animals accomplished the reaching task. Only analysis of limb movements during reaching and, similarly, of the forces and timing of the limbs during overground locomotion, could reveal the differences between the lesioned and unlesioned animals that persisted at 8 weeks after surgery. These findings, and those of others, underlie the assertion that afferent sensory information in the dorsal columns appears to contribute to both skilled movements and to relatively unskilled locomotor movements in a manner that can be detected using methods with sufficient sensitivity (McKenna et al., 1999). This emphasizes the importance of using sensitive behavioural measurements to assess sensorimotor behaviour after experimental spinal cord injury (Muir et al., 2000a).

Chapter 5. THE DIFFERENTIAL EFFECTS OF CERVICAL AND THORACIC DORSAL FUNICULUS LESIONS IN RATS*

(*manuscript published- Kanagal and Muir, 2008. Behavioural Brain Research, 187; 379-386)

5.1 Abstract

The purpose of this research was to compare the locomotor abilities of rats with cervical dorsal spinal funicular (DF) lesions to those of rats with the same lesion at the mid-thoracic level. The dorsal funiculus, consisting of ascending sensory fibers and the main component of the corticospinal tract, was transected either at spinal level C2 or at T8. We examined limb force generation and limb timing and coordination during overground locomotion, as well as foot placement errors during locomotion over a horizontal ladder. At 6 weeks post-surgery, bilateral lesions of the cervical DF caused subtle but persistent changes in the generation of ground reaction forces and limb timing during overground locomotion, and caused persistent forelimb, but not hindlimb, errors during ladder crossing. In contrast, the same lesion at the mid-thoracic level did not affect overground locomotion and caused only minor forelimb and hindlimb errors during ladder walking at 2 weeks post-lesion which recovered to pre-surgical levels by 6 weeks post-lesion. DF lesions at cervical versus thoracic levels thus have differential effects on locomotor abilities in rats. We compare these results with previous work and suggest that the differential response to DF transection might be related to both functional distinctions between the fore- and hindlimbs and to anatomical differences in the dorsal funiculi at different spinal levels. These findings have

implications for the mechanisms of recovery as well as the types of behavioural tests which can be practically used to measure functional changes in different lesion models.

5.2 Introduction

Animal models for CNS disorders such as spinal cord injury (SCI) have most commonly been rodents and cats. The use of quadrupedal animals as behavioural models for human SCI presents some interesting issues when comparing the functional effects of injuries. In intact animals, the use of all four limbs during locomotion normally permits a larger variety of limb coordination patterns (i.e. gaits) compared to that available to bipeds. After SCI, this capability allows more varied and subtle methods of compensation for dysfunction of one or more limbs when compared to the same injury in bipeds (Muir et al., 1998; Webb and Muir, 2002). Furthermore, lesions at different levels of the spinal cord in animal models would be expected to affect locomotion in qualitatively different ways. For example, rats with thoracic lesions can generally use the relatively unaffected forelimbs to compensate for dysfunction of one or both hindlimbs. In contrast, animals with cervical lesions would be expected to have some dysfunction of both forelimbs and hindlimbs and so make compensatory changes which are different from animals with the same lesion at the thoracic level. This has important implications for the types of behavioural tests which can be practically used to measure functional changes in different lesion models.

Although there are many studies describing functional deficits and recovery after either thoracic or cervical lesions in rodent models, there are few which directly compare the two. We have previously shown that lateral hemi-sections at the cervical or thoracic spinal level

in rats caused some predictable functional changes, in reflex responses for example, but also some less expected ones (Webb and Muir, 2002). For instance, while the hindlimbs are much less affected by cervical spinal hemi-sections during overground locomotion compared to the same lesion at the thoracic level, the locomotor forces exerted by the ipsilateral forelimb are altered in a similar manner for both cervical and thoracic lesions (Webb and Muir, 2002). Clearly, different levels of spinal cord lesions cause different deficits and compensations.

With this in mind, we proposed to compare the locomotor abilities of rats with cervical lesions of the dorsal spinal funiculus to those of rats with the same lesion at the mid-thoracic level. The dorsal funiculus is part of the spinal cord that is invariably lesioned during most experimental spinal injury studies. This is due in part to the accessibility of the dorsal cord for surgical approaches. Additionally, the dorsal funiculus in rodents is comprised of both ascending sensory pathways and the descending CST (Brosamle and Schwab, 1997). Because of its functional importance in humans, lesioning of CST in the rat dorsal funiculus has become a popular model in experimental SCI studies. Nevertheless, studies involving specific injury to CST axons at the level of the medulla have failed to show persistent deficits during overground locomotion, suggesting that the loss of CST is not as debilitating in rats, at least, as it is in humans (Metz et al., 1998; Muir and Whishaw, 1999). Recent work from our lab supports these findings and demonstrates that the dorsal component of the CST appears to contribute to more challenging tasks, such as locomotion over a ladder (Chapter 4, this thesis).

In the present study, we assessed locomotor abilities in rats both overground and while crossing a horizontal ladder. During overground locomotion, we used force platforms to measure the ground reaction forces exerted through each limb, as well as to measure limb timing and step lengths. We initially hypothesized that rats with bilateral lesions of the cervical dorsal funiculus would display deficits involving all four limbs, while the rats with bilateral lesions of thoracic dorsal funiculus would display deficits involving only the hindlimbs. We found instead that cervical lesions produced subtle but persistent effects on all four limbs during overground locomotion but only affected forelimb placement during ladder crossing, whereas thoracic lesions had no effect on overground locomotion and only transient effects on limb placement during ladder crossing.

5.3 Materials and Methods

5.3.1 Animals

This experiment was conducted using 12 adult female Long-Evans rats (225-250g) obtained from Charles River Laboratories (Que., Canada). The rats were housed in a light controlled room (12h light/ 12h dark) with in the Animal Care facility in the Department of Veterinary Biomedical Sciences at the University of Saskatchewan. All rats were fed with rat chow but were food restricted such that their body weights were maintained below 300g. Their body weights were recorded every week. All rats were examined daily for their health and were cared for according to the regulatory standards set out by the Canadian Council on Animal Care.

5.3.2 Training

All rats were trained on a runway and ladder walking as described in Section 3.4.1 in this thesis.

5.3.3 Surgery

All animals underwent standard anaesthesia, analgesia and surgical procedure as described in Sections 3.3.1 and 3.3.2. In this study, lesions were made bilaterally to either the cervical (C2; cervical DF: n=6) or mid thoracic (T8; thoracic DF: n=6) dorsal funiculus (dorsal columns with dorsal corticospinal tract).

5.3.4 Behavioural assessment

Prior to surgery, all rats were assessed behaviourally using kinetic, kinematic and endpoint measurements. Kinetic parameters consisted of measurement of ground reaction forces during overground locomotion. Kinematic parameters ie., step lengths, stance durations and limb timing, were also measured during overground locomotion. Endpoint measurements were used to count the number of footslips made while crossing horizontal ladder (skilled locomotion). Data was collected prior to surgery (pre-surgery) and again after surgery at weeks 2 and 6.

5.3.4.1 Overground locomotion

5.3.4.1.1 Measurement of ground reaction forces

Ground reaction forces (kinetics) were recorded and analyzed as described in Section 3.4.4.1 in this thesis.

5.3.4.1.2 Measurement of stride parameters

The onset and offset timings of individual limbs from all the 3 force plates were used to calculate kinematic measurements as described in Section 3.4.4.2 in this thesis.

5.3.4.2 Skilled locomotion -Horizontal ladder

Skilled locomotor abilities of rats were tested as described in Section 3.4.3 in this thesis.

5.3.5 Histology

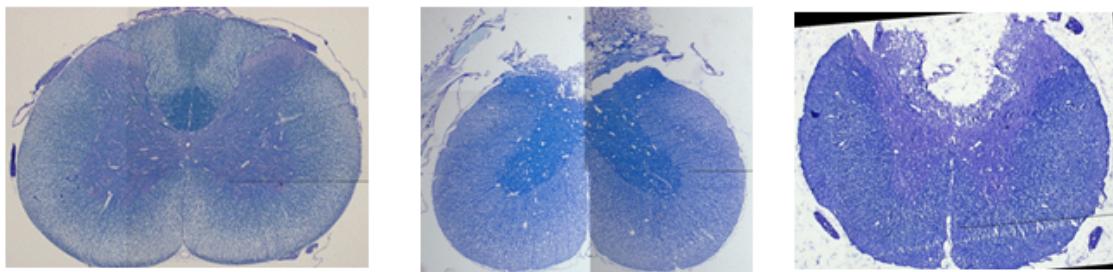
Histological processing and staining was done at 6 weeks following spinal cord injury as described in Section 3.5 of the thesis.

5.4 Results

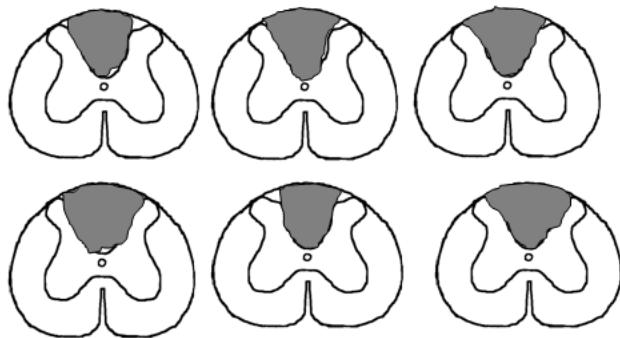
5.4.1 Histology

In the cervical DF group, all 6 rats met the criterion of combined damage to the sensory ascending and descending CST axons (Fig. 5.1B, 5.1D). In the thoracic DF group, 5 rats had consistent damage to dorsal funicular axons (Fig. 5.1C, 5.1E), while one rat was eliminated from the study as it had sustained extensive damage to dorsal horns. For the final analyses, there were 6 rats in cervical DF group and 5 rats in thoracic group. In both the groups, the damage was restricted to the dorsal funiculus in that there was slight to no damage to the dorsal horns and no damage to the central canal, or to the dorsolateral funiculus (Fig. 5.1).

A) Control spinal cord B) Cervical DF lesion- C2 C) Thoracic DF lesion- T8



D) Schematic representation of cervical DF damage



E) Schematic representation of thoracic DF damage

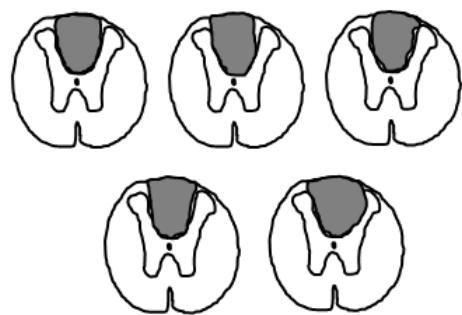


Fig. 5.1: Representative photomicrographs of spinal cords from (A) Sham, (B) Cervical DF and (C) Thoracic DF lesioned rats stained with Luxol-Fast Blue and Cresyl Violet. In both cervical DF and thoracic DF lesioned groups, ascending sensory fibers and CST were damaged. Schematic representation of lesion epicenter in (D) 6 cervical DF rats and (E) 5 thoracic DF rats.

5.4.2 Behavioural assessment

5.4.2.1 Overground locomotion

5.4.2.1.1 Analysis of ground reaction forces (GRF)

During trotting, normal rats alternatively bear their weight on diagonal limb pairs (i.e. right forelimb-left hindlimb alternating with left forelimb-right hindlimb). Both forelimbs and hindlimbs produce similar vertical forces (Fig. 5.2A). In the fore-aft direction, when a limb touches the ground, an initial braking force is generated (negative fore-aft forces) followed by a propulsive force (positive fore-aft forces). In a trot, forelimbs generate most of the braking force, while the hindlimbs generate predominantly propulsive forces (Fig. 5.3A). The displacement of the limbs in the lateral direction is small and hence very small medio-lateral forces are generated. Ground reaction forces were bilaterally symmetrical in both pre- and post-operative animals, in that forces generated by both forelimbs (right and left) or both hindlimbs (right and left) were not different from each other.

Cervical DF lesions induced changes in the generation of ground reaction forces which persisted for the entire duration of the study (6 weeks post-surgery). After surgery, rats in this group showed reduced generation of peak vertical forces by both the forelimbs (right and left) and the hindlimbs (right and left) ($p<0.05$; Fig. 5.2B, 5.2D) compared to their pre-surgical performance. In the fore-aft direction, the forelimbs generated increased propulsive force ($p<0.05$; Fig. 5.3B, 5.3D) whereas the hindlimbs generated increased braking force ($p<0.05$; Fig. 5.3B, 5.3D) both at 2 and 6 weeks post surgery, when compared to their pre-surgical values. In contrast, thoracic DF rats showed no changes

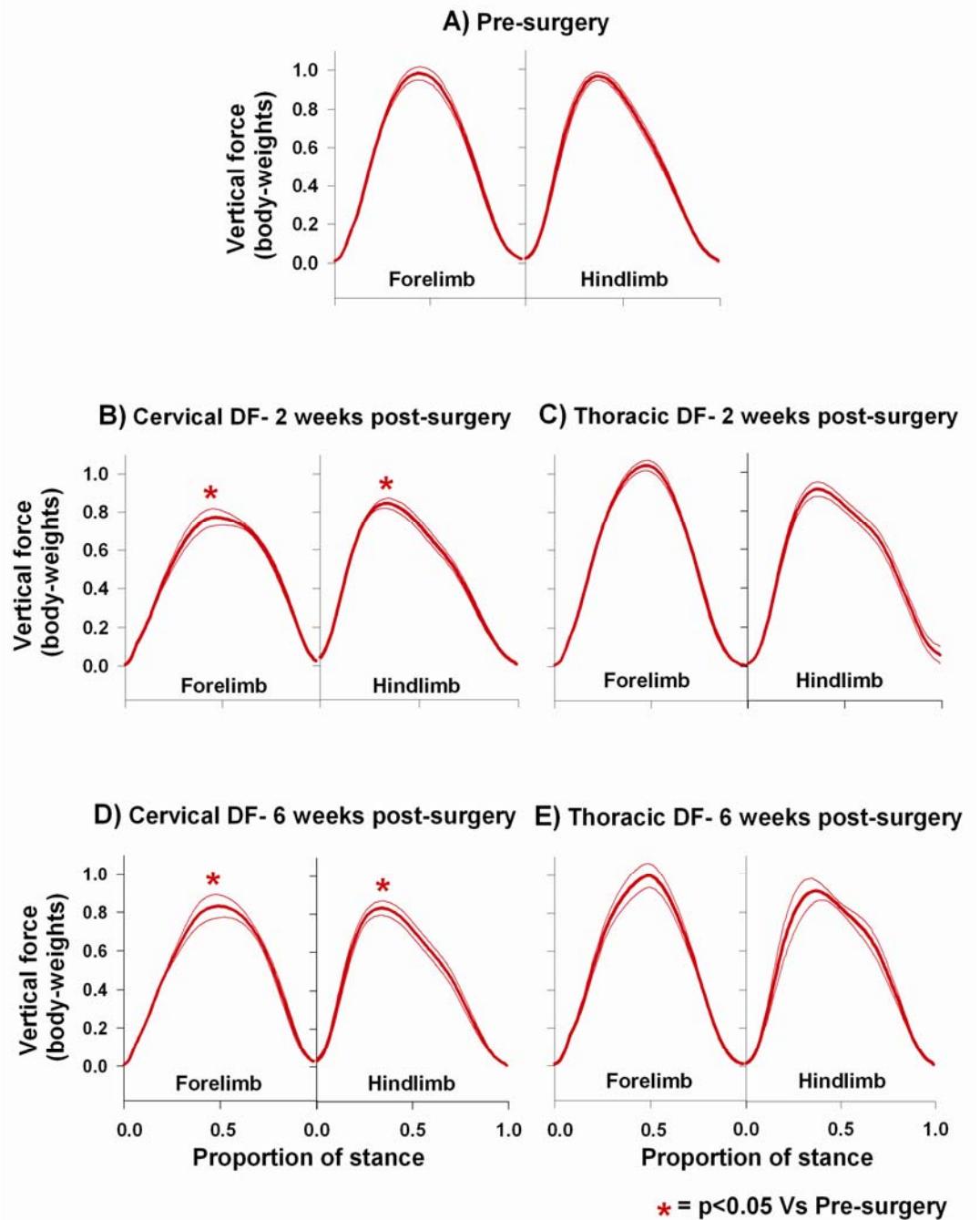


Fig. 5.2: Vertical forces produced by the forelimbs and hindlimbs for (A) pre-surgical, (B) cervical DF-2 weeks post-surgery (C) thoracic DF-2 weeks post-surgery (D) cervical DF-6 weeks post-surgery and (E) thoracic DF-6 weeks post-surgery rats. At both 2 and 6 weeks after surgery, cervical DF rats produced reduced vertical forces in both the forelimbs and the hindlimbs ($p < 0.05$) compared to pre-surgical rats. After surgery, thoracic DF rats did not show any changes in vertical forces produced by either the forelimbs or the hindlimbs compared to the pre-surgical rats. Thick lines represent mean data for each group of animals, thin lines represent \pm standard error of the mean.

either in generation of peak vertical forces, vertical impulse (Fig. 5.2C, 5.2E) or fore-aft forces (Fig. 5.3C, 5.3E) both at 2 and 6 weeks post-surgery, compared to their pre-surgical values. Comparison between the groups revealed differences in generation of the fore-aft forces ($p<0.05$; Fig. 5.3B). Cervical DF rats generated increased propulsive force by the forelimbs and increased braking force by the hindlimbs when compared with thoracic DF rats.

5.4.2.1.2 Step lengths and stride parameters

In both the cervical and thoracic DF groups, there were no differences either in stride length or stride duration compared to pre-surgical values. Consistent with this finding, there were no differences in the trotting speeds of the rats after surgery compared to pre-surgical speeds in both cervical DF group (pre-surgery: 72.75 cm/s, week 2: 69.2 cm/s, week 6: 73.5 cm/s; p value =0.114) and thoracic DF (pre-surgery: 76.5 cm/s, week2: 74.7 cm/s, week 6: 75 cm/s; p value=0.369) rats. There were also no differences in step distance parameters for any limb in either cervical or thoracic DF rats compared to pre-surgical values. For limb timing parameters, we found differences in stance duration only in the cervical DF rats. Post-surgery (both at 2 and 6 weeks), stance duration of the forelimbs were increased in the cervical DF rats ($p<0.05$; Table 5.1; Fig. 5.4B, 5.4E) compared to pre-surgical values. In contrast, we found no differences in any limb timing parameters in the thoracic DF rats either at 2 weeks (Fig. 5.4C) or at 6 weeks (Fig. 5.4E) post-surgery compared to pre-surgical values.

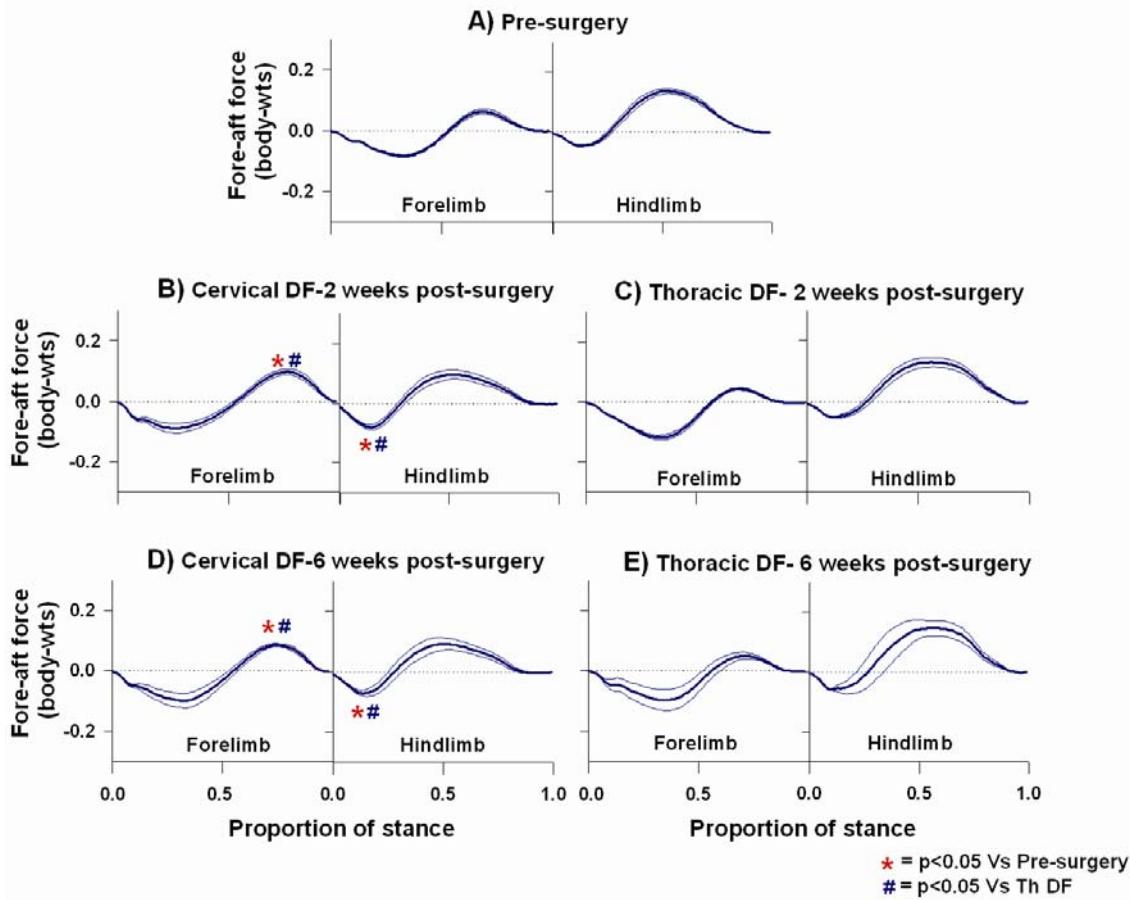


Fig. 5.3: Fore-aft forces generated in (A) pre-surgical, (B) cervical DF-2 weeks post-surgery (C) thoracic DF- 2 weeks post-surgery, (D) cervical DF-6 weeks post-surgery and (E) thoracic DF-6 weeks post-surgery rats. At both 2 and 6 weeks post-surgery in cervical DF rats, the forelimbs generated increased propulsive forces ($p < 0.05$) and the hindlimbs generated increased braking forces ($p < 0.05$) compared to the pre-surgical and thoracic DF rats. After surgery, thoracic DF rats did not show any changes in the fore-aft forces compared to the pre-surgical rats. Thick lines represent mean data for each group of animals, thin lines represent \pm standard error of the mean.

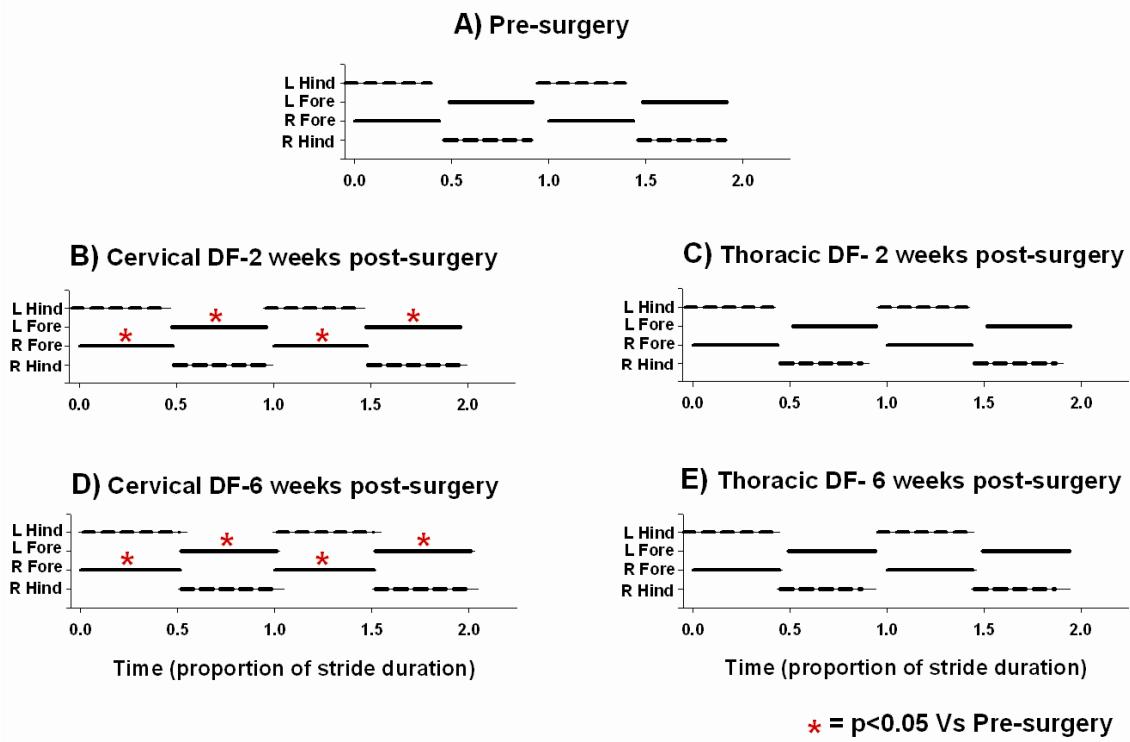


Fig. 5.4: Forelimb (solid lines) and hindlimb (dashed lines) contact timing for (A) pre-surgical, (B) cervical DF-2 weeks post-surgery, (C) thoracic DF-2 weeks post-surgery, (D) cervical DF-6weeks post-surgery and (E) thoracic DF-6weeks post-surgery rats. The lengths and positions of the thick solid and dashed bars represent mean limb contact times for each group of animals, while the thin lines at either end of each bar represent the standard error of the mean. During trotting, diagonal limb pairs (i.e. right fore and left hind or left fore and right hind) are in ground contact at the same time. Both cervical DF and thoracic DF rats moved at a trot, similar to pre-surgical rats. At both 2 and 6 weeks post-surgery, cervical DF rats showed increased stance duration of the forelimbs compared to pre-surgical values (pre-surgery mean \pm SEM: right forelimb 0.445 ± 0.01 , left forelimb 0.429 ± 0.006 ; 2 wks post-surgery :right forelimb 0.501 ± 0.01 , left forelimb 0.487 ± 0.002 ; 6 wks post-surgery right forelimb 0.510 ± 0.01 , left forelimb 0.488 ± 0.01) In thoracic DF rats, there was no change in the stance duration of the limbs at either time point compared to pre-surgical values.

	Pre-surgery	Cervical DF- 2 weeks post-surgery	Cervical DF-6 weeks post-surgery
Right forelimb	0.445±0.01	0.501±0.001* (12.5% increase vs. pre-surgery)	0.510±0.01* (14.5% increase vs pre-surgery)
Left forelimb	0.429±0.006	0.487±0.002* (13.5% increase vs pre-surgery)	0.488±0.01* (13.75% increase vs pre-surgery)

Table 5.1: Stance duration of the forelimbs expressed as proportion of stride duration in cervical DF lesioned rats. After cervical DF lesions, stance duration of the forelimbs increased by about 1.3 times their pre-surgical values. (* =p<0.05 vs pre-surgery). There were no changes in the stance durations of hindlimbs in cervical DF lesioned rats or in the stance durations of any limbs in thoracic DF lesioned rats (data not shown). All values reported as mean ± SEM.

5.4.2.2 Skilled locomotion: horizontal ladder

Cervical DF rats made fewer correct steps with the forelimbs after surgery compared to pre-surgical performance ($p<0.001$: Fig. 5.5A). The forelimb deficit persisted even at 6 weeks post-surgery. However there were no hindlimb errors at any time point after surgery in this group (Fig. 5.5B). In the thoracic DF group, there was a slight reduction in both the forelimb (Fig. 5.5A) and the hindlimb (Fig. 5.5B) correct steps at 2 weeks after surgery compared to pre-surgical performance ($p<0.05$). This transient reduction however recovered by 6 weeks after surgery.

5.5 Discussion

Our results demonstrate that bilateral lesions of the cervical dorsal funiculus in rats caused subtle but persistent alterations in limb force and coordination during overground locomotion, and persistent forelimb, but not hindlimb, deficits during ladder crossing. The same lesion at the mid-thoracic level did not affect overground locomotion, and caused only minor transient fore- and hindlimb deficits during ladder crossing. We compare these results with previous work and suggest some possible explanations for our findings.

While the altered forces generated by both fore- and hindlimbs during overground locomotion after bilateral cervical DF lesions are generally consistent with our earlier results after unilateral DF lesions, it is interesting that the bilateral lesions in the current study produced smaller changes in forelimb forces compared to those after unilateral injury. This difference can likely be ascribed to the different response to bilateral vs

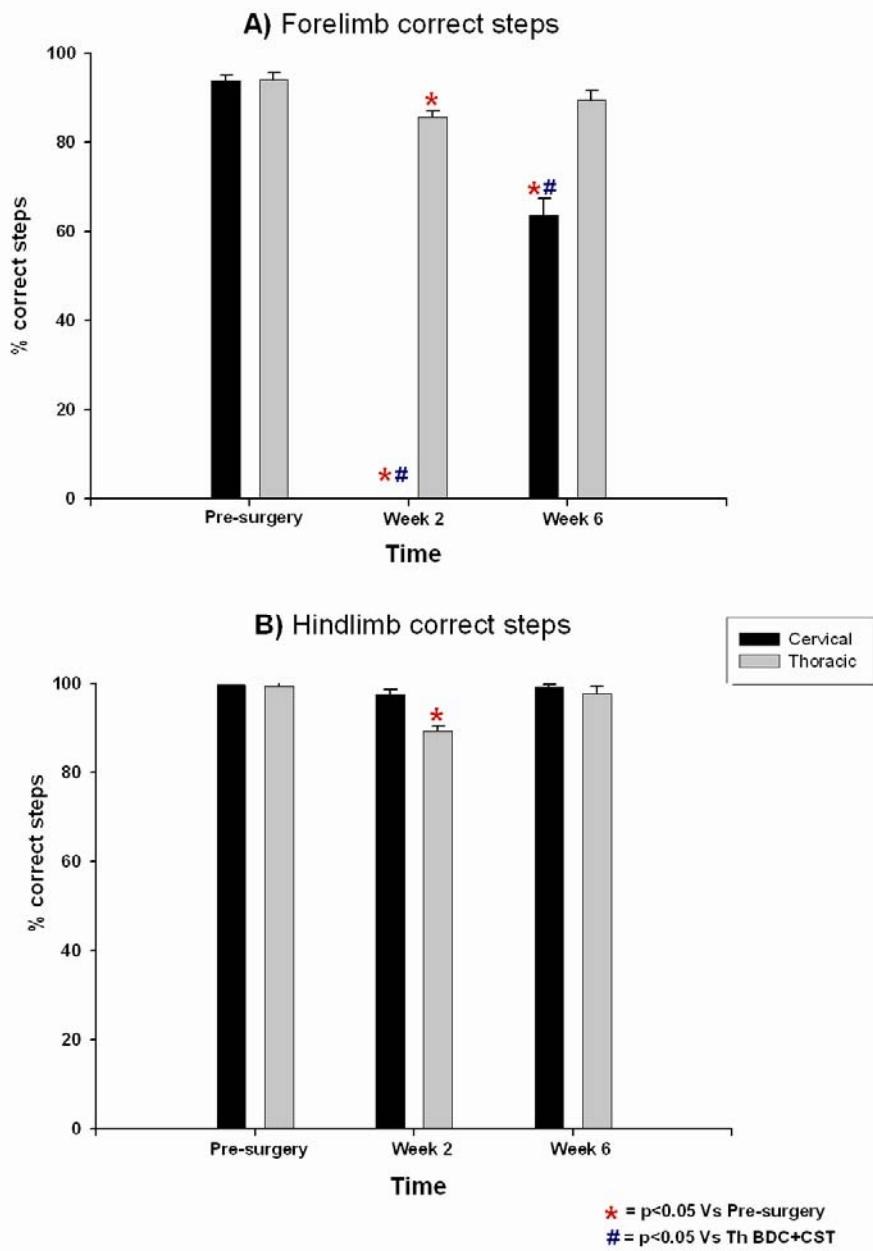


Fig. 5.5: Graphs representing percentage of (A) forelimb and (B) hindlimb correct steps while crossing a horizontal ladder, by cervical DF (black bars) and thoracic DF rats (gray bars). Cervical DF rats persistently made more forelimb errors (week 2: $p<0.001$ and week 8: $p<0.05$) but no hindlimb errors at all the time points compared to pre-surgery. Thoracic DF rats made forelimb and hindlimb errors at only week 2 ($p<0.05$) compared to pre-surgery, and recovered to pre-surgical performance by week 6 post-surgery.

unilateral lesions, in that animals with unilateral lesions can still use the limbs contralateral to the lesion to compensate for ipsilateral deficits (Webb and Muir, 2002; Muir and Whishaw, 1999; Webb and Muir, 2003; Webb and Muir, 2004; Chan et al., 2005; Klapka et al., 2005). Thoracic DF lesions, in contrast to cervical lesions, had no effect on limb contact patterns, or on vertical or fore-aft forces, which is also consistent with work from other groups. Transection lesions of the thoracic DF (both sensory fibers and CST) did not cause alterations in stepping patterns or in locomotor rating scores during overground locomotion in rats in several studies (Hendricks et al., 2006; Ballermann et al., 2006; Schucht et al., 2002). Of course, more severe thoracic spinal lesions do result in impairments, in both limb movement and interlimb coordination during overground locomotion (Collazos-Castro et al., 2006; Basso et al., 1995).

The finding that cervical DF lesions cause persistent forelimb foot faults with no hindlimb foot faults during ladder crossing is also consistent with findings from others. Forelimb foot slips are more frequent than hindlimb foot slips after cervical lesions of different severities (Onifer et al., 2005; Gensel et al., 2006). In contrast, several studies involving thoracic lesions of the dorsal spinal cord have resulted in hindlimb deficits on the horizontal ladder, unlike the results demonstrated here (Klapka et al., 2005; Schucht et al., 2002; Bolton et al., 2006; McEwen and Springer, 2006; Metz et al., 2000). These contrasting results can, however, be explained by the use of different methodologies. In most studies, spinal lesions were not limited to the DF, resulting in damage to the lateral funiculi in particular (Klapka et al., 2005; McEwen and Springer, 2006). The lateral funiculi are known to contain brainstem-spinal pathways important for motor control in

rats (Muir et al., 2007; Loy et al., 2002; Steeves and Jordan, 1980; Ballermann and Fouad, 2006). Thoracic level lesions limited to the DF did produce measurable increases in hindlimb foot faults in one study, but this study also used a more challenging ladder task in which the ladder rungs were randomly spaced (Bolton et al., 2006). This differs from our ladder in which the rungs were consistently spaced 1 cm apart (see methods). Thus the lack of hindlimb foot fault errors after thoracic DF lesions in the present study is not absolute, but depends, in part, upon the method of assessment.

There are several possible explanations for the differential effects of cervical vs thoracic lesions. The first is that, while we have shown that the descending CST pathway in the DF is important for the correct placement of forelimbs on the ladder task (Chapter 4, this thesis), the hindlimbs might rely less on this input. Hindlimb placement, either on the ground or on ladder rungs, could be influenced more by proprioceptive information arising from the limb and torso, and thus would be controlled more at the spinal level compared to the forelimbs (Grillner, 1981; Pearson, 2000; Pearson, 2000b). Of course, the hindlimbs are not completely independent of information transmitted through DF pathways, in that hindlimb footslips do occur when thoracic DF rats are challenged with a ladder with randomly spaced rungs, as described above (Bolton et al., 2006).

Another explanation for the differential effects of cervical and thoracic lesions is that the pathways composing the DF differ between cervical and thoracic regions. In particular, axons in the cervical DF transmit some, though not all, of the proprioceptive information from the forelimbs via the cuneocerebellar tract and these axons would be damaged after

cervical DF lesions (Snyder et al., 1978). In contrast, comparable proprioceptive information from the hindlimbs travels largely in the dorsal part of the lateral funiculus, and thus would remain undamaged by DF lesions (Poppele et al., 2003; Matsushita, 1999). That proprioceptive information contributes to limb action during locomotion is supported by evidence that cervical lesions of the dorsal part of the lateral funiculus affect the hindlimbs more severely than forelimbs during both overground and ladder locomotion (Muir et al., 2007).

One final explanation for the apparent lack of effect of thoracic lesions on hindlimb action is that the forelimbs could be effectively compensating for, and thus masking, hindlimb deficits. Recently, it has been shown that rats compensate for thoracic spinal cord injury by enhancing the forelimb extensor activity as measured by EMG recordings (Ballermann et al., 2006). We have also demonstrated previously that the limbs contralateral to unilateral spinal lesions in rats play an important role in compensating for deficits of the limbs ipsilateral to the lesion (Webb and Muir, 2002; Webb and Muir, 2003; Webb and Muir, 2004; Muir and Whishaw, 1999; Webb and Muir, 2005). Thus, animals use both the affected and unaffected limbs differently after spinal lesions in order to produce effective overground and/or ladder locomotion.

An important issue that emerges from our findings is the limited number of tests available to measure hindlimb function. Unlike tests for the forelimb, such as skilled reaching tasks, grip strength meter, or sticker removal, assessments of hindlimb function invariably involve all 4 limbs, including runway/ladder locomotion, inclined plane or

rope climbing tests (for reviews of behavioural assessment after SCI, see Webb and Muir , 2005; Metz et al., 2000; Muir and Webb, 2000; Kesslak and Keirstead, 2003). In addition to being unable to assess hindlimb function separately from forelimb function in these tasks, the situation is further complicated by the fact that the hindlimbs and forelimbs do not have the same functional contributions to these tasks and also appear to rely differently on supraspinal vs intersegmental and propriospinal inputs. One of the effects is that, as we have shown, forelimbs and hindlimbs appear to be differentially sensitive to loss of ascending and descending inputs. The behavioural tests used to assess hindlimb function after specific experimental injury models therefore need to be chosen with these limitations in mind.

Both the altered use of the limbs as well as any post surgical recovery after spinal DF lesions is potentially accompanied by plastic changes within circuitry of the brain and spinal cord. In the spinal cord, these changes have been shown to include re-organization of the spared white matter pathways (for reviews refer Jeffery and Blakemore, 1999; Maier and Schwab, 2006) through mechanisms such as collateral sprouting (Fouad et al., 2001; Weidner et al., 2001) and/or formation of new intraspinal circuits in response to injuries (Bareyre et al., 2004). Additionally, a multifaceted pattern of change in synaptic size, synaptic strengths and membrane properties of spinal neurons has been shown to accompany alterations in motor skills in rats and other animals (Chen et al., 1999; Wolpaw and Carp, 2006).

5.6 Conclusions

In conclusion, the behavioural deficits and compensations which rats display after the loss of dorsal funicular pathways differ significantly based on the spinal level of the injury.

These differences are likely related to both functional distinctions between the fore- and hindlimbs and anatomical differences in the dorsal funiculi at different spinal levels. These findings have important implications for the mechanisms of recovery as well as the types of behavioural tests which can be practically used to measure functional changes in different lesion models.

Chapter 6. EFFECTS OF COMBINED DORSOLATERAL AND DORSAL FUNICULAR LESIONS ON SENSORIMOTOR BEHAVIOUR IN RATS.

6.1 Abstract

The purpose of this research was to investigate the compensatory role of undamaged spinal pathways after partial spinal injury in rats. We have previously shown that bilateral lesions of the dorsal funiculus (DF) at the cervical level caused changes in overground and skilled locomotion that affected the forelimbs more than the hindlimbs. The same lesions also caused fore-paw deficits during a skilled pellet retrieval task (Chapter 4, this thesis). In contrast, bilateral cervical lesions of the dorsolateral funiculus (DLF) caused alterations in overground and skilled locomotion that were most marked in the hindlimbs rather than the forelimbs, but also caused fore-paw deficits during skilled pellet retrieval (Muir et al., 2007). We hypothesized that the relative lack of forelimb deficits during locomotion after DLF lesions was due to compensatory input arising from intact pathways in the DF. We tested this hypothesis in the present study by performing bilateral DF lesions in animals in which both DLFs had been transected 6 weeks previously. These secondary DF lesions involved either only ascending sensory pathways (DLF+ASP group) in the DF, i.e. sparing the corticospinal tract (CST), or involved both the ASP and the CST (DLF+DF group). All animals were assessed during overground locomotion, while crossing a horizontal ladder and during a pellet retrieval task. During overground locomotion, both groups moved with slightly altered forces and timing in both forelimbs and hindlimbs. During both ladder crossing and reaching, secondary lesions to DF (with or without CST) exacerbated the deficits seen after initial

DLF lesions and additionally caused changes in the manner in which the rats used their forelimbs during reaching. Nevertheless, the relative magnitude of the deficits indicates that DF pathways in rats likely do not compensate for loss of DLF pathways during the execution of locomotor tasks, though there is indirect evidence that DLF-lesioned rats might rely more on ascending sensory pathways in the DF during skilled forelimb movements. The plastic changes mediating recovery are therefore necessarily occurring in other regions of the CNS, and, importantly, need time to develop, because animals with DLF+DF lesions performed simultaneously displayed marked functional deficits and were unable to use their forelimbs for skilled locomotion or reaching.

6.2 Introduction

After any type of spinal injury, reorganization of neural circuitry occurs at multiple levels in the CNS. Within the spinal cord, altered connectivity of pre-existing pathways and formation of new connections, including sprouting from both lesioned and un-lesioned pathways, have all been demonstrated (for detailed reviews refer Raineteau and Schwab, 2001). Some of these processes are thought to contribute to recovery of function (Weidner et al., 2001; Fouad et al., 2001; Bareyre et al., 2004; Courtine et al., 2008). Thus, behavioural capabilities after partial spinal cord injuries are likely the result of (1) deficits due to loss of damaged pathways and (2) compensatory input from intact pathways. In order to effectively interpret behavioural abilities and also to design new rehabilitation treatments after partial injuries, we have to better understand the compensatory roles of spared spinal pathways in spontaneous recovery.

As a first step toward understanding the compensatory roles of spared pathways, it will be necessary to distinguish the functional contributions of various spinal tracts. In the past, behavioural recovery in spinal injury models was frequently correlated to proportions of spared white matter after injury. Several studies have attempted to associate function with different spinal pathways, making broad distinctions between the important locomotor contributions of pathways in the ventral half of the spinal cord compared to the role of dorsally located pathways in more skilled movements (Loy et al., 2002a; Loy et al., 2002b; Schucht et al., 2002). We, and others, have further focussed our investigations to examining the specific contributions of various dorsal spinal pathways, in part because dorsally located axons are injured in most spinal models (Anderson et al., 2005; Schrimsher and Reier, 1993; Chapter 4, this thesis).

In previous studies, we showed that lesions of pathways in the cervical dorsal funiculus (DF) affected forelimbs but not hindlimbs during both overground and skilled locomotion (Chapter 4, this thesis). In contrast, lesions of the cervical dorsolateral funiculi (DLF) in rats affected the hindlimbs more severely than the forelimbs during the same tasks (Muir et al., 2007). The differential effects of damage to DF or DLF pathways in preferentially affecting forelimbs or hindlimbs respectively, led us to hypothesize that the lack of forelimb deficits in rats with DLF lesions might be due to amelioration by intact pathways in the dorsal funiculus (DF). We tested this hypothesis in the present study by removing the effect of DF pathways after animals had recovered from DLF lesions. We initially damaged the DLF pathways on both sides and allowed the rats to recover for 6 weeks. We then performed bilateral DF lesions (with or without including the CST) on

the same rats and compared their behavioural capabilities to those of rats with simultaneous DLF and DF lesions. We show that pathways in the dorsal funiculus do not compensate for loss of DLF axons during locomotion in rats with pre-existing DLF lesions, although there is indirect evidence that animals with DLF lesions might rely more on ascending sensory pathways in the DF during skilled forelimb use. We discuss the present results in light of previous work and suggest some probable explanation for our findings.

6.3 Materials and Methods

6.3.1 Animals

Twenty two adult female Long-Evans rats weighing 250-275g obtained from Charles River Laboratories (QC, Canada) were used for the experiment. The rats were housed in a light controlled room (12h light/12h dark) with in the Animal care facility at the Department of Veterinary Biomedical Sciences, Western College of Veterinary Medicine at the University of Saskatchewan. All animal procedures were approved by the University of Saskatchewan's Committee on Animal Care and Supply. All rats were fed with rat chow but were food restricted through out the experiment, such that their body weights were maintained at 90% of *ad lib* feeding weight. All animals were examined daily for their health by a veterinarian and cared for according to the regulatory guidelines set out by the Canadian Council on Animal Care.

6.3.2 Training

All animals were trained according to the procedure described in Section 3.4.1 in this thesis

6.3.3 Lesion groups

At the beginning of the experiment we had 2 groups of animals. Rats in the first group received bilateral lesions to the dorsolateral funiculi (DLF group; n=16) while the rats in the second group received simultaneous bilateral lesions to dorsolateral and dorsal funiculi (simultaneous DLF and DF group; n=6), in which both the ascending sensory fibers and the dorsal corticospinal tract in the dorsal funiculus were damaged. Six weeks after the DLF lesions in the first group, rats were again randomly assigned to receive a second surgery of either (1) bilateral transection of the ascending sensory fibers (ASP) in the dorsal columns, without any damage to the underlying dorsal corticospinal tract (DLF+ASP group; n=9) or (2) bilateral transection of all the fibers in the dorsal funiculus (DLF+DF group; n=7) including ascending sensory fibers and the dorsal corticospinal tract. At the end of 6 weeks after the initial lesion, we thus had 3 groups- (1) DLF+ASP group (n=9), (2) DLF+DF group (n=7) and (3) simultaneous DLF and DF group (n=6).

6.3.4 Surgery

All animals underwent standard anesthesia, analgesia and cervical cord lesions as described in Sections 3.3.1 and 3.3.2 of this thesis.

6.3.5 Behavioural assessment

Prior to surgery, all rats were assessed behaviourally using endpoint, kinetic and kinematic measurements as we have described (Chapter 3; Muir et al., 2007). Endpoint measures included scoring reaching success during skilled reaching and counting number of footslips made while crossing the horizontal ladder (skilled locomotion). Kinetic parameters consisted of measurement of ground reaction forces during overground locomotion. Kinematic parameters like step lengths, stance durations and overlaps of limbs were also measured during overground locomotion. Data was collected once prior to DLF surgery (pre-surgery) and again at weeks 2, 6, 8 and 10 weeks after DLF surgery.

6.3.5.1 Overground locomotion

6.3.5.1.1 Kinetics and kinematics

Ground reaction forces were recorded and analyzed as described in Section 3.4.4.1 in this thesis.

6.3.5.1.2 Measurement of stride parameters

As described in Section 3.4.4.2 in this thesis.

6.3.5.2 Skilled locomotion -Horizontal ladder

A horizontal ladder was used to test the skilled locomotor abilities of rats, as described in Section 3.4.3 in this thesis

6.3.5.3 Skilled reaching- single pellet reaching

Skilled reaching is used as a test to assess fine voluntary forelimb movement as described in Section 3.4.2 in this thesis.

6.3.6 Histology

As described in Section 3.5

6.3.7 Statistical analysis

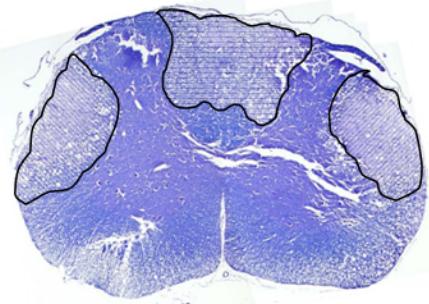
As described in Section 3.6

6.4 Results

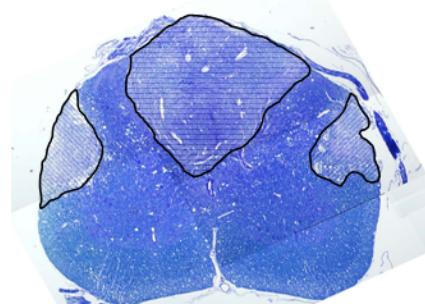
6.4.1 Histology

In the DLF+ASP group, seven out of the nine rats met the criterion of inclusion- they all had bilateral damage to dorsolateral funiculus and damage to ascending sensory fibers with complete or near complete sparing of the ventrally located dorsal component of CST in the dorsal funiculus (Fig. 6.1A, 6.1C). In this group, 2 rats were eliminated as they had sustained incomplete lesions to CST. In the DLF+DF group, all seven rats met the criterion of complete lesions to DLF and DF without any damage to the ventrally located central canal or extensive lesions of the dorsal horn (Fig. 6.1B, 6.1D). In the simultaneous DLF and DF lesions, four out of the six rats had lesions of DLF and DF, with some degree of dorsal gray matter damage, and the other 2 rats had dorsal hemisection lesions and therefore were not included in this group (Fig 6.1E). For the final

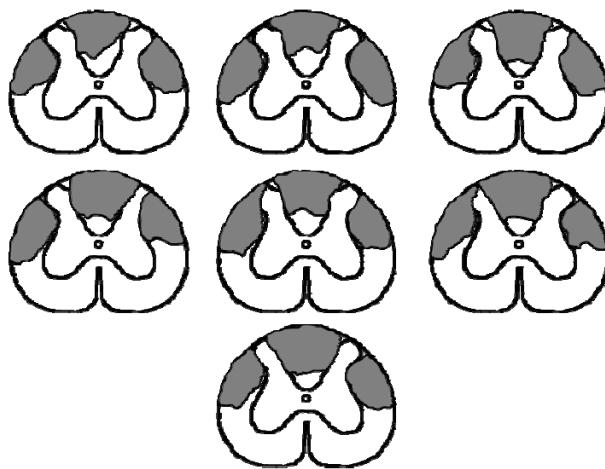
A) DLF followed by ASP lesions



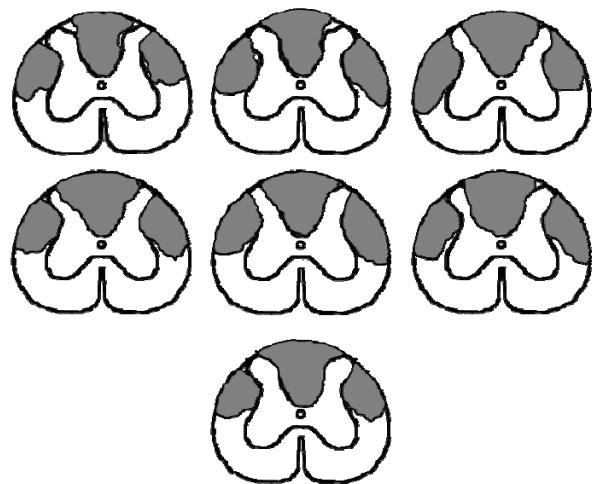
B) DLF followed by DF lesions



C) DLF followed by ASP lesions



D) DLF followed by DF lesions



E) Simultaneous DLF and DF lesions

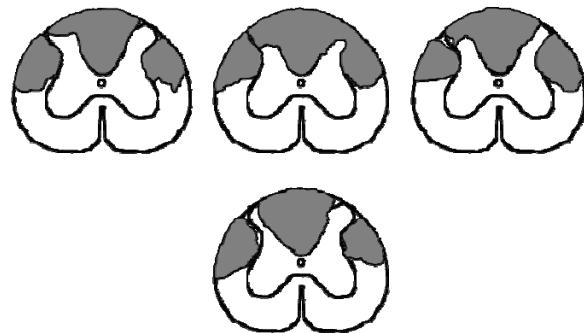


Fig. 6.1: Representative photomicrographs of spinal cords from (A) DLF followed by ASP and (B) DLF followed by DF lesioned rats stained with Luxol-Fast Blue and Cresyl Violet. Hatched areas in the pictures show the areas of damage in dorsal and dorsolateral funiculi. Schematic drawings representing the lesion epicenter (cervical level C2-C3) in (C) DLF followed by ASP lesioned rats, (D) DLF followed by DF lesioned rats, and (E) simultaneous DLF and DF lesioned rats. In each drawing, the grey shaded region represents the extent of damaged tissue. All rats sustained bilateral damage to the dorsolateral funiculus. In DLF followed by ASP-lesioned rats, the ascending sensory fibers in the dorsal funiculus were damaged with minimal damage to the dorsal CST. In DLF followed by DF lesioned rats, both the ascending sensory fibers and the dorsal CST in dorsal funiculus were damaged. In simultaneous DLF and DF rats, both DLF and DF were damaged at the same time.

analysis there were seven rats each in the DLF+ASP and DLF+DF groups and four rats in the combined DLF and DF group.

6.4.2 Behavioural assessment

6.4.2.1 Overground locomotion

6.4.2.1.1 Analysis of ground reaction forces (GRF)

Pre-surgical rats

Rats trotted at an average speed of 74.34 ± 2.6 cm/sec (Table 6.1). Ground reaction forces produced by all pre-surgical animals were similar to those from our previous studies (Chapters 4 and 5 this thesis; Muir and Whishaw, 1999a; Muir and Whishaw, 1999b; Muir and Whishaw, 2000; Muir et al., 2007; Poulton and Muir, 2005; Webb and Muir, 2002; Webb and Muir, 2003; Webb et al., 2003; Webb and Muir, 2004). During trotting rats alternatively bear the weight on diagonal limb pairs (right fore-left hind, left fore-right hind; Fig. 6.4A). Both the forelimbs and the hindlimbs produced similar peak vertical forces (Fig. 6.2A). Forelimbs produced most of the braking forces (negative fore-aft forces) and hindlimbs produced most of the propulsive forces (positive fore-aft forces) (Fig. 6.3A). The medio-lateral forces were small (data not shown).

Post-surgical rats- DLF+ASP group and DLF+DF group

As we performed bilateral lesions, the changes in both forelimbs (right and left) and both hindlimbs (right and left) were similar. After the initial DLF lesions, rats in both groups were travelling at similar speeds as before surgery (Table 6.1). Analysis of forces revealed significantly reduced peak vertical forces produced by both the forelimbs and

DLF+ASP						DLF+DF					
Time	Mean Speed (cm/s)	RF stance	RH stance	LF stance	LH stance	Time	Mean Speed (cm/s)	RF stance	RH stance	LF stance	LH stance
Pre-surgery	74.34	0.43±0.01	0.43±0.01	0.43±0.01	0.42±0.028	Pre-surgery	76.55	0.43±0.01	0.43±0.01	0.42±0.01	0.43±0.01
W2	74.46	0.45±0.01	0.49±0.01*	0.46±0.01	0.49±0.01* (19.5%)	W2	73.55	0.46±0.01	0.48±0.01*	0.44±0.01 (12.5%)	0.49±0.01* (14.7%)
W6	76.24	0.45±0.01	0.49±0.01* (14.3%)	0.46±0.01	0.48±0.01* (17.1%)	W6	73.81	0.46±0.01	0.50±0.01*	0.44±0.01 (16.8%)	0.48±0.01* (10.8%)
W8	73.21	0.48±0.01*	0.49±0.01* (11.3%)	0.49±0.02*	0.49±0.06* (13.9%)	W8	73.2	0.49±0.01*	0.51±0.01*	0.49±0.01* (18%)	0.51±0.01* (17.06%)
W10	74.68	0.48±0.01*	0.49±0.01* (11.5%)	0.49±0.02*	0.50±0.02* (12.4%)	W10	75.68	0.49±0.01*	0.51±0.02*	0.49±0.01* (13.2%)	0.52±0.02* (20%)

Table 6.1: Stance duration of limbs (means±S.E.M) expressed as proportion of stride duration and mean trotting speeds in DLF+ASP and DLF+DF lesioned rats. In both the groups, there were no differences in the speeds at which rats were trotting before and at any time after surgery. After initial DLF lesions in both groups, stance durations of the hindlimbs were increased compared to their pre-surgical times (* $p<0.05$). After either ASP or DF re-lesion, the stance durations of the forelimbs increased compared to their pre-surgical times (* $p<0.05$) although hindlimb stance durations were no different than after DLF lesions alone. The percentage increase in stance durations of limbs compared to their pre-surgical times are indicated in brackets below the actual numbers.

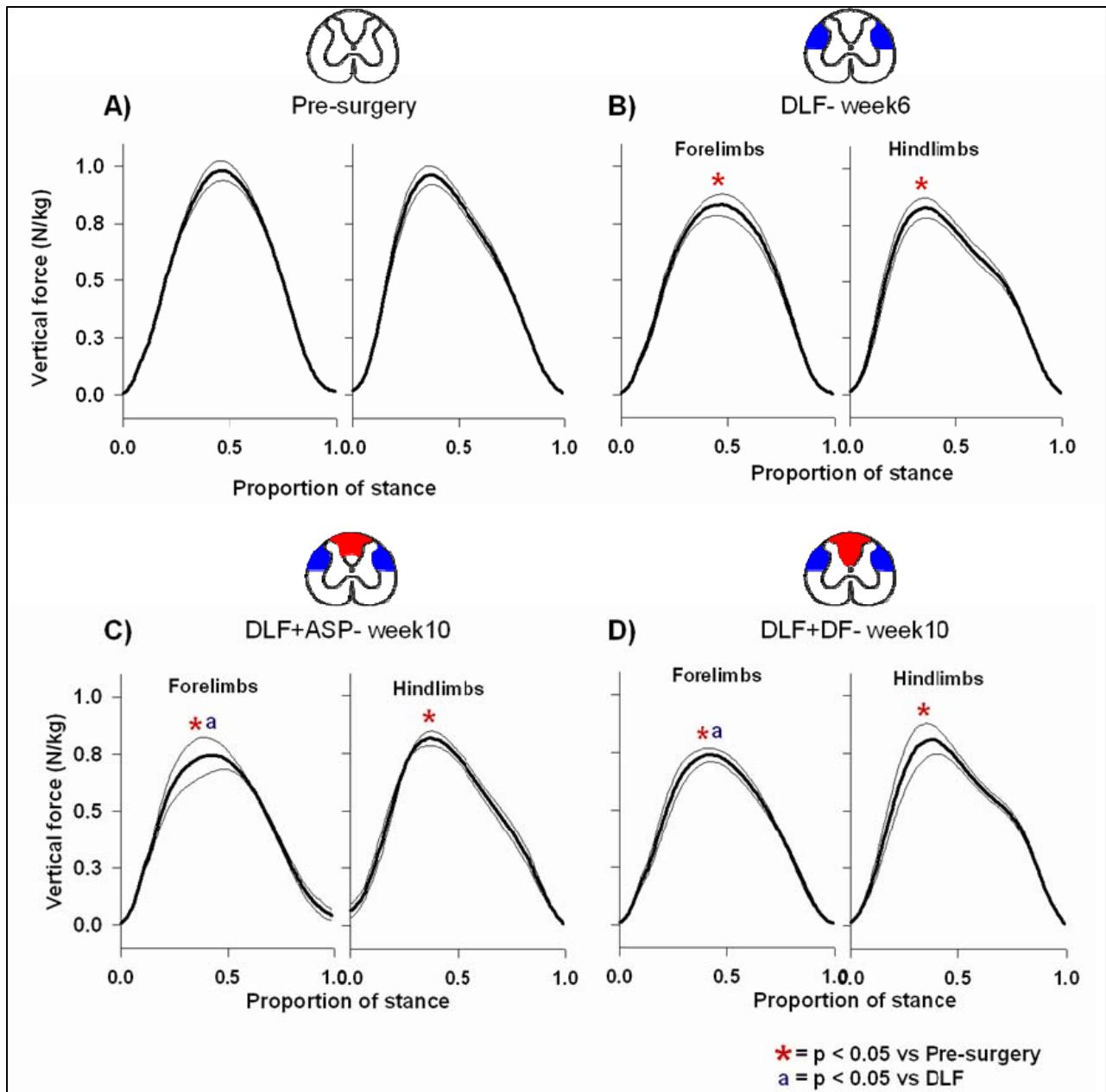


Fig. 6.2: Vertical forces produced by the forelimbs and the hindlimbs for (A) pre-surgical, (B) DLF-6 weeks post-surgery, (C) DLF+ASP-10 weeks post-surgery, (D) DLF+DF-10 weeks post-surgery rats. After DLF surgery, rats produced reduced peak vertical forces in both the forelimbs and the hindlimbs ($p<0.05$) compared to the pre-surgical rats. Six weeks after the initial DLF surgery, subsequent ASP or DF lesions produced further reduction in the peak forelimb vertical forces ($p<0.05$) compared to DLF rats but the peak hindlimb vertical forces were similar to those of DLF rats. Animals with simultaneous DLF and DF lesions could not trot on the runway and hence no forces could be recorded. In all the graphs, thick lines represent mean data for each group of animals and thin lines represent \pm standard error of the mean.

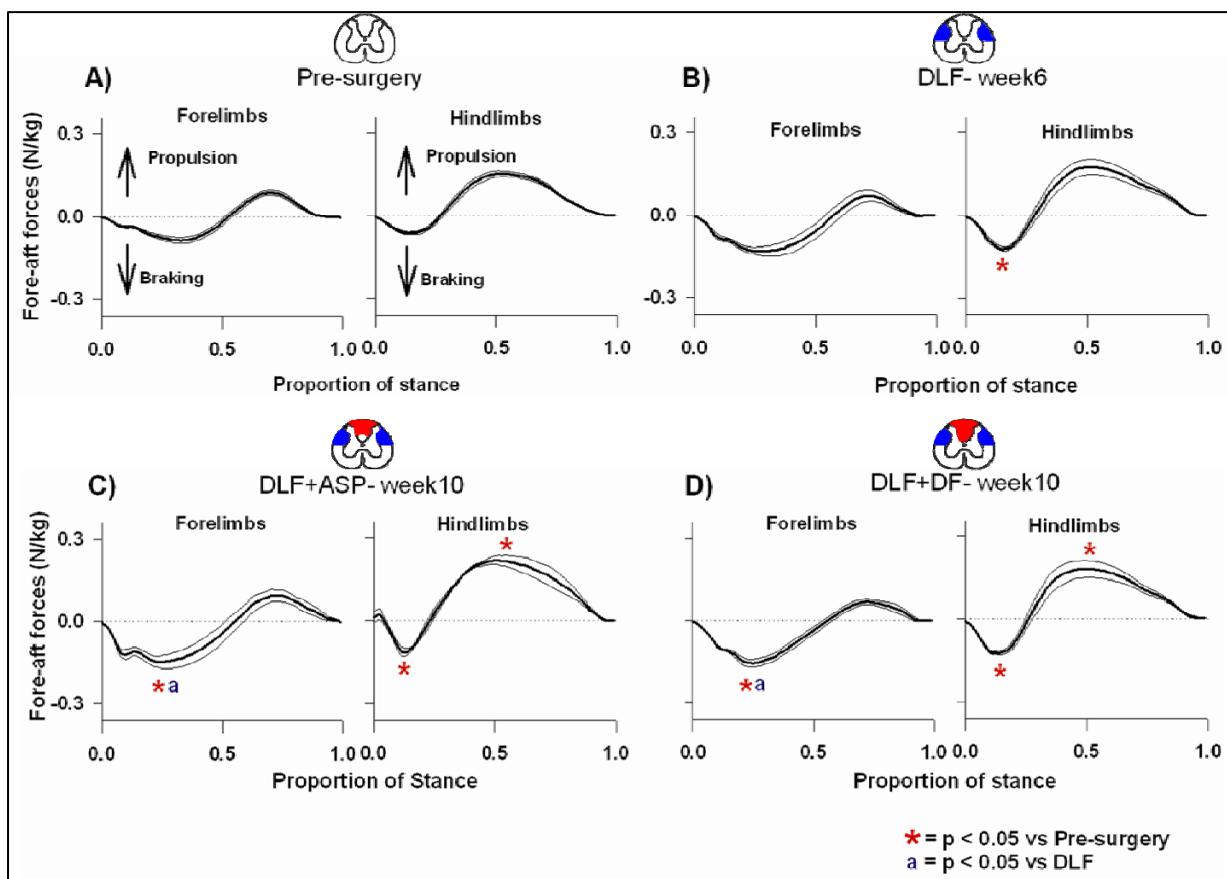


Fig. 6.3: Fore-aft forces generated in (A) pre-surgical, (B) DLF- 6 weeks post-surgery, (C) DLF+ASP-10 weeks post-surgery, and (D) DLF+DF-10 weeks post-surgery rats. After DLF surgery, the hindlimbs generated increased peak braking forces ($p<0.05$) compared to the pre-surgical rats. Six weeks after the initial DLF surgery, subsequent ASP or DF lesions caused increased braking forces by the forelimbs ($p<0.05$) compared to pre-surgical and DLF rats. Additionally, the hindlimbs generated increased propulsive forces ($p<0.05$) compared to pre-surgical rats, although these forces were not different from those of DLF lesioned animals. In all the graphs, thick lines represent mean data for each group of animals and thin lines represent \pm standard error of the mean.

the hindlimbs (Fig. 6.2B, $p<0.05$ for both groups). In the fore-aft direction, hindlimbs produced increased braking forces (Fig. 6.3B, $p<0.05$ for both groups). For all vertical forces, impulses were no different than pre-surgical rats (data not shown). In both groups, addition of either ASP or DF lesions 6 weeks after DLF lesions produced identical changes in the ground reaction forces. There was an additional reduction in the forelimb peak vertical forces (Fig. 6.2C, 6.2D, $p<0.05$) but the hindlimb peak vertical forces were similar to those with DLF lesions alone. In the fore-aft direction, addition of either ASP or DF lesions on top of the DLF lesions resulted in increased braking forces by the forelimbs compared to pre-surgical values and to DLF lesions alone, and increased propulsive forces by the hindlimbs compared to pre-surgical values (Fig. 6.3C, 6.3D, $p<0.05$). Comparisons between the two groups did not reveal any differences in ground reaction forces.

Post-surgical rats -Combined DLF and DF group

Interestingly, rats with the combined DLF and DF lesions could not trot on the runway at any time post-surgery, hence no ground reaction forces could be recorded. The rats could move overground but did so in a crouched fashion with their forelimbs splayed. This behaviour was similar to that of rats which had sustained dorsal hemisection lesions.

6.4.2.1.2 Step lengths and stride parameters

In both groups, there were no differences either in the stride length or stride duration at any time point compared to the pre-surgical values. Consistent with this, there were no differences in the trotting speeds of rats after surgery (Table 6.1). There were also no

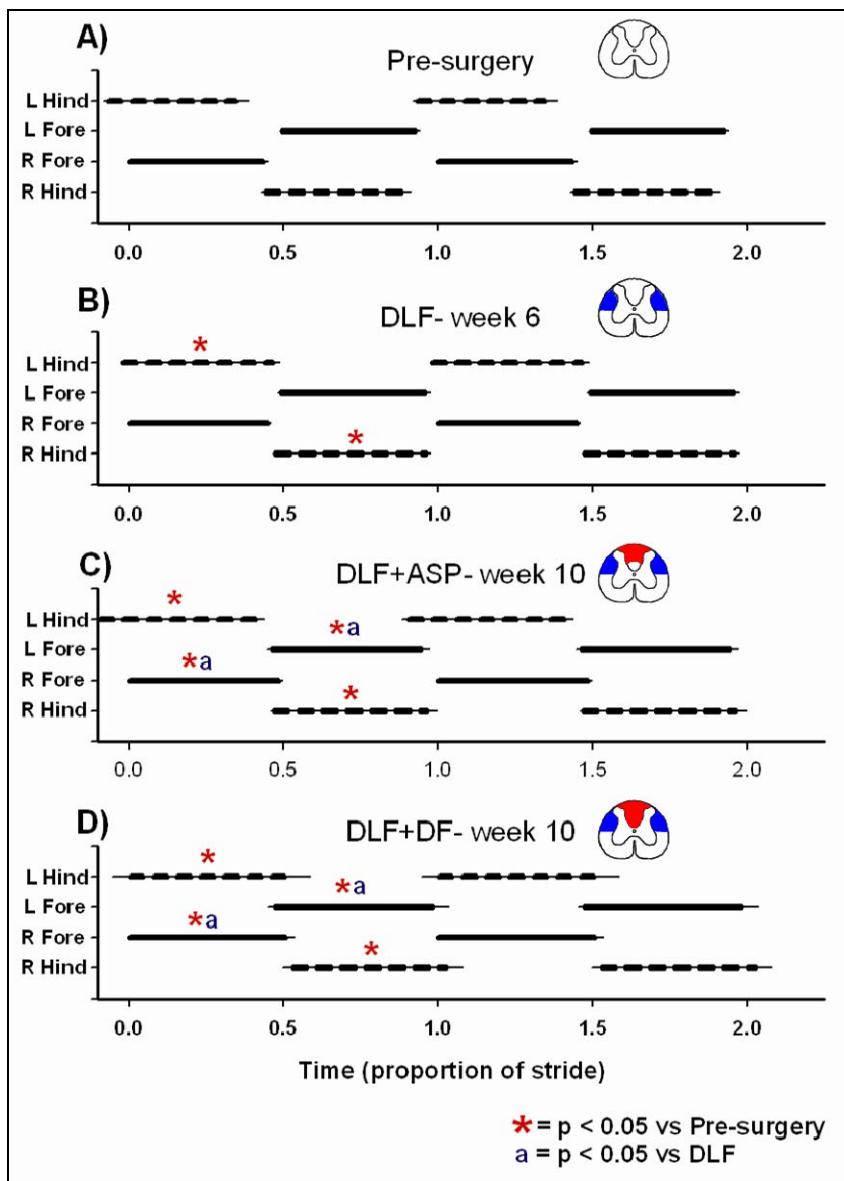


Fig. 6.4: Forelimb (solid lines) and hindlimb (dashed lines) contact timing for (A) pre-surgical, (B) DLF-6 weeks post-surgery, (C) DLF+ASP-10 weeks post-surgery, (D) DLF+DF-10 weeks post-surgery rats. The length and position of the thick and dashed lines represent mean limb stance times for each group of animals, while the thin lines at either end represent the standard error of the group means. When the rats are trotting, diagonal limb pairs (i.e. right fore and left hind or left fore and right hind) are in ground contact at the same time. All post-surgical rats in the present experiment were trotting on the runway, similar to pre-surgical rats. After DLF surgery, only the hindlimbs showed increased stance durations ($p < 0.05$) compared to pre-surgical rats. Six weeks after the initial DLF surgery, subsequent ASP or DF lesions caused increased forelimb stance durations ($p < 0.05$) compared to both pre-surgical and DLF rats, while the hindlimb stance durations were similar to those of DLF rats.

differences in step distance parameters for any limb in either group after surgery.

Analysis of limb timing parameters revealed significant differences only in the stance duration of the limbs (Fig 6.4 and Table 6.1). In both the groups after the initial DLF lesions, the stance durations of the hindlimbs were increased compared to the pre-surgical values (Fig. 6.4B, Table 6.1, $p<0.05$) but there were no differences in the forelimb stance durations. Addition of either ASP or DF on top of DLF lesions produced the same changes, in that forelimb stance durations in both groups were increased compared to both pre-surgical and DLF lesion values (Fig.6.4C, 6.4D, Table 6.1, $p<0.05$). Hindlimb stance durations remained unchanged from animals with DLF lesions alone. Comparison between the groups did not reveal any differences in stance durations.

6.4.2.2 Skilled locomotion- horizontal ladder

Prior to surgery, rats were proficient at walking over the ladder - the forelimbs slipped or missed the rungs for about 5% of the total steps and hindlimbs made no errors. In both the groups after the initial DLF lesions, both forelimb and the hindlimbs made errors compared to pre-surgery ,although hindlimb errors were more frequent than forelimb errors (Fig. 6.5A, 6.5B, $p<0.05$). Six weeks after the initial DLF lesions, both ASP and DF secondary lesions caused additional increases in both the forelimb and the hindlimb errors compared to DLF lesions (Fig. 6.5A, 6.5B, $p<0.05$). Forelimb placement was more severely affected by the secondary lesions compared to hindlimb placement. Comparison between the groups revealed no significant differences at any time points. Nevertheless, rats with DLF+ASP lesion at week 10 had slightly more hindlimb correct placements (Mean \pm SEM: hindlimbs: 46.6 ± 3.2) compared to those with DLF+DF lesions

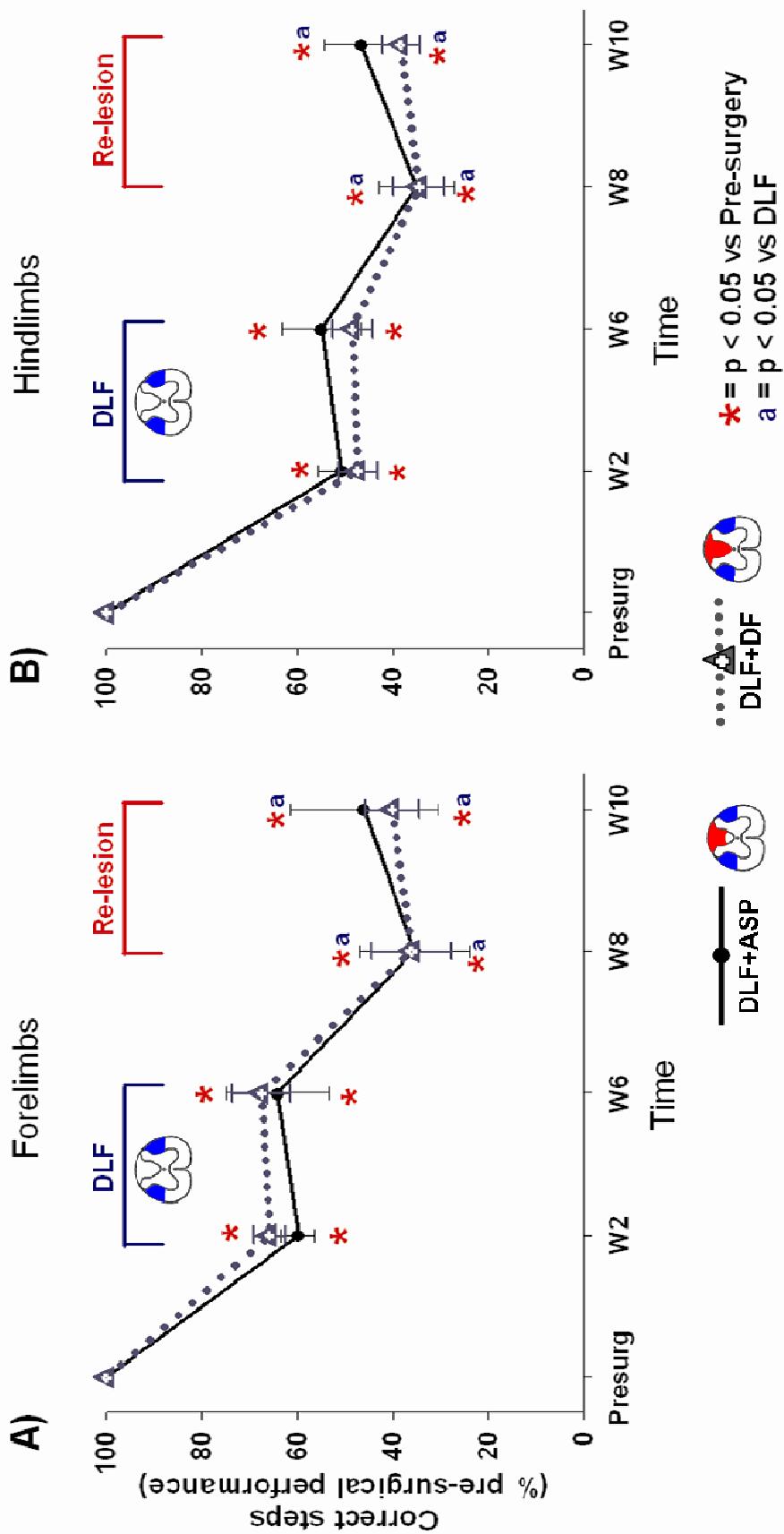


Fig. 6.5: Correct steps during ladder walking as a percentage of pre-surgical performance for DLF+ASP (black solid lines) and DLF+DF (dotted lines) rats. Graphs represent (A) forelimb and (B) hindlimb correct steps for each group. After DLF surgery, rats in both groups made more forelimb and hindlimb errors compared to pre-surgical rats ($p<0.05$). Six weeks after the initial DLF surgery, sequential ASP or DF lesions increased both forelimb and hindlimb errors compared to pre-surgical and DLF rats ($p<0.05$).

(hindlimbs: 38.32 ± 1.6) though they were not statistically significant (hindlimbs: $p=0.079$).

Simultaneous DLF and DF group

After surgery, rats with simultaneous DLF and DF lesions could not walk on the horizontal ladder even at 10 weeks, the latest time point tested. This was also the case in rats with dorsal hemisection lesions. Hence no data from horizontal ladder could be recorded in these rats.

6.4.2.3 Skilled fore-paw usage- single pellet reaching

Prior to surgery, all rats were successfully retrieving 70% of the pellets in their first attempt. After surgeries, reaching ability in rats was reduced at all time points compared to pre-surgery. In both the groups after the initial DLF lesions, rats showed reduced ability to reach for the food pellets (Fig. 6.6) compared to pre-surgical performance. Secondary lesions to either ASP or DF further reduced the reaching ability in rats with pre-existing DLF lesions (Fig.6, $p<0.05$ compared to pre-surgery, DLF). Post-hoc analysis revealed that by week 10, reaching ability in rats with DLF+ASP lesions ($48.82 \pm 2.5\%$ of pre-surgical performance) was better than that of animals with DLF+DF lesions ($26.21 \pm 3.04\%$ of pre-surgical performance) (Fig.6.6, $p<0.05$).

Qualitative analysis revealed changes in the individual components of the reach. After initial DLF lesions, the components affected were supination type-2 movements and arpeggio, consistent with our previous studies (Fig. 6E, 6G, $p<0.05$ and Muir et al, 2007).

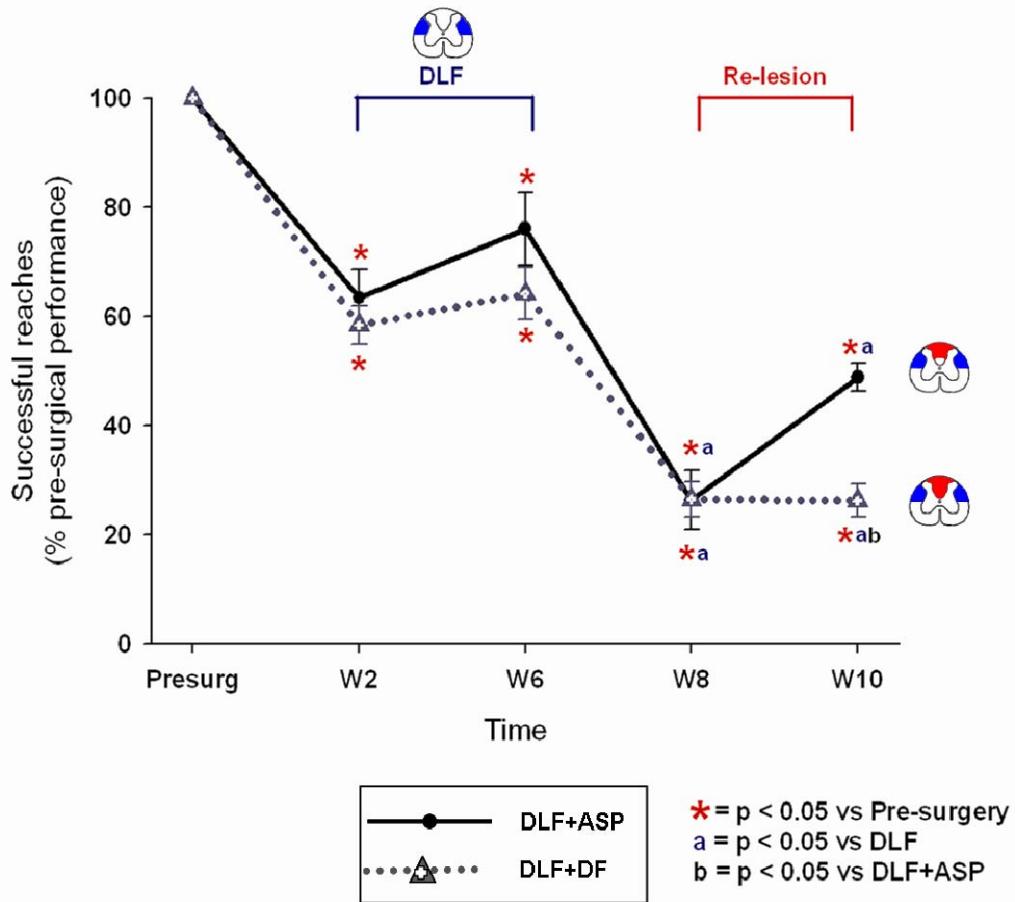


Fig. 6.6: Reaching ability as a percentage of pre-surgical performance in rats with DLF+ASP lesions (solid black line) and DLF+DF lesions (dotted line). After DLF surgery, the reaching abilities of the rats in both groups were reduced ($p<0.05$) compared to those of pre-surgical rats. Six weeks after the initial DLF surgery, addition of either ASP or DF damage caused further reduction in the reaching ability of the rats ($p<0.05$) compared to both pre-surgical and DLF rats. At week 10, DLF+ASP rats had slightly better reaching ability ($p<0.05$) than those with DLF+DF damage.

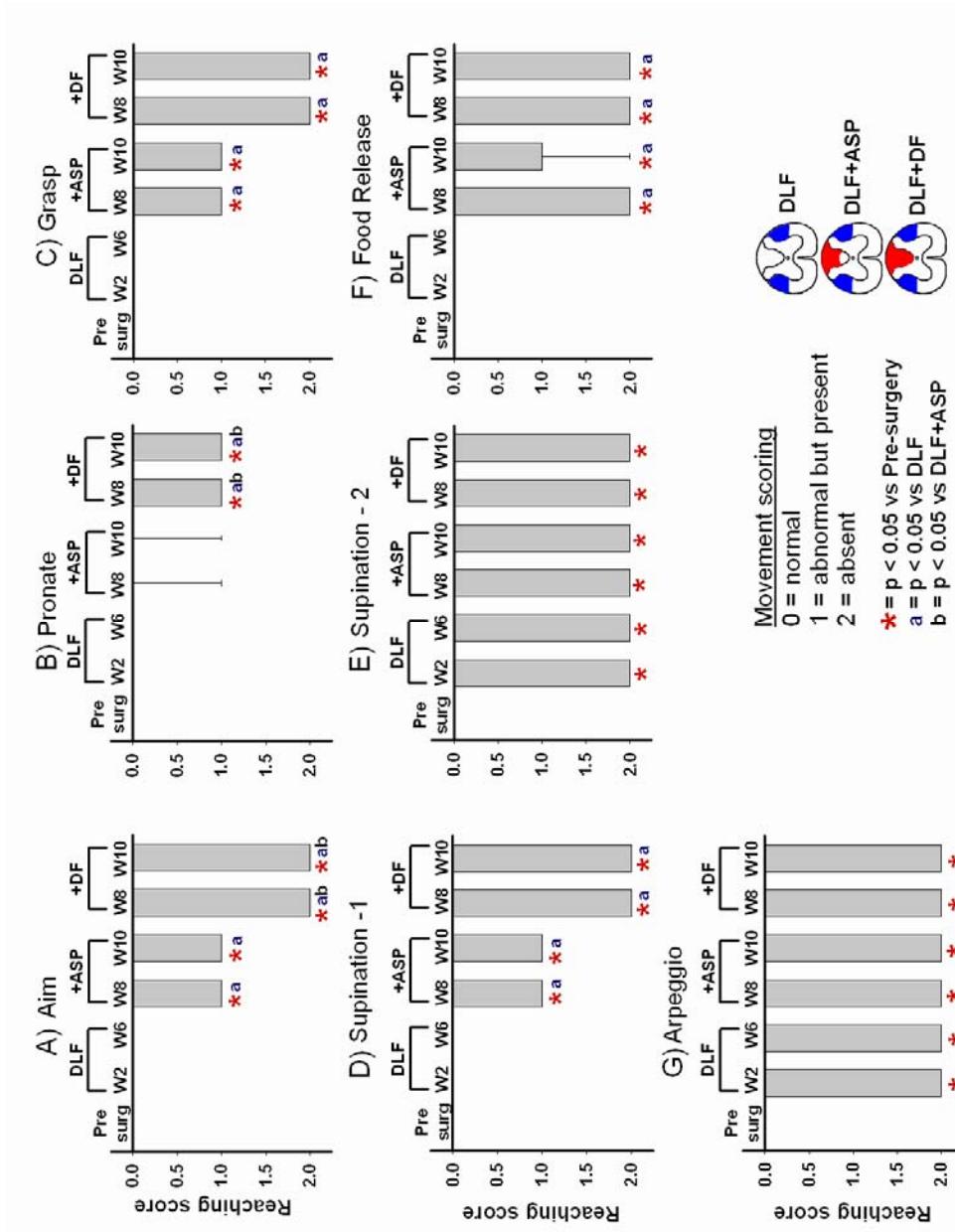


Fig. 6.7: Qualitative reaching scores in DLF-, DLF+ASP- and DLF+DF-lesioned rats. After DLF lesions, rats showed an absence of supination type 2 and arpeggio movements compared to pre-surgical rats (E, G, p<0.05). Sequential ASP or DF damage in DLF lesioned rats caused additional impairments in aim, grasp, supination type 1, food release movements compared to both pre-surgical and DLF rats (A, C, D, F, p<0.05). In addition, ASP+DF rats showed abnormal pronation movement compared to pre-surgical, DLF and DLF+ASP rats (B, p<0.05). Bars represent median values ± 95% confidence limits.

After secondary ASP or DF lesions were added on top of the DLF lesions, the additional components affected included aim, grasp, supination type-1 and food release (Fig. 6A, 6C, 6D, 6F, $p<0.05$). Additionally, in DLF+DF group, pronation was also affected after the secondary DF lesions (Fig. 6B, $p<0.05$). DLF+DF rats could grasp the pellet, but they rotated their heads and used their opposite paw to aid in eating the pellet. Comparison between the groups revealed that animals with damage to the CST (DLF+DF group) had more severe deficits in both aim and pronation (Fig. 6A, 6B, $p<0.05$).

Combined DLF and DF group

After surgery, rats with combined DLF and DF lesion could not reach for the food pellets at any time point. Despite their many attempts, rats in this group could not extend their paw through the horizontal slit to reach for the pellets and therefore no reaching data could be recorded from these rats. Rats in this group also adopted a curled paw posture at rest and while reaching but not during weight support. This persisted even at 10 weeks after the combined lesions. Behaviourally, rats with combined DLF and DF lesions were indistinguishable from rats with dorsal hemisection lesions.

6.5 Discussion

Similar to our previous work, rats in the present study demonstrated both forelimb and hindlimb deficits during overground and skilled locomotion after cervical DLF lesions, although hindlimb deficits were more pronounced. DLF-lesioned rats were also less proficient at retrieving food pellets during a skilled forepaw task. Six weeks after DLF damage, lesions of the DF (with or without CST damage) exacerbated forelimb deficits

<i>Lesion type</i>	<i>Overground Locomotion</i>	<i>Skilled (ladder) locomotion</i>	<i>Skilled pellet retrieval</i>
DLF 	Altered peak forces, increased stance durations, Hindlimbs more affected than forelimbs	Increased footslip errors Hindlimbs > forelimbs	Reduced reaching success 60% pre-surgical levels
DLF followed by ASP 	Additional altered peak forces and stance durations of forelimbs	Additional forelimb footslips so that hindlimb errors = forelimb errors	Further reduction in reaching success to 20 % pre-surgical levels.
DLF followed by DF 	Same as above	Same as above	Same as above
Concurrent DLF-DC 	No overground trotting	No ladder locomotion	0% success
ASP * 	Altered peak forces, increased stance durations. Only forelimbs affected.	Slightly increased errors, only forelimbs affected.	Reduced reaching success to 70% of pre-surgical levels
ASP + CST * 	Same as above	Increased errors, only forelimbs affected	Reduced reaching success 30% pre-surgical levels

Table 6.2: Summary of behavioural outcomes associated with dorsal spinal lesions. See Figure 6.1 for explanation of lesions.

*descriptions of functional outcomes arise from data presented in Chapter 5 of this thesis (Kanagal and Muir, 2007) and are presented herefor comparison only.

more than hindlimb deficits during both overground and skilled locomotion. During skilled forepaw usage, these secondary lesions to DF (with or without CST) also exacerbated the reaching deficits seen after initial DLF lesions and additionally caused changes in the manner in which the rats used their forelimbs during reaching. Interestingly, rats with simultaneous lesions to DLF and DF could not perform any of the behavioural tasks employed. We discuss these results in relation to previous studies and suggest some possible explanations for our findings.

Pathways in the dorsal funiculus do not compensate for loss of DLF axons during locomotion in rats with pre-existing DLF lesions

We originally hypothesized that the relative lack of forelimb deficits in DLF-lesioned rats during overground and skilled locomotion was due to compensatory input from intact DF pathways. Thus, in DLF lesioned rats, DF pathways should make a greater contribution toward maintaining forelimb locomotor performance compared to DF pathways in rats with intact DLF. Lesions of DF pathways in DLF-lesioned rats should therefore produce forelimb deficits that are more severe than in animals with lesions of DLF pathways or of DF pathways, and also should be more severe than those with DF and DLF pathways lesioned simultaneously. Our hypothesis was not supported by the results of the present study, in that input from the DF did not appear to make a greater contribution to maintenance of limb function in DLF-lesioned animals. This evidence is discussed below.

During overground locomotion, DLF lesions mainly altered hindlimb forces and timing, and the secondary ASP or DF lesions simply added changes to forelimb forces and timing that were similar in magnitude to those caused by ASP or DF lesions alone (Figs 6.2, 6.3, 6.4; Chapter 4, this thesis). This suggests that, during overground locomotion in DLF-lesioned animals, pathways in the DF were not compensating for, i.e. masking, forelimb deficits. Instead, the small effect of DLF lesions on forelimb forces and timing could have been due simply to lack of significant influence of DLF pathways on forelimb function during overground locomotion. This result extends the findings of other studies, which albeit use less specific lesions and less sensitive assays, demonstrating that lesions of dorsal thoracic spinal cord have minor effects on overground locomotion (Loy et al., 2002a; Loy et al., 2002b; Schucht et al., 2002; Basso et al., 1996; Basso et al., 2002).

The effect of dorsal spinal lesions on skilled locomotion was more marked compared to the effects on overground locomotion, similar to our previous findings and the findings of others (Chapter 4, this thesis; Muir et al 2007; Loy et al, 2002a; Loy et al., 2002b; Schucht et al., 2002). Nevertheless, we still did not see evidence for extra compensatory input from the DF pathways. Instead, changes in limb function during skilled ladder locomotion in rats with staggered lesions could be attributable to the combined effects of the individual lesions. This is most evident in the hindlimbs, where secondary ASP or DF lesions only slightly reduced the number of correct steps in DLF-lesioned animals (Fig 6.5). The forelimbs were less affected by initial DLF lesions, but ASP or DF lesions resulted in a decrease in forelimb correct steps such that forelimb and hindlimb deficits were comparable at weeks 8 and 10 (Fig. 6.5). This is consistent with our previous work

showing that ASP or DF lesions affect forelimbs more severely than the hindlimbs during skilled ladder locomotion (Chapter 4, this thesis)

In spite of these differences in forelimb and hindlimb performance on the ladder, it should be pointed out that the actions of these limbs are not independent of each other. Forelimb slips can destabilize the animal on the ladder so as to increase the likelihood of hindlimb slips in subsequent steps, and vice versa (see also Onifer et al., 2005). We have tried to minimize this dependence partly by using footslip measurements from only one stride for each pass along the ladder, such that each stride used is independent of the other. Furthermore, we have shown that forelimb errors can occur without any hindlimb errors, as in animals with DF lesions at 4 wks post-surgery (Fig 6.6; Chapter 4, this thesis). Alternatively, animals can make more hindlimb errors than forelimb errors, as for DLF lesions (Fig 5 and 6; Muir et al., 2007). Nevertheless, it is possible that the slight increase in hindlimb errors after secondary ASP or DF lesions in the present study is related to the increase in forelimb slips in the same animals (Figs 6.5B and 6.6B).

Even though our initial hypothesis concerned the differential effects of DLF lesions on fore- and hindlimb function during locomotion, we also examined skilled forelimb movement during pellet retrieval. Again, we found no direct evidence that DF pathways make a greater contribution during skilled pellet retrieval when DLF pathways are absent compared to when they are present. DF lesions in previously DLF lesioned rats resulted in reaching success rates of 20% of pre-surgical performance, comparable to DF lesions alone (Fig 6.7 and Chapter 4, this thesis). Qualitative analysis of reaching movements

revealed that sequential DF lesions (with or without CST) in rats with pre-existing DLF lesions altered all the components of the reach which were individually affected by any of the lesions alone. This pattern is similar to that of rats with combined pyramidal tract and red nucleus lesions (Whishaw et al., 1998). Interestingly, there were several components of the reach, namely grasp and food release, that were unaffected by ASP, DF or DLF lesions individually but were abnormal or missing altogether in DLF-lesioned animals which underwent secondary ASP or DF lesions (Fig. 6.7, and Chapter 4, this thesis). This suggests that the normal execution of grasp and food release in animals with DLF-lesions alone might be the result of novel contributions by either ASP or CST or both in the intact DF.

Animals with DLF lesions might rely more on ascending sensory pathways in the DF during skilled forelimb use.

An interesting finding in the present study is that secondary lesions to the DF in rats with pre-existing DLF lesions caused similar deficits regardless of whether the CST was intact. This is less surprising for overground locomotion, because we and others have shown that the CST does not have a major role during stereotyped behaviour like runway locomotion (Metz et al., 1998; Chapter 4, this thesis; Muir and Whishaw, 1999a). In contrast, the CST plays an important role during tasks requiring skilled sensorimotor integration, such as ladder walking and skilled forepaw usage (Chapter 4, this thesis; Metz and Whishaw, 2002; Whishaw and Metz, 2002). It might be expected that, for these skilled tasks, inclusion of the CST in the secondary DF lesions would produce deficits which were more severe compared to those of animals with only ASP damage after DLF

lesions. Instead, rats in both groups had similar abilities during ladder walking and skilled reaching (Fig 6.5, 6.6). It is possible that more challenging tasks, such as a ladder task with irregularly spaced rungs, might have differentiated between animals with an intact CST and animals without CST (Metz and Whishaw, 2002).

The lack of distinct differences between ASP and DF lesions in DLF-lesioned animals is in contrast to the greater functional differences seen after ASP or DF lesions alone (Chapter 4, this thesis). In particular, ASP lesions alone produced relatively small decrements in forelimb skilled movements for both ladder locomotion and pellet retrieval (80% and 70% of pre-surgical performance, respectively) compared to the deficits produced by ASP lesions in previously DLF-lesioned animals (55% and 40% of DLF-lesioned performance, respectively) (Fig. 6.5 and 6.6; Chapter 4, this thesis). This dramatic loss of function after secondary ASP lesions does suggest, albeit indirectly, that animals rely more on ASP input after DLF damage compared to when the DLF is intact.

There were some differences between ASP and DF lesions in DLF-lesioned animals. Longer term recovery after secondary lesions did differ between the groups, at least for reaching (50% vs 25% success rate, respectively; Fig 6.6). Additionally, the manner in which the rats used their forepaws for reaching was not identical in the two groups. Animals with damage to the CST (DLF + DF group) showed impairments in both aim and pronation which were significantly less affected or unaffected in animals with the CST intact (DLF+ASP group) (Fig 6.7A and 6.7B). This is more consistent with the pattern of recovery after ASP or DF lesions alone in which damage to the CST along with

the ASP caused severe and permanent reaching deficits, whereas ASP lesions alone result in transient deficits (Chapter 4, this thesis). Still, recovery after ASP lesions is much better in animals with intact DLF pathways compared to animals with ASP lesions after DLF damage. It is possible that the more proficient use of the forelimbs in animals with ASP lesions alone is due to contributions from alternate sensory pathways, such as the spinocerebellar tracts, in the DLF (Chapter 4, this thesis; Tracey, 2004; Onifer et al., 2005). It is not inconceivable that, in addition to the possible increased reliance on ASP pathways after DLF lesions for maintenance of reaching performance, that there is a corresponding reliance on DLF pathways after ASP lesions.

Rats with sequential lesions perform better than those with simultaneous lesions

A distinctive finding in this study was that rats with simultaneous lesions to DLF and DF could not perform any of the behavioural tasks tested, unlike rats with the same lesions performed sequentially. Other studies have shown that rats with dorsal hemisection lesions, although at the mid-thoracic level, are capable of performing skilled locomotor tests like horizontal ladder (Piantino et al., 2006; Metz et al., 2000; Gulino et al., 2007), narrow beam (Metz et al., 2000), rope-walking (Hendriks et al., 2006), although with deficits. Of course, lesions at thoracic level have different effects on behavioural abilities compared to cervical lesions, even if the damage involves same spinal funiculi (Chapter 5, this thesis). Nevertheless, in contrast to the present results, rats with dorsal C4 hemisections have been reported to be able to walk on a grid surface (Onifer et al., 2005). Although the reasons for these different results are not clear, there are dissimilarities in methodology between the studies. First, movement over a grid is conceivably less

challenging than movement over a ladder, in that there are more positions for the paws to contact on a grid compared to a ladder. Second, lesions at C4 might preserve more functional capabilities of the forelimbs compared to the C2 level lesions in the present study.

In spite of this discrepancy with the work of Onifer, et al (2005), it is clear within the present study that there are marked behavioural differences between animals with simultaneous C2 dorsal spinal lesions and those with staggered lesions. The higher functional capabilities in the latter group is likely due to the sparing of more pathways after the initial DLF lesion, which allowed the behavioural tasks under examination to be carried out, though with deficits. Better functional recovery after staggered CNS lesions compared to simultaneous lesions has been described previously as the *serial lesion effect* (Finger et al., 1973; Alstermark et al., 1987). According to this hypothesis, if CNS structures are lesioned in two different surgeries, separated by a period of time, there are fewer functional deficits than if the same structures are lesioned in one session (Finger et al., 1973). This effect has been demonstrated in several studies, but most recently in spinal-injured rats (Courtine et al., 2008). Rats which received 2 lateral hemisections, contralateral to each other and 5 segments apart, could not locomote when the lesions were performed simultaneously. If the lesions were performed separately, 10 weeks apart, animals were able to locomote on a treadmill (Courtine et al., 2008). Time-dependent changes, involving spared connections relayed through propriospinal neurons, were shown to mediate motor recovery in that study (Courtine et al, 2008). It is likely that similar changes are involved in the present study.

One possible underlying mechanism for the serial lesion effect is that the ongoing use of the limbs in motor tasks after the first lesion, in our case the DLF lesion, would have presumably resulted in plastic changes throughout the CNS, including in propriospinal neurons. Exercise and activity have beneficial effects after spinal lesions (Cha et al., 2007; Edgerton et al., 2004; Wolpaw and Tennissen, 2001; Van-Meeteren et al., 2003), including increases in neurotrophins and their receptors (Ying et al., 2003; Ying et al., 2005), which can influence sprouting (Schnell et al., 1994) and strengthen the spared connections (Brus-Ramer et al., 2007; Courtine et al., 2008). This has been demonstrated behaviourally after dorsal quadrant lesions in rats during pellet reaching, where training after lesions improved their reaching ability compared to their injured untrained counterparts (Girgis et al., 2007). During ladder walking, early initiation of training immediately after lesions had beneficial effects compared to late training (Norrie et al., 2005). In the present study, these training- and activity-dependent mechanisms would have involved many regions of the CNS, including DF pathways as we have discussed, but also would have necessarily involved connections associated with ventrally located spinal pathways (e.g. reticulospinal, vestibulospinal and ventral corticospinal tracts) which were spared even after secondary lesions (Little et al., 1988; Weidner et al., 2001; You et al., 2003; Ballermann and Fouad, 2006). In contrast to the staggered lesion group, the rats with simultaneous lesions had only ventrally located pathways remaining after surgery. These pathways were clearly insufficient, in the present study, to allow execution of the tasks immediately after surgery, and thus training-induced plastic changes could not occur.

6.6 Conclusions

In conclusion, our evidence suggests that rats with DLF lesions do not necessarily rely more on DF pathways during the execution of various sensorimotor tasks, although there is indirect evidence suggesting that these rats might rely more on ascending sensory pathways in the DF during skilled forelimb movements. The plastic changes mediating compensatory effects are therefore necessarily occurring in other regions of the CNS, and, importantly, take time to develop.

Chapter 7. TASK-DEPENDENT COMPENSATION AFTER PYRAMIDAL TRACT AND DORSOLATERAL SPINAL LESIONS IN RATS

7.1 Abstract

The purpose of this research was to investigate whether pathways in the dorsal part of the lateral spinal funiculus (DLF) can compensate for loss of corticospinal input (CST) to the spinal cord. The CST is known to control skilled limb movements in rats. The DLF contains several different pathways, including the rubrospinal tract (RST) which is also thought to influence limb movements. After lesions of either the corticospinal or the rubrospinal system, it is unclear how much of the remaining forelimb function is due to the presence of the alternate pathway. To begin to address this issue, the present study investigates the compensatory role of pathways in the DLF, including the rubrospinal tract, after bilateral lesions of the pyramidal tract (PT). We initially performed bilateral PT lesions in rats, which effectively removed the CST input to the spinal cord. We tested these rats during overground locomotion, skilled locomotion and skilled forelimb usage. After a six week recovery period, we then performed bilateral DLF lesions and compared the behavioural abilities of these rats to those of animals which underwent simultaneous PT and DLF lesions. If DLF pathways do compensate for PT lesions, then animals with PT lesions would rely more on DLF pathways than animals without PT lesions. Thus we hypothesized that animals with DLF lesions which were performed six weeks after PT lesions would exhibit more deficits on several behavioural tasks compared to animals which received PT and DLF lesions simultaneously. Our hypothesis was supported only for skilled pellet retrieval. Hence some DLF pathways, including the RST, were able to

compensate for loss of CST input during skilled reaching but not during overground or skilled locomotion in PT-lesioned rats. These differential responses suggest that behavioural tasks vary in their reliance on specific pathways after injury, and, furthermore, that compensation for loss of specific connections can arise from numerous sources.

7.2 Introduction

After injuries to the spinal cord, reorganization occurs at multiple levels in the central nervous system (CNS) (Raineteau and Schwab, 2001). This reorganization conceivably involves the re-routing of the signals from brain to spinal cord or, conversely, from spinal cord to brain along uninjured pathways, especially if the injured and uninjured pathways have overlapping functions. In rats, the corticospinal tract (CST) and rubrospinal tract (RST) send projections to the spinal cord and are thought to have collaborative roles in control of forelimb movements (Whishaw et al., 1998; Whishaw et al., 1990).

Interestingly, it is also thought that corticospinal and rubrospinal systems can substitute for each other's role during the execution of a skilled locomotor task such as walking on a rotating bar (Kennedy, 1990). The switch between the two systems may involve normally present intermediate circuits, which re-route descending motor commands after injury to the uninjured system (Kennedy 1990; Fanardjian et al., 2000a,b,c;).

We have been interested in the possible complementary and compensatory roles of these two systems. Our previous studies, and those of others, have shown that damage to the corticospinal system in the brain or spinal cord alters forelimb function during skilled

movements (Whishaw et al., 1993; Whishaw et al., 1998; Whishaw and Metz, 2002; Piecharka et al., 2005; Schrimsher and Reier, 1993; Anderson et al., 2005; Chapter 4, this thesis; Chapter 5, this thesis). Similarly, lesions of the rubrospinal system, either of the red nucleus in the midbrain or the rubrospinal tract in the spinal dorsolateral funiculus (DLF), alter forelimb function during skilled movements (Muir et al., 2007; Whishaw et al., 1998; Whishaw and Gorny, 1996; Whishaw et al., 1992; Whishaw et al., 1990; Schrimsher and Reier, 1993). It is unclear how much of the remaining forelimb function after lesions of either system is due to the presence of the alternate pathway. In part, this is because many studies involve unilateral lesions, such that the contralateral axons from the same system might provide some compensatory input toward functional recovery. Bilateral lesions, as applied in the present study, reduce this possibility and allow investigation of the compensatory role of different, but functionally related, pathways. A related issue is whether compensatory input from particular pathways is limited to specific behaviours or can be generalized to different uses of the limbs. Many studies have focussed on skilled limb use during ladder or grid walking, or during a pellet retrieval task. In the current study, we examine skilled limb use during both ladder walking and pellet retrieval as well as relatively stereotyped limb use during overground locomotion. Earlier studies have shown that overground locomotor abilities in rats are unaffected by unilateral lesions to the pyramidal tract (PT), which removes corticospinal input to one side of the spinal cord (Metz et al., 1998; Muir and Whishaw, 1999a). On the other hand, damage to the DLF, which includes the rubrospinal tract, causes persistent changes in hindlimb action during overground locomotion (Muir et al., 2007; Webb and

Muir, 2003). It is uncertain whether either of these systems compensates for loss of the other during overground locomotion.

To begin to address this issue, the present study investigates the compensatory role of pathways in the DLF, including the rubrospinal tract, after bilateral lesions of the pyramidal tract. We initially performed bilateral PT lesions in rats, which effectively removed the corticospinal input to the spinal cord. We tested these rats during overground locomotion, skilled locomotion and skilled forelimb usage. After a six week recovery period, we then performed bilateral DLF lesions and compared the behavioural abilities of these rats to those of animals which underwent simultaneous PT and DLF lesions.. If animals with PT lesions rely more on DLF pathways to perform behavioural tasks, then they will be more severely affected by DLF lesions compared to uninjured animals. We thus hypothesized that animals with DLF lesions which were performed six weeks after PT lesions would exhibit more deficits on several behavioural tasks compared to animals which received PT and DLF lesions simultaneously. We show that compensation from DLF pathways does appear to occur, but is task specific and limited to skilled forelimb usage during pellet retrieval. Interestingly, we found that rats with combined damage to PT and DLF pathways could still perform all of the behavioural tasks, suggesting that there are other pathways besides DLF which are involved in compensation after PT lesions.

7.3 Materials and Methods

7.3.1 Subjects

Twenty-four adult female Long-Evans rats (body weights between 250-280g) obtained from Charles River Laboratories (Quebec, Canada) were used in this experiment. The rats were housed as pairs and the cages were kept inside a 12h light/12 h dark cycle controlled room at the Animal Care Facility, Western College of Veterinary Medicine at the University of Saskatchewan. The rats were fed rodent chow and their body weights were maintained below 300 g during the entire experimental period. All rats were handled and examined daily by a veterinarian and a log was maintained to record their body weights every week. The rats were cared for according to the guidelines prescribed by the Canadian Council on Animal Care.

7.3.2 Behavioural training

Animals were trained according to the protocols described in Section 3.2 of this thesis

7.3.3 Experimental plan

Three experiments were conducted in this study. After training and collection of pre-surgical behavioural data, the animals were randomly assigned to one of the following groups: (1) staggered pyramidal tract and dorsolateral funicular lesion group (PT followed by DLF), (2) dorsolateral funicular lesion group (DLF) and (3) simultaneous pyramidal tract and dorsolateral funicular lesion group (PT-DLF).

Experiment 1: Staggered pyramidal tract and dorsolateral funicular lesion group (n=10)

In this group, rats first underwent bilateral pyramidotomy (PT lesions) at the level of the caudal medulla. Six weeks after the initial PT lesions, these rats received bilateral dorsolateral funicular lesions (DLF) at the cervical spinal cord level C3. In these rats, behavioural data (*see behavioural assessment*) was collected once pre-surgery and every 2 week for 10 weeks (10 weeks after initial PT lesions = 4 weeks after staggered DLF lesions).

Experiment 2: Dorsolateral funicular lesion group (n=6)

In this group, rats received DLF lesions at cervical spinal level C3. Behavioural data was collected once pre-surgery and every 2 weeks for 8 weeks.

Experiment 3: Simultaneous pyramidal tract and dorsolateral funicular lesions (n=8)

In this group, rats underwent both PT lesions (at caudal medulla) and DLF lesions (at cervical spinal cord C3) in the same surgery. In these rats, behavioural data was collected once pre-surgery and every 2 weeks for a period of 10 weeks.

7.3.4 Surgeries

All animals underwent standard anaesthesia and analgesia as described in Section 3.3.2. The pyramidal tract and spinal surgeries as described in Section 3.3.2 in this thesis.

7.3.5 Behavioural measurements

Prior to surgery, all rats were assessed behaviourally using endpoint, kinetic and kinematic measurements as we have described (Chapter 3, this thesis; Muir et al., 2007). Endpoint measures included scoring reaching success during skilled pellet retrieval and counting number of footslips made while crossing the horizontal ladder (skilled

locomotion). Kinetic parameters consisted of measurement of ground reaction forces during overground locomotion. Kinematic parameters such as step lengths, stance durations and overlaps of limbs were also measured during overground locomotion.

7.3.5.1 Overground locomotion

7.3.5.1.1 Kinetics and kinematics

Ground reaction forces were recorded and analyzed as described in Section 3.4.4.1 in this thesis.

7.3.5.1.2 Measurement of stride parameters

As described in Section 3.4.4.2 in this thesis.

7.3.5.2 Skilled locomotion -Horizontal ladder

A horizontal ladder was used to test the skilled locomotor abilities of rats, as described in Section 3.4.3 in this thesis

7.3.5.3 Skilled reaching- single pellet reaching

Skilled reaching is used as a test to assess fine voluntary forelimb movement as described in Section 3.4.2 in this thesis.

7.3.6 Histology

As described in Section 3.5

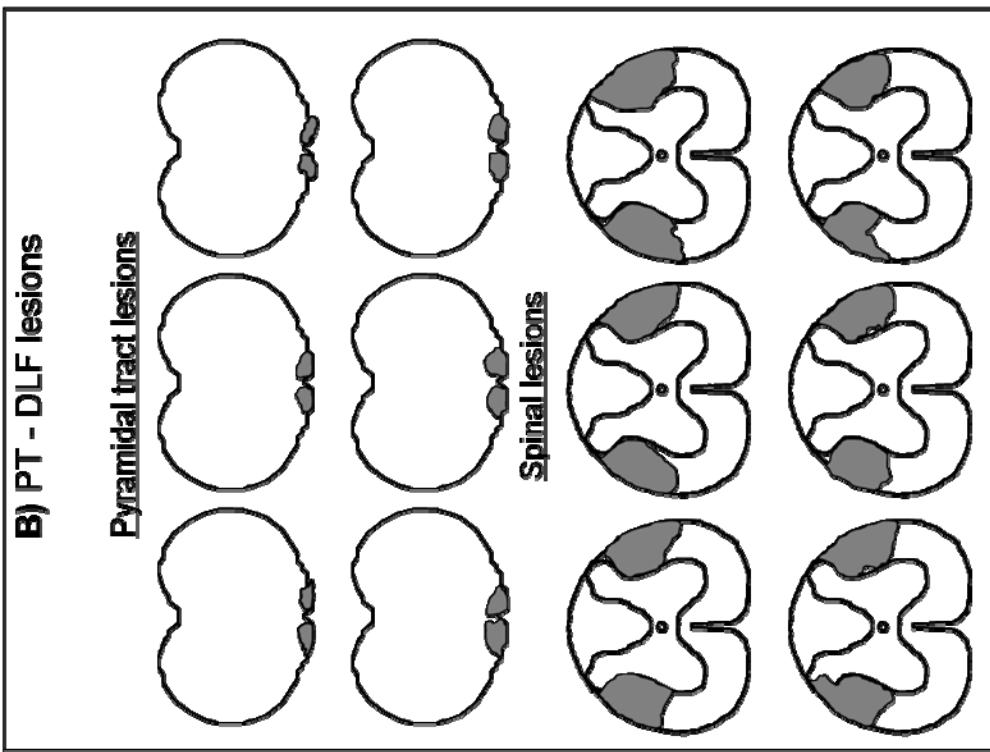
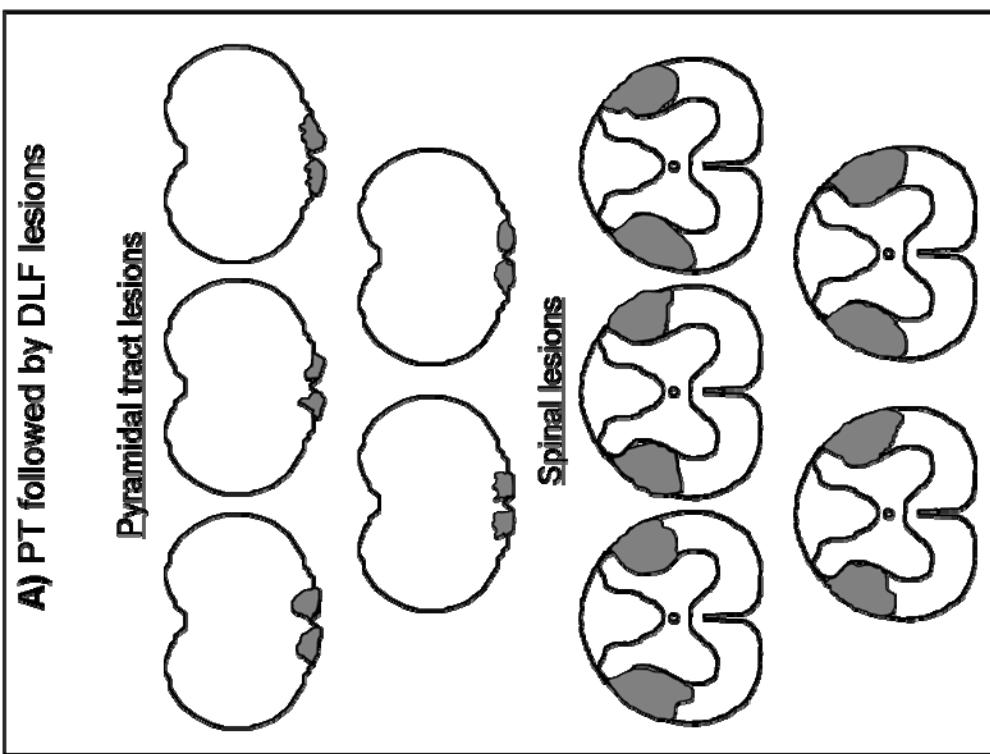
7.3.7 Statistical analysis

As described in Section 3.6

7.4 Results

7.4.1 Histology

In the present experiment, assignment of the rats into respective groups was based on light microscopic evaluation of histological sections for completeness of the lesions (both PT and DLF). In the staggered lesion group, 2 rats died under anesthesia due to surgical complications after PT lesions. The experiment was continued with the remaining 8 rats. After the end of experiment, microscopic evaluation of histological section revealed that 5 of 8 rats in the PT followed by DLF group, had similar and near complete and consistent damage of pyramidal tracts (left and right; Fig. 7.1A) and consistent bilateral DLF lesions in the spinal cord (Fig. 7.1A). The remaining 3 rats (V11, KS16, and KS20) were eliminated from this group as they had sustained additional damage to ascending sensory fibers in the dorsal funiculus (Fig. 7.1D). Of these 3 rats, one rat had a complete dorsal funicular damage (V11, Fig. 7.1D) and the other 2 had some sparing of ascending sensory pathways (KS 16 and KS 20; Fig. 7.1D). In the DLF group, all 6 rats had sustained consistent bilateral damage to the dorsolateral funiculus (Fig. 7.1C). In the simultaneous PT-DLF group, two rats were eliminated as one had sustained extensive damage to spinal cord and no behavioural data could be collected and the other had incomplete damage of the pyramidal tract (data not shown). The remaining 6 of the 8 rats were included in the study and had sustained near complete, similar sized lesions of the pyramidal tract (Fig. 7.1B) and consistent lesions of DLF (Fig. 7.1B).



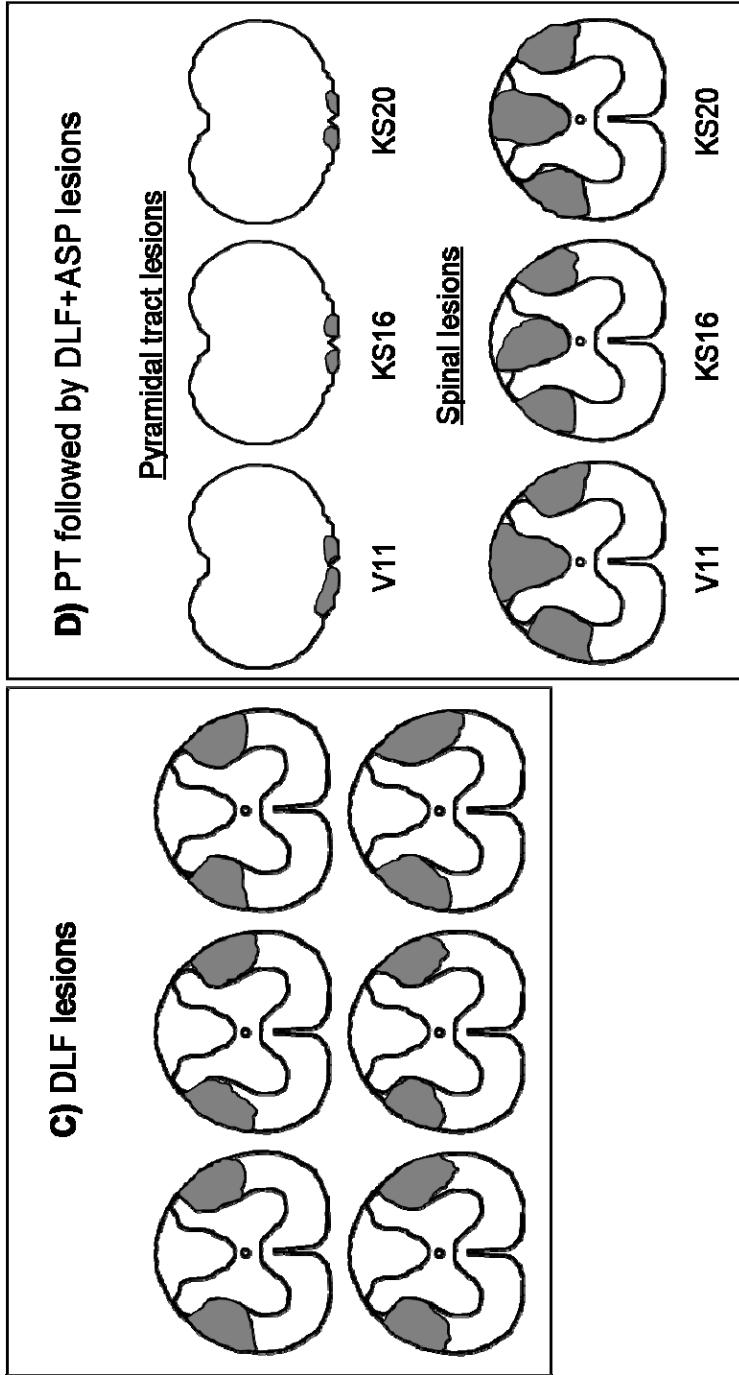


Fig. 7.1: Schematic drawing representing the epicenter of pyramidal tract (caudal medulla) and spinal lesions (cervical level C3) in (A) PT followed by DLF rats, (B) Simultaneous PT-DLF rats, (C) DLF rats and (D) PT followed by DLF+ASP rats. In each drawing, grey shaded region represents the extent of damaged tissue. Rats in PT followed by DLF group sustained bilateral damage to PT which was followed 6 weeks later by bilateral DLF lesions. In simultaneous PT-DLF rats, both PT and DLF were damaged at the same time. In DLF group, only dorsolateral pathways were damaged bilaterally. In PT followed by DLF+ASP group, initial PT damage was followed by DLF and additional ASP damage 6 weeks later. Among the three rats in this group V11 had complete damage to ASP while KS16 and KS20 had incomplete damage to ASP.

In all the rats, PT lesions were restricted to pyramidal tract without extending deeper to cause damage to inferior olfactory nucleus. The DLF lesions were identical to those from our previous study (Muir et al., 2007). In all the spinal lesions, damage was restricted to dorsolateral funiculus and there was no sign of damage extending ventral to the level of the central canal. In a few rats, there was evidence of little damage to the lateral side of the dorsal horns, but never completely damaging the dorsal horns. Within each group at any particular time point, behavioural data for the rats did not vary amongst each other, suggesting consistent lesions within a group of rats.

7.4.2 Experimental groups

After histological evaluation and grouping, there were 5 rats in the PT followed by DLF group (Fig. 7.1A), 6 rats in DLF group (Fig. 7.1C) and 6 rats in the simultaneous PT-DLF group (Fig. 7.1B). The 3 rats (V11, KS16 and KS20) eliminated from PT followed by DLF group due to additional ASP damage were grouped together as PT followed by DLF+ASP group (Fig. 7.1D). Though data from the last group was not included in any of the statistical comparisons, individual rat data from ladder and reaching tests are presented in the results.

7.4.3 Behavioural assessment

7.4.3.1 Overground locomotion

7.4.3.1.1 Analysis of ground reaction forces (GRF)

Pre-surgical rats

Ground reaction forces produced by all rats before surgery were identical to those from our previous studies (Chapters 4, 5 and 6 this thesis; Muir and Whishaw, 1999a; Muir and Whishaw, 1999b; Muir and Whishaw, 2000; Muir et al., 2007; Poulton and Muir, 2005; Webb and Muir, 2002; Webb and Muir, 2003; Webb et al., 2003; Webb and Muir, 2004). When the rats are trotting, diagonal limb pairs (i.e. right fore and left hind or left fore and right hind) are in ground contact at the same time (Fig. 7.4A, D, G). In the vertical direction, both the forelimbs and hindlimbs produced similar peak forces (Fig. 7.2A, D and G). In the fore-aft direction, the forelimbs produced most of the braking forces (negative fore-aft forces), while the hindlimbs produced most of the propulsive forces (positive fore-aft forces; Fig. 7.3A, D and G). Medio-lateral forces were small and laterally directed (data not shown). Rats in all the groups were trotting at an average speed of 73.29 ± 0.9 cm/s (Table 7.1).

Post-surgical rats-PT followed by DLF group

As we performed bilateral lesions, the changes in right and left forelimbs and right and left hindlimbs were similar. After PT lesions, rats did not show any changes in generation of forces or trotting speeds compared to pre-surgical rats (Fig. 7.2, 7.3, Table 7.1). Both at weeks 2 and 6 after surgery, PT rats produced similar forelimb and hindlimb vertical (Fig. 7.2B) and fore-aft forces (Fig. 7.3B) as they did prior to surgery.

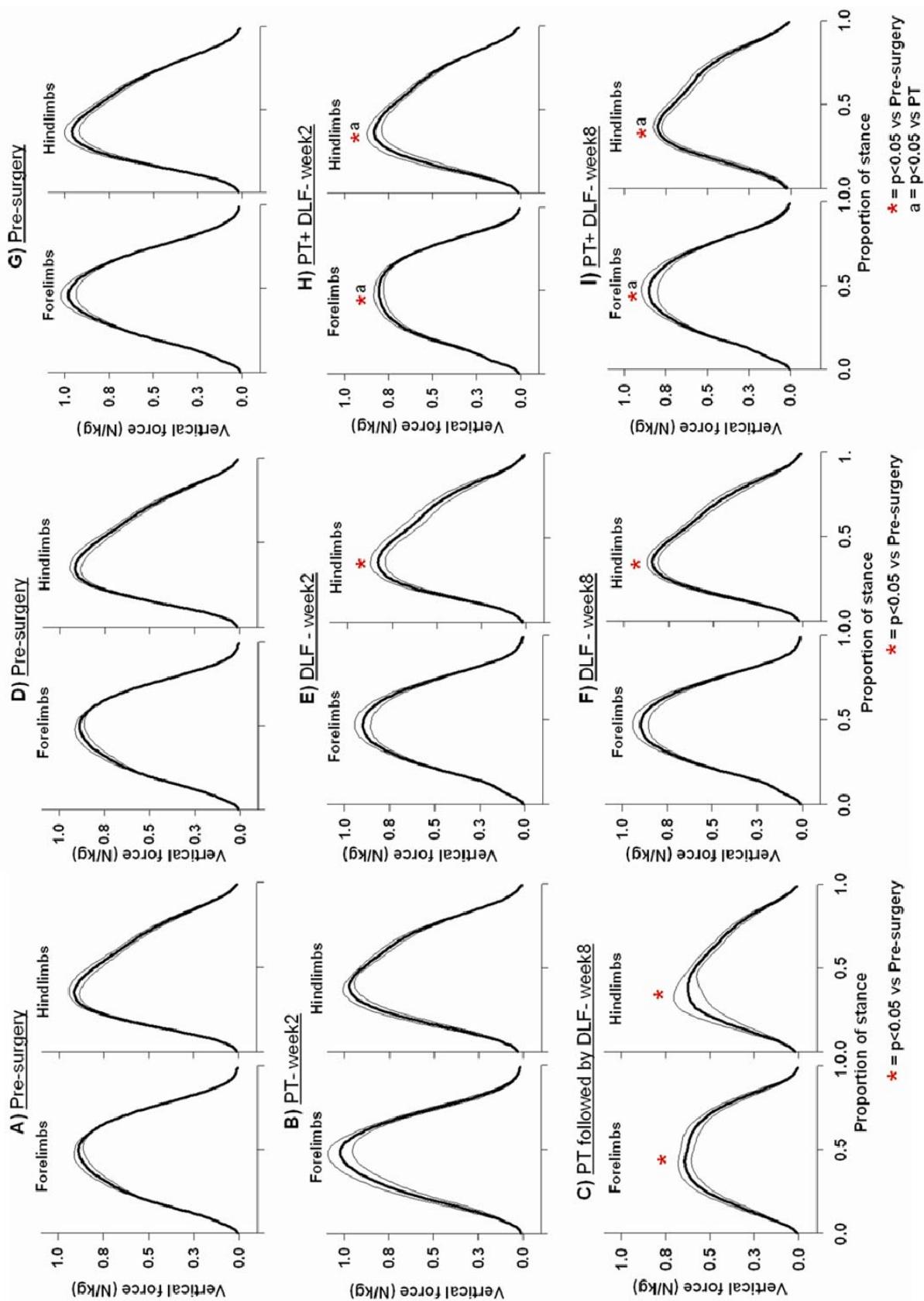


Fig. 7.2: Vertical forces produced by the forelimbs and the hindlimbs for (A, B, C) PT followed by DLF, (D, E, F) DLF and (G, H, I) simultaneous PT-DLF rats. PT rats did not show any changes in the generation of vertical forces (B) but addition of DLF lesions in PT rats caused the rats to move slowly preventing comparisons with rats from other groups. DLF lesions in PT rats reduced peak vertical forces in both the forelimbs and the hindlimbs when compared to speed-matched slower pre-surgical runs ($p<0.05$; C). DLF rats showed reduced hindlimb vertical forces at all time points after surgery (E, F). After surgery, PT-DLF rats showed reduced peak vertical forces in both the forelimbs and the hindlimbs ($p<0.05$; H, I) compared to pre-surgical and PT rats, but were no different from DLF rats. In all graphs, thick lines represent mean data for each group of animals and thin lines represent \pm standard error of mean.

For simplicity, only week 2 data are shown here. Addition of DLF lesions in PT rats changed the generation of forces, but also reduced the trotting speeds (Table 7.1) compared to both pre-surgical and PT rats. As trotting velocities have an effect on generation of ground reaction forces in animals (Khumsap et al., 2001, Khumsap et al., 2002), we compared the week 8 and week 10 data with that from the slower pre-surgical runs (average; 61.47 cm/s) from these rats.

Even after locomotor velocity was accounted for, DLF lesions in PT rats reduced the generation of peak vertical forces by both the forelimbs and the hindlimbs ($p<0.001$ vs pre-surgery; Fig. 7.2C). In the fore-aft direction, the forelimbs generated increased braking forces ($p<0.001$ Vs pre-surgery; Fig. 7.3C) and the hindlimbs generated increased propulsive forces ($p<0.001$ Vs pre-surgery; Fig. 7.3C) compared to pre-surgery (only week 8 data is shown). There were no differences in any of the impulses suggesting that rats were travelling at the same speed as in the slow pre-surgical runs. In these rats we found no changes in medio-lateral forces either after PT lesion or after sequential DLF lesion (data not shown). Because of the difference in trotting speeds between the post-surgical time points, inter-week comparisons were not done in this group.

DLF group

DLF lesions did not change the trotting speeds in the rats (Table 7.1). Generation of forces in DLF rats were similar to those seen in our previous experiment (Muir et al., 2007). In DLF rats, generation of vertical forces by the hindlimbs were reduced as

	PT followed by DLF group				DLF group				PT-DLF group	
	Pre-surgery	Slower pre-surgical runs	W2	W8	Pre-surgery	W2	W8	Pre-surgery	W2	W8
RF stance	0.42±0.01	0.45±0.00	0.42±0.01	0.52±0.01* (15%)	0.43±0.01	0.45±0.02	0.44±0.02	0.42±0.00	0.47±0.00*	0.47±0.01*
RFI stance	0.43±0.02	0.47±0.02	0.43±0.03	0.55±0.03* (17%)	0.43±0.01	0.45±0.01	0.44±0.01	0.42±0.00	0.49±0.00*	0.49±0.01*
LF stance	0.42±0.01	0.45±0.01	0.45±0.03	0.51±0.02* (13.3)	0.44±0.02	0.44±0.02	0.45±0.02	0.42±0.00	0.47±0.00*	0.48±0.00*
LFI stance	0.43±0.00	0.48±0.02	0.42±0.02	0.55±0.03* (15.25%)	0.43±0.01	0.45±0.01	0.45±0.01	0.42±0.00	0.49±0.01*	0.49±0.01*
Trotting speed (cm/s)	74.25	61.47 ^Δ	70.42	59.95* ^Δ	73.12	70.72	76.73	72.51	68.37	73.5

Table 7.1: Stance durations of limbs (means ± S.E.M) expressed as proportion of stride duration and average trotting speeds in PT followed by DLF-, DLF- and PT-DLF-lesioned rats. PT lesions and DLF lesions did not cause any changes in the stance durations of the limbs or trotting speed. Addition of either simultaneous or staggered DLF lesions in PT rats increased the stance durations of both the forelimbs and the hindlimbs (* $p<0.05$) compared to pre-surgical rats. Staggered but not simultaneous DLF lesions in PT rats reduced the trotting speeds in rats (* $p<0.05$ vs pre-surgery), therefore week 8 data from PT followed by DLF group was compared to slower pre-surgical runs from this group (Δ). The percentage increase in stance durations of limbs compared to their pre-surgical times are indicated in brackets below the actual numbers. In PT followed by DLF group, percentage increase in stance durations are as compared to slower pre-surgical runs.

compared to pre-surgery ($p<0.05$; Fig. 7.2E, F). We did not observe any changes in the forelimb vertical forces (Fig. 7.2E, F). There were also no changes in the generation of fore-aft forces at any time point (Fig. 7.3E, F). The changes in ground reaction forces after DLF lesions remained unchanged for the entire duration of the study (Fig. 7.2E, F and Fig. 7.3E, F).

PT+DLF group

Rats with simultaneous PT and DLF lesions showed reduction in generation of peak vertical forces by both the forelimbs and the hindlimbs ($p<0.001$ vs pre-surgery; Fig. 7.2H, I). In the fore-aft direction, the generation of forces were similar to pre-surgical performance, except an increase in the braking forces produced by the forelimb and in the propulsive forces produced by the hindlimbs (Fig. 8.3H, I) compared to pre-surgery ($p<0.001$). The changes in generation of forelimb and hindlimb forces after simultaneous PT+DLF surgery remained unchanged throughout the entire experimental period (Fig. 7.2H, I and Fig. 7.3H, I).

Group comparisons

Comparison of peak forces between the groups revealed slight differences in vertical and fore-aft forces. In the simultaneous PT-DLF group, both the forelimb and the hindlimb vertical forces were reduced compared to PT rats ($p<0.05$; Fig. 7.2H, I), the forelimb braking forces were increased compared to PT rats and to DLF rats ($p<0.05$; Fig. 7.3H, I) and the hindlimb propulsive forces were increased compared to PT lesions alone ($p<0.05$; Fig. 7.3H, I). Apart from the difference in forelimb braking forces, there were no other

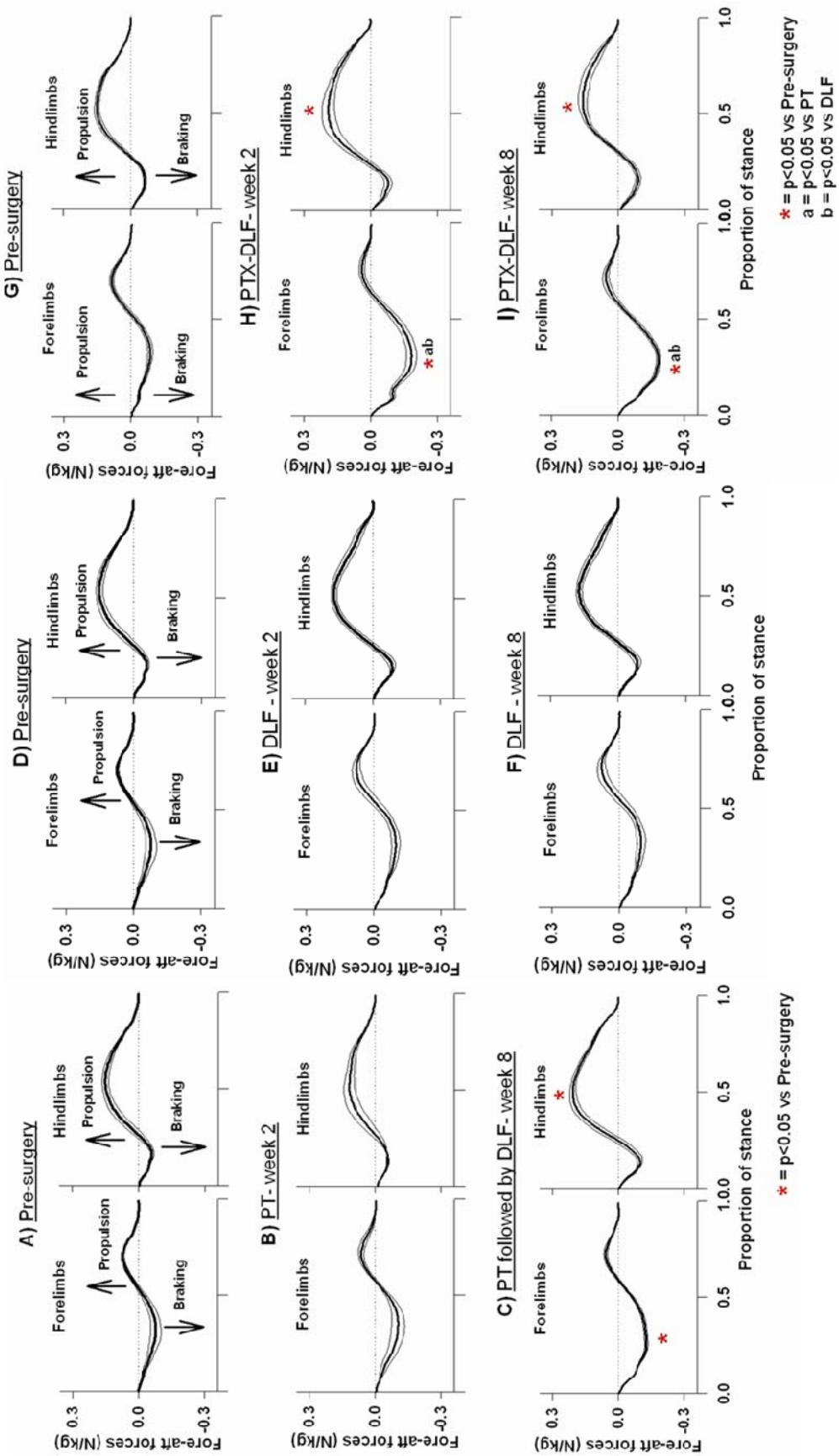


Fig. 7.3: Fore-aft forces produced by the forelimbs and the hindlimbs for (A, B, C) PT followed by DLF, (D, E, F) DLF and (G, H, I) simultaneous PT-DLF rats. PT rats did not show any changes in the generation of fore-aft forces (B) but addition of DLF lesions in PT rats caused the rats to move slowly preventing comparisons with rats from other groups. DLF lesions in PT rats produced increased braking forces in the forelimbs and increased propulsive forces in the hindlimbs compared to the speed-matched slower pre-surgical runs (C). DLF rats showed no changes in generation of fore-aft forces at time points ($p < 0.05$; E, F). Simultaneous PT-DLF lesions produced increased braking forces in the forelimbs compared to pre-surgical, DLF and PT rats and increased propulsive forces in the hindlimbs ($p < 0.05$; H, I) compared to pre-surgical rats. In all graphs, thick lines represent mean data for each group of animals and thin lines represent \pm standard error of mean.

differences between simultaneous PT-DLF group and DLF group (Fig. 7.2H, I and Fig. 7.3H, I).

PT followed by DLF+ASP group

In all 3 rats, GRF data after initial PT lesions was similar to other PT rats from PT followed by DLF group (data not shown). After secondary lesions, rats in this group could walk but not trot; hence no GRF data could be recorded. All three rats walked in a crouched fashion with splayed forelimbs similar to rats with simultaneous DLF+DF lesions from our previous study (Chapter 6 this thesis). Among the 3 rats V11 was more severely affected. Data from this group was not statistically analyzed or used for group comparisons

7.4.3.1.1 Step lengths and stride parameters

When the rats are trotting, diagonal limb pairs (i.e. right fore and left hind or left fore and right hind) are in ground contact at the same time (Fig. 7.4A, D, G). All post-surgical rats in the present experiment were trotting on the runway, similar to pre-surgical rats (Fig. 7.4). For comparison and analysis, all the post-surgical runs were speed matched with pre-surgical runs.

PT followed by DLF group

In this group, after PT lesions, there were no differences in any of the stride parameters or stance durations (Fig. 7.4B; Table 7.1). Addition of DLF lesions, however, increased the stance duration of both the forelimbs and the hindlimbs compared to pre-surgery

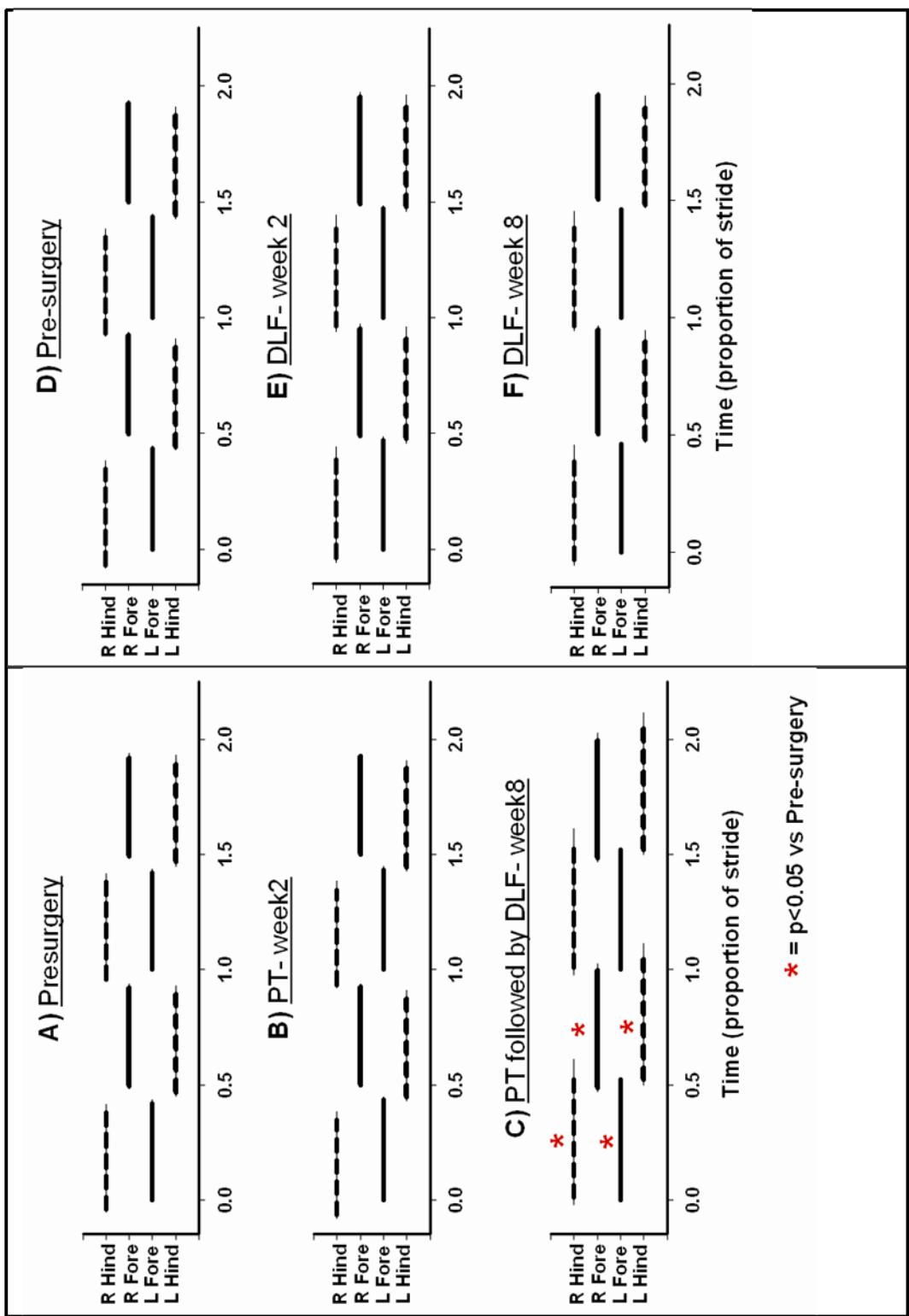
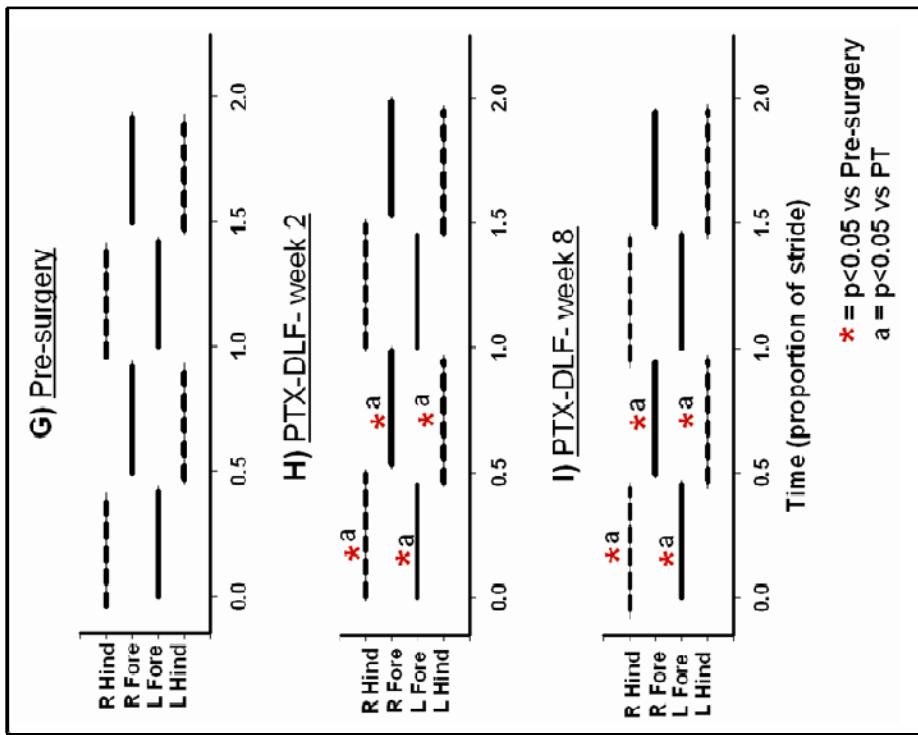


Fig. 7.4: Forelimb (solid lines) and hindlimb (dashed lines) contact timing in (A, B, C) PT followed by DLF, (D, E, F) DLF and (G, H, I) PT-DLF rats. The length and position of the thick and thin lines represent mean limb stance times for each group of animals, while thin lines at either end represent standard error of the group means. When the rats are trotting, diagonal limb pairs (i.e. right fore and left hind or left fore and right hind) are in ground contact at the same time. All post-surgical rats in the present experiment were trotting on the runway, similar to pre-surgical rats. PT rats showed no changes in the stance duration or timing compared to pre-surgical rats (B). Addition of DLF lesions in PT rats caused increased stance durations by both the forelimbs and the hindlimbs ($p<0.05$; C) compared to speed-matched slower pre-surgical runs. DLF lesion caused no changes in the stance durations or limb timings compared to pre-surgery (E, F). Simultaneous PT-DLF lesions caused increased forelimb and hindlimb stance durations ($p<0.05$) compared to both pre-surgical and PT rats (H, I). There were no differences between DLF and PT-DLF rats at any time point.



($p<0.001$; Fig. 7.4C; Table 7.1). Comparisons could not be made in this group between PT lesions alone and after the addition of staggered DLF lesion as the rats were moving at different speeds.

DLF group

In this group there were no differences in any of the stride parameters tested compared to pre-surgical data (Fig. 7.4E, F; Table 7.1).

PT-DLF group

After simultaneous PT-DLF lesions, there was an increase in stance durations of both the forelimbs and the hindlimbs (Fig. 7.4H, I; Table 7.1) compared to pre-surgery ($p<0.001$) and these changes persisted for the entire duration of study.

Group comparisons

Comparison between the groups revealed differences between PT rats and simultaneous PT-DLF rats. At weeks 2 and 6, simultaneous PT-DLF rats showed increased stance duration compared to PT rats ($p<0.05$; Fig. 7.4H, I; Table 7.1). Post-surgical week 8 and 10 data from PT followed by DLF rats could not be compared with rats from other 2 groups because of differences in trotting speed. However, no differences were found between PT rats and DLF rats or between DLF rats and simultaneous PT-DLF rats (Fig. 7.4H, I).

PT followed by DLF+ASP group

After initial PT lesions, there were no changes in the step lengths, similar to the PT rats in the PT followed by DLF group (data not shown). After secondary lesions, the rats could not trot, so step length and stride parameter data could not be recorded. Data from this group was not statistically analyzed or used for group comparisons.

7.4.3.2 Skilled locomotion- Horizontal ladder

Prior to surgery, all rats were walking proficiently over the ladder. The forelimb correct steps (85%) were consistently lower than the hindlimb correct steps (98%).

PT followed by DLF group

Initial PT lesions caused increased forelimb errors compared to pre-surgery (38% increase from pre-surgery, $p<0.05$; Fig. 7.5A), but had no effect on the placement of hindlimbs (Fig. 7.5B). Addition of DLF lesions in PT rats, caused a slight increase in forelimb errors (50% increase from pre-surgery, 18% increase from PT lesions; $p<0.05$; Fig. 7.5A) but a severe increase in the errors of hindlimbs (65% increase from pre-surgery, 60% increase from PT lesions; $p<0.05$; Fig. 7.5B) compared to both pre-surgery and PT lesions alone.

DLF group

Results from DLF rats were similar to those from our previous study (Muir et al., 2007). DLF lesions caused increase in both the forelimb and the hindlimb errors compared to pre-surgery ($p<0.05$; Fig. 7.5A and Fig. 7.5B). The effects, however, were more severe

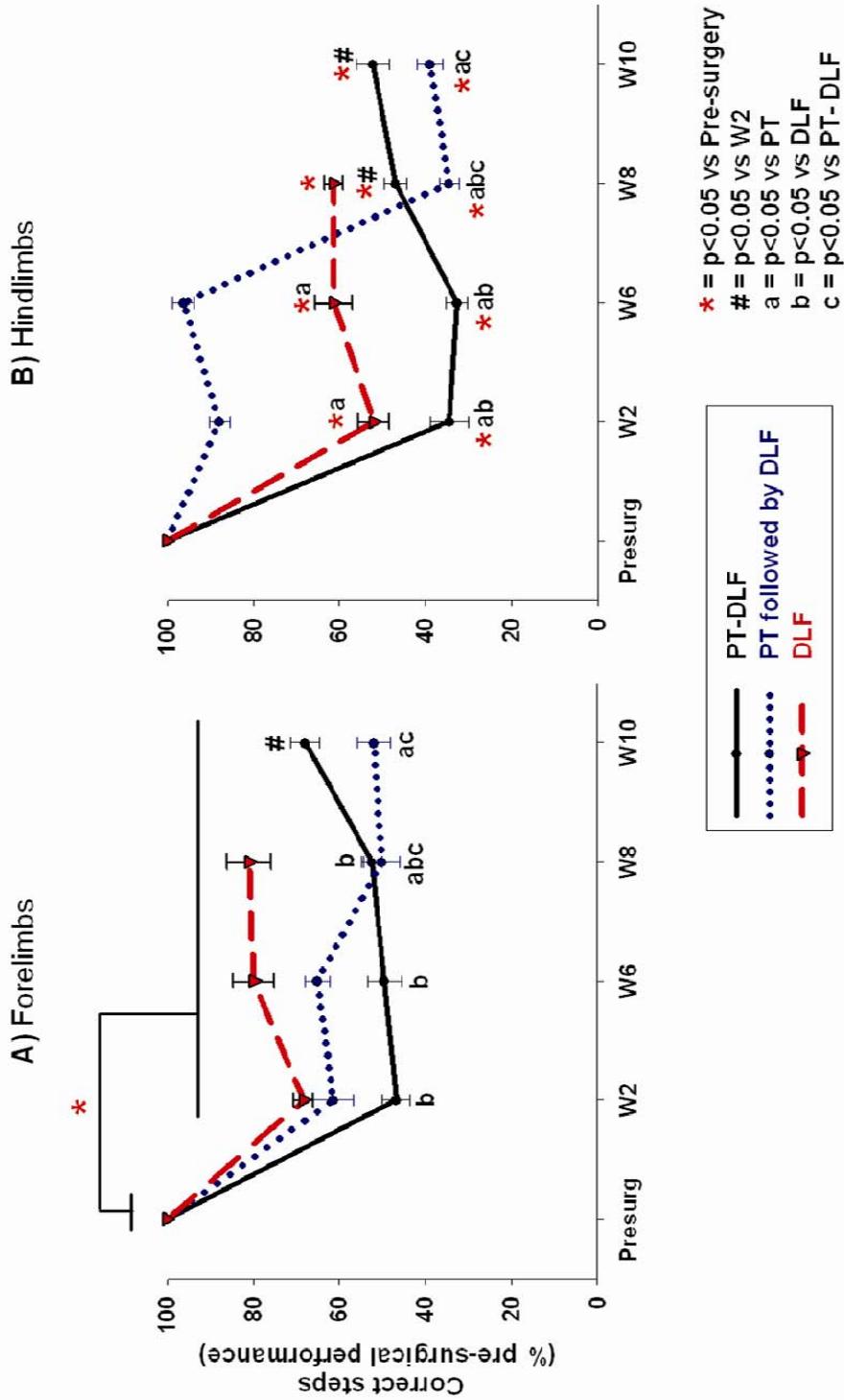


Fig. 7.5: Forelimb (A) and hindlimb (B) correct steps as percentage pre-surgical ability during ladder walking, in PT followed by DLF (dotted lines), DLF (dashed lines) and PT-DLF (solid lines) rats. After PT lesions, rats made more forelimb errors ($p<0.05$) compared to pre-surgical rats. After 6 weeks, addition of DLF lesions in PT rats increased both forelimb and hindlimb errors ($p<0.05$) compared to pre-surgical, PT and DLF rats. Sequential DLF lesions affected hindlimb placement more severely than forelimbs. DLF lesions caused increased forelimb and hindlimb errors ($p<0.05$) compared to pre-surgical rats. Simultaneous PT-DLF lesions caused increased forelimb and hindlimb errors ($p<0.05$) compared to pre-surgical, PT and DLF rats.

in the hindlimbs (Fig. 7.5B) than in the forelimbs (Fig. 7.5A). The deficits persisted even at 8 weeks after surgery.

PT-DLF group

Simultaneous PT-DLF lesions caused additive increases in both the forelimb errors (Fig. 7.5A; 43% increase compared to pre-surgery) and hindlimb errors (Fig. 7.5B; 68% increase compared to pre-surgery) compared to pre-surgical rats ($p<0.05$). The forelimb and hindlimb errors persisted even at 10 weeks after the lesions. However, there was a slight improvement in the limb placements on the ladder rungs at week 10 compared to week 2 (Fig. 7.5A, 7.5B).

Group comparisons

Comparison of groups revealed differences in placement of both the forelimbs and the hindlimbs. DLF rats showed more hindlimb, but not forelimb errors compared to PT rats ($p<0.05$; Fig. 7.5A and Fig. 7.5B). Simultaneous PT-DLF rats made more forelimb and hindlimb errors compared to DLF rats ($p<0.05$; Fig. 7.5A and Fig. 7.5B), and made more hindlimb errors compared to PT rats ($p<0.05$; Fig. 7.5B). Addition of DLF lesions in PT rats (staggered lesion group) caused increased forelimb and hindlimb errors compared to DLF rats ($p<0.001$; Fig. 7.5A and Fig. 7.5B). The PT followed by DLF group also showed increased forelimb errors at week 10 and hindlimb errors at weeks 8 and 10 compared to simultaneous PT-DLF group ($p<0.05$; Fig. 7.5A and Fig. 7.5B).

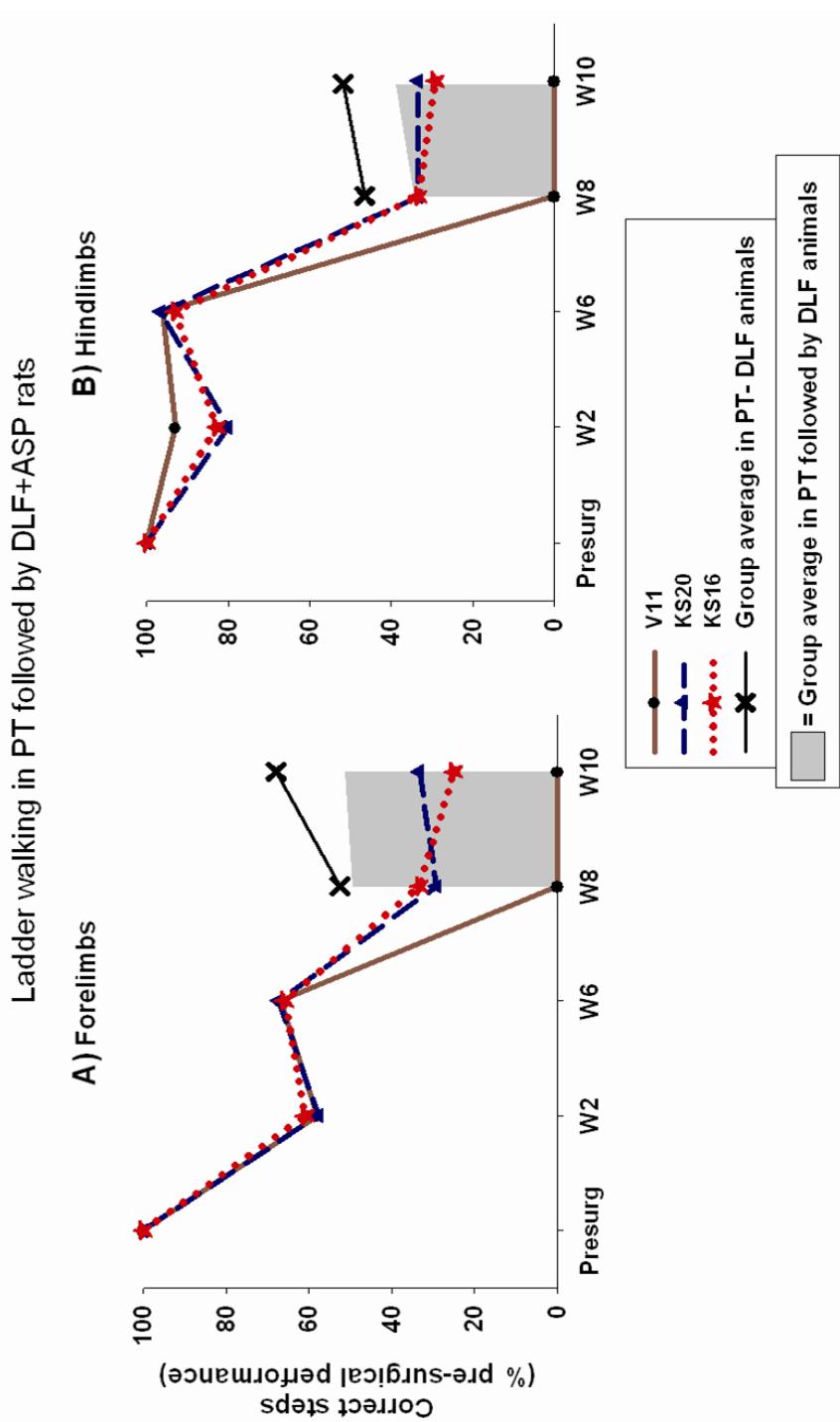


Fig. 7.6: Individual ladder walking data expressed as percentage pre-surgical performance from V11, KS16 and KS20. (A) forelimb correct steps and (B) hindlimb correct steps in rats with PT followed by DLF+ASP lesions. Group averages from PT followed by DLF (shaded area-W8 and W10) and simultaneous PT-DLF (black solid line above W8 and W10) groups are also shown for comparison. After PT lesions, rats V11, KS16 and KS20 were similar to PT rats from PT followed by DLF group. After sequential lesions, rat V11 which had sustained the maximum ASP damage, could not walk over ladder. However rats KS16 and KS20 had spared ASP and could walk over ladder, but showed both forelimb and hindlimb errors that were severe than rats with either staggered or simultaneous PT and DLF lesions. Effect of ASP damage seemed to be more evident in the forelimb than hindlimb placement.

PT followed by DLF+ASP group

After PT lesions, the forelimb and hindlimb errors were similar to those of PT rats in the PT followed by DLF group. After secondary lesions to spinal cord which included both DLF and ASP, V11 could not walk on the ladder, while KS16 and KS20 showed increased forelimb and hindlimb errors (Fig. 7.6A and Fig. 7.6B). Importantly, additional damage to ASP seemed to affect the forelimb placement more than the hindlimb placement (Fig. 7.6A). Data from this group was not statistically analyzed or used for group comparisons.

8.4.3.3 Skilled fore-paw usage- single pellet reaching

PT followed by DLF group

PT lesions reduced the successful pellet retrievals in rats (Fig. 7.7). At 2 weeks, PT rats were reaching at approximately 60% of their pre-surgical success and by 6 weeks they showed a slight improvement (65% of pre-surgical ability), but their reaching ability was still significantly lower than pre-surgical rats ($p<0.05$; Fig. 7.7). Analysis of reaching movements in PT rats revealed differences in aim, pronation, supination-1 and supination-2 movements compared to pre-surgical rats ($p<0.05$; Figs. 7.9A, B, D and E). After 6 weeks, addition of DLF lesions in PT rats caused a marked reduction in the reaching ability compared to both pre-surgery and PT lesioned animals ($p<0.05$; Fig. 7.7). After staggered DLF lesions, PT rats were retrieving about 18% of their pre-surgical ability and there was no improvement at the last time point tested (Fig. 7.7). Among the reach components, in addition to abnormalities after PT lesions, grasp, supination-2, food

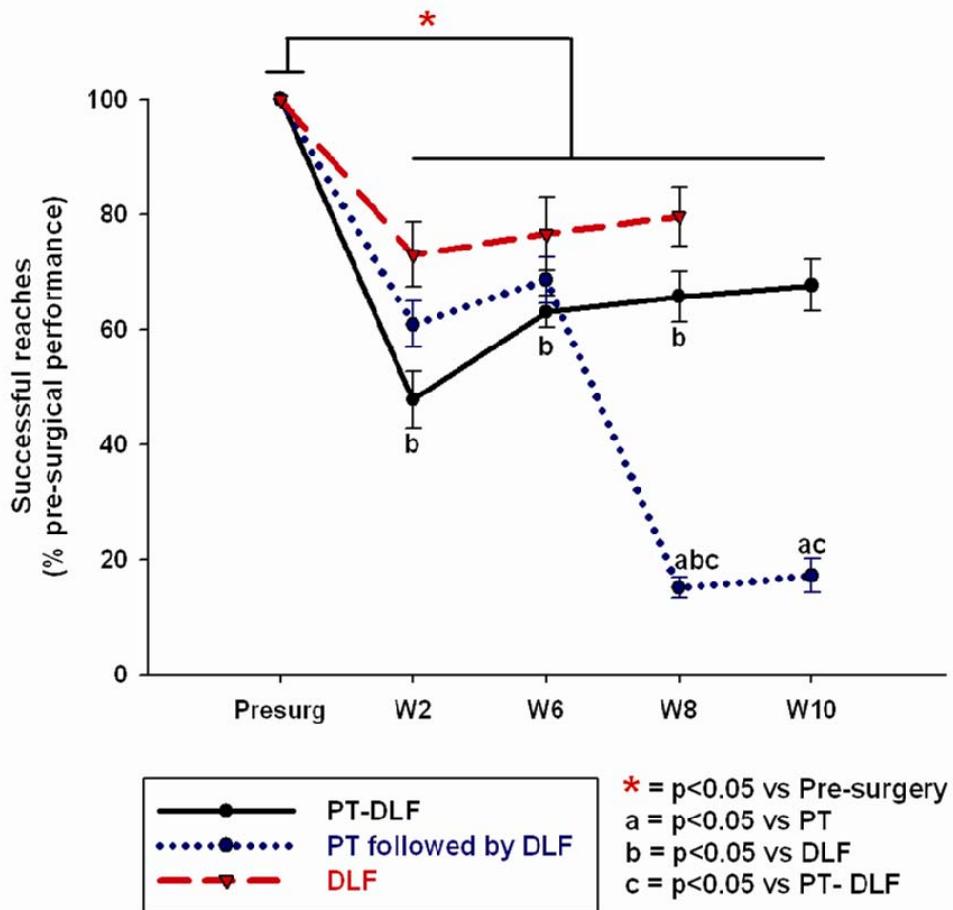


Fig.7.7: Reaching ability as a percentage of pre-surgical performance in rats with PT followed by DLF- (dotted line), DLF- (dashed line) and simultaneous PT-DLF (solid black line) lesions. PT rats showed reduced ability in retrieving pellets ($p<0.05$) compared to pre-surgical rats. Six weeks later addition of DLF lesions in PT rats caused further reduction in reaching success ($p<0.05$), which was more severe compared to pre-surgical, PT, DLF and DLF-PT rats. DLF rats showed reduced reaching ability ($p<0.05$) compared to pre-surgical rats at all time points. Simultaneous PT-DLF lesions reduced the reaching ability in rats ($p<0.05$) compared to pre-surgical and DLF rats.

release and arpeggio movements were also affected compared to pre-surgery and PT lesions ($p<0.05$; Figs. 7.9A-G).

DLF group

DLF lesions also permanently reduced the reaching ability in rats compared to pre-surgical rats ($p<0.05$; Fig. 8.7). Analysis of reaching movement in DLF rats showed consistent absence of supination type 2 and arpeggio movements after DLF lesions ($p<0.05$; Figs. 7.9E and 7.9G), similar to those seen in our previous study (Muir et al., 2007).

PT-DLF group

Simultaneous PT-DLF lesions reduced reaching ability in rats ($p<0.05$; Fig. 7.7). PT+DLF rats showed impairments in all most all the reach components, particularly aim, pronation, grasp, supination-1, supination-2, food release and arpeggio movements ($p<0.05$; Figs. 7.9A-G) compared to pre-surgical rats. In these rats, the reduced pellet reaching ability and the abnormalities during reaching persisted through the entire period of experiment.

Group comparisons

There were no differences in reaching success between DLF rats and PT rats, but the addition of DLF lesions in PT rats severely reduced the reaching ability compared to rats with either PT lesions or DLF lesions ($p<0.001$; Fig. 7.7). Importantly, reaching abilities in PT followed by DLF rats were also far more impaired compared to those of animals

with PT and DLF lesions performed simultaneously ($p<0.001$; Fig. 7.7). Simultaneous PT-DLF rats did show reduced reaching abilities compared to PT rats and to DLF rats (Fig. 7.7). In the qualitative reach analysis, PT lesions caused abnormalities in aim, pronation, supination type 1 movements (Figs. 7.9A, 7.9B and 7.9D) unlike DLF lesions. In contrast, DLF lesions caused abnormalities in arpeggio, unlike PT lesions, and also caused more severe deficits in supination type 2 movements (Fig 7.9G and E). After the addition of DLF lesions in PT rats, grasp and food-release were abnormal, both of which had remained relatively unaffected after either PT lesions or DLF lesions alone ($p<0.05$; Figs. 7.9C and 7.9F). There were no differences in the qualitative aspects of reaching movements between animals which received DLF lesions six weeks after PT lesions and those which received simultaneous PT-DLF lesions.

PT followed by DLF+ASP lesions

After initial PT lesions these rats were similar to PT rats in the staggered lesion group both in reaching ability and reaching movements. But after secondary lesions, only 2 rats (KS16 and KS20) could reach for food pellets and both of them showed drastically reduced successful reaches (Fig. 7.8). Both rats retrieved pellets after multiple attempts and almost always used both the paws to hold onto pellets and also rotated their heads to eat the pellets unlike rats from other groups. Almost all the reaching elements were affected (data not shown) in these 2 rats. Data from this group was not statistically analyzed or used for group comparisons.

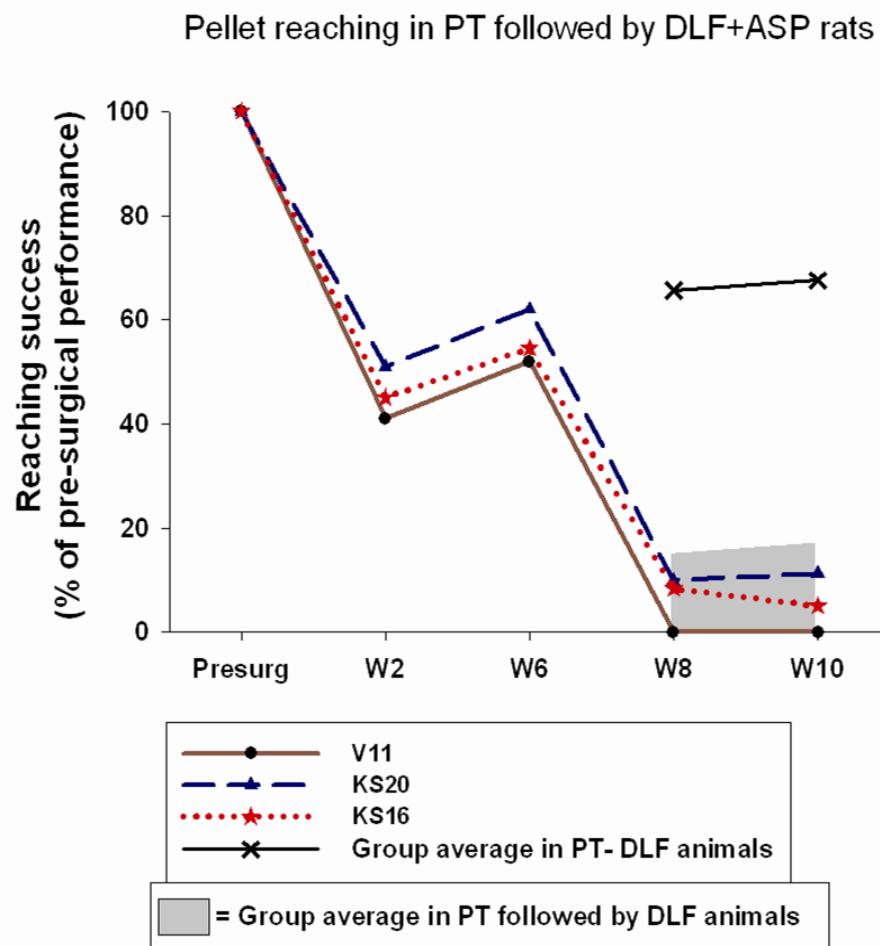


Fig. 7.8: Individual pellet reaching data expressed as percentage pre-surgical performance from V11, KS16 and KS20. Group averages from PT followed by DLF (shaded area-W8 and W10) and simultaneous PT+DLF (black solid line above W8 and W10) groups are also shown for comparison. After PT lesions, rats V11, KS16 and KS20 were similar to PT rats from PT followed by DLF group. After sequential lesions, rat V11 which had sustained the maximum ASP damage could not retrieve any food pellet and both KS16 and KS20 showed severely reduced reaching success which was lower than PT followed by DLF or PT+DLF rats.

7.5 Discussion

The results of the present study reveal that bilateral PT lesions caused no discernable deficits during overground locomotion, caused deficits of forelimbs and not hindlimbs during ladder locomotion, and altered forelimb movements and success during skilled pellet retrieval. Consistent with our previous studies, bilateral DLF lesions caused mild changes in the hindlimbs during overground locomotion, affected hindlimbs more severely than forelimbs during ladder locomotion and also altered forelimb movements and success during skilled reaching. When DLF lesions were added to the PT lesions, animals were generally more severely affected compared to either lesion alone. During overground and skilled locomotion, however, there were no differences between animals which received DLF lesions six weeks after PT lesions compared to animals which received the lesions simultaneously. In contrast, the reaching deficits were much more severe in animals which received DLF lesions six weeks after PT lesions compared to animals with simultaneous PT-DLF lesions. Thus our original hypothesis, that DLF pathways can compensate for PT lesions, is partially supported in that this appears to occur for skilled pellet retrieval but not during skilled or overground locomotion. We discuss our results in relation to previous studies and suggest explanations for our findings.

Bilateral pyramidal lesions cause deficits similar to unilateral pyramidal lesions

Bilateral transection of the pyramidal tract effectively removes all the corticospinal input to the spinal cord. Earlier studies have shown that unilateral damage to pyramidal tract does not affect overground locomotor abilities in rats (Metz et al., 1998; Muir and

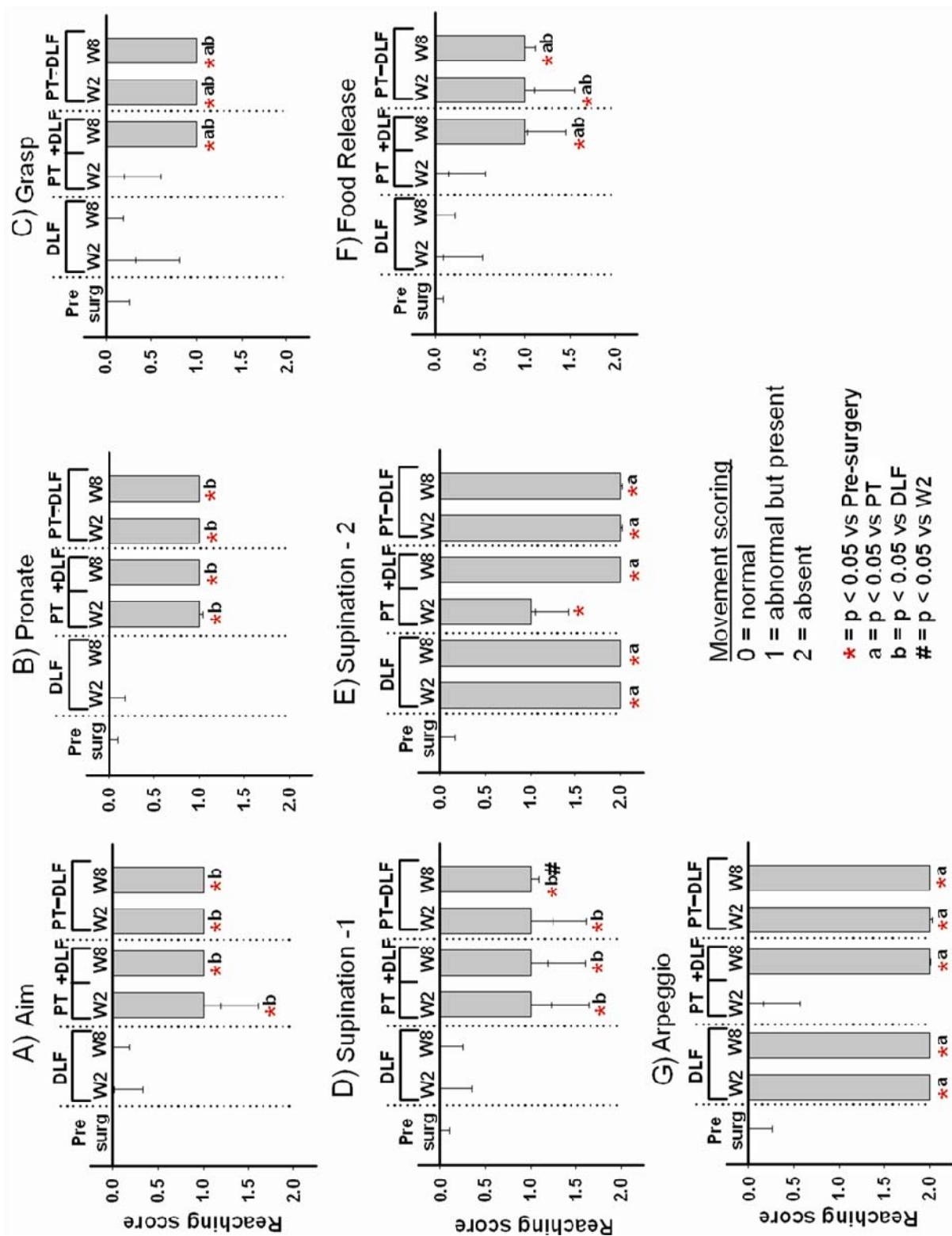


Fig. 7.9: Qualitative reaching scores in DLF-, PT followed by DLF- and PT-DLF-lesioned rats. Bars represent median values \pm 95% confidence limits. After PT lesions rats showed impairments in aim, pronation, supination type 1, supination type 2 movements (A, B, D, E; $p<0.05$) compared to pre-surgical rats. Addition of DLF lesions in PT rats caused further impairments in grasp, Supination type 2, food release and arpeggio movements (C, E, F, G; $p<0.05$) compared to pre-surgical and PT rats. DLF rats showed absence of supination type 2 and arpeggio movements (E, G; $p<0.05$) compared to pre-surgical rats. Simultaneous PT-DLF rats showed impairments in aim, pronation, grasp, supination type 1, supination type 2, food release and arpeggio (A-G; $p<0.05$) compared to pre-surgical rats.

Whishaw, 1999a). After unilateral damage to PT, Metz et al. found transient locomotor deficits which recovered by the first week of lesion, except for hypermetria which persisted at 4 weeks (Metz et al., 1998). By measuring ground reaction forces during overground locomotion, Muir and Whishaw confirmed the lack of deficits during overground locomotion after unilateral PT lesions (Muir and Whishaw, 1999a). As unilateral lesions spare the pyramidal tract on one side, we initially thought that bilateral PT lesions might cause more severe deficits than unilateral lesions. Instead, we found no changes in either the generation of limb forces (Figs.7.2 and 7.3) or limb timing (Fig.7.4) after bilateral PT lesions. The results from the present study unequivocally confirm and extend the previous reports that lesions of PT do not cause measurable deficits during overground locomotion.

In contrast to its minimal role during overground locomotion, the pyramidal tract is important in tests requiring skilled movements of the limbs, such as skilled locomotion and single pellet reaching, and our results are consistent with these findings (Metz and Whishaw, 2002; Whishaw et al., 1993; Whishaw et al., 1998). During ladder walking, rats with unilateral PT lesions committed more foot faults with both the forelimbs and the contralateral hindlimb (Metz and Whishaw, 2002). In our study with bilateral lesions, we observed no hindlimb deficits but did see deficits involving both forelimbs which were more severe compared to the forelimb deficits after unilateral PT lesions (Metz and Whishaw, 2002). It is possible that the lack of hindlimb deficits in the present study compared to the results of Metz and Whishaw (2002) could be due to differences in the ladder type, in that we used a ladder with regularly spaced rungs, which is less

challenging than that used by Metz and Whishaw (2002) (for detailed discussion see Chapter 4, this thesis). Interestingly, sparing of even some PT fibers is enough to maintain reaching ability in rats. In that study, reaching success in rats with incomplete lesion of PT was indistinguishable from that of pre-surgical rats. However, rats with incomplete PT lesions showed alterations in the reaching movements (Piecharka et al., 2005). In bilateral PT lesioned rats in the present study, the reduction in reaching success and the alterations in reaching movements (aim, pronation and supination type-1 movement; Fig.9) were similar to those of animals with complete unilateral PT lesions (Whishaw et al., 1993; Whishaw et al., 1998; Piecharka et al., 2005).

DLF pathways do not compensate for the loss of corticospinal input during overground or skilled locomotion

We originally hypothesized that lack of deficits after PT lesions during overground locomotion might be due to the compensation from intact DLF pathways. If DLF pathways were compensating after PT lesions, we would have seen more severe deficits after secondary DLF lesions in PT lesioned animals compared to animals with simultaneous PT-DLF lesions. Unfortunately, the two groups cannot be directly compared, because of the differences in locomotor velocity. PT lesioned rats with secondary DLF lesions trotted more slowly on the runway than did simultaneous PT-DLF lesioned animals. At slower velocities, animals spend more time in stance duration and show reduced generation of peak forces by the limbs (McLaughlin et al, 1996; Khumsap et al., 2001; Khumsap et al., 2002). This could be erroneously interpreted as the effect of the lesions, when in reality it is the effect of speed. We addressed the issue by matching

the trotting speeds of runs for comparison. Nevertheless, when compared to slower pre-surgical runs, secondary DLF lesions in PT rats showed reduced vertical forces (Fig. 7.2C) and increased stance duration in the limbs (Fig. 7.4C). It is possible that these changes are the result of the secondary DLF lesions - the addition of DLF damage in PT lesioned rats did not make the rats any worse than those with only DLF damage. Instead it was apparent that, because animals with DLF lesions performed six weeks after PT lesions had comparable limb forces and timing as those with simultaneous PT-DLF lesion, DLF pathways do not appear to compensate for the loss of corticospinal input during overground locomotion. (Figs. 7.2 and 7.3). These results are also consistent with our previous work describing the persistent effect of DLF lesions on limb action during overground locomotion (Muir et al, 2007).

Compared to overground locomotion, ladder locomotion revealed more obvious deficits after lesions, but again, our results suggest that DLF pathways do not compensate during ladder walking in rats with pre-existing PT lesions. If DLF pathways were providing compensatory input after PT lesions alone, we would have expected to see more severe deficits in the staggered lesions group compared the simultaneously lesioned PT-DLF animals. Simultaneous PT and DLF lesions caused decreases in forelimb (43% compared to pre-surgical rats) and hindlimb (68% compared to pre-surgical rats) correct steps which could be seen as simply an additive effect of both lesions (Fig. 7.5). DLF lesions inflicted 6 weeks after PT lesions caused increases in forelimb and hindlimb errors comparable to those of animals with simultaneous PT-DLF lesions (forelimbs 50% and hindlimbs 65% correct steps compared to pre-surgical values).

DLF pathways do compensate for the loss of corticospinal input during skilled reaching

In contrast to overground or skilled locomotion, DLF pathways do appear to mediate reaching abilities in rats with PT lesions. Our results demonstrate that simultaneous PT-DLF lesions produced reaching success rates that are comparable to those after PT lesions alone (Fig. 7.7). Similarly, reaching movements in animals with simultaneous lesions were a combination of those impaired by PT lesions and by DLF lesions although, grasp and food release were also affected (Fig. 7.9). These results are consistent with those from rats with combined PT and red nucleus lesions (Whishaw et al., 1998). In contrast to the mainly additive effects of PT and DLF damage in the simultaneous lesion (PT-DLF) group, DLF lesions inflicted 6 weeks after PT lesions severely potentiated the reaching deficits, to 15% of presurgical success rates (Fig. 7.7). These results suggest that there was a greater contribution of DLF pathways in PT-lesioned animals toward maintaining reaching success compared to DLF pathways in animals with intact PT. Thus, after PT-lesions, pathways in the DLF provide compensatory input during skilled pellet retrieval, a finding which supports our hypothesis. Interestingly, the qualitative analysis of reaching movements showed that the same elements are affected in animals with simultaneous PT-DLF lesions and those with secondary DLF lesions 6 weeks after PT lesions. Thus, the dramatic reduction in reaching success after secondary DLF lesions is not due to a qualitative difference in the movements used to perform the reach.

The question arises as to which DLF pathways might be providing this compensatory input. Pathways which travel in the DLF include the dorsal spinocerebellar tract, the

rubrospinal tract, the lateral component of CST and reticulospinal pathways (Yamada et al., 1991; Brown 1974; Antal et al., 1992; Terashima, 1995; Brosamle and Schwab, 1997; Houle and Jin, 2001). The dorsal spinocerebellar tract transmits sensory information from the tail, trunk and a portion of hindlimbs and likely has lesser role in forelimb function. The lateral CST would be damaged after pyramidal lesions. This leaves the rubrospinal tract and possibly the reticulospinal pathways as a likely source of compensatory input after PT lesions.

During skilled reaching in rats, both the corticospinal and rubrospinal tract act concertedly to bring about skilled forelimb movements (Hermer-Vazquez et al., 2004; Whishaw et al., 1998; Whishaw and Gorny, 1996; Whishaw et al., 1993). The idea that the rubrospinal tract could substitute after damage to corticospinal system during skilled forelimb movement was first proposed by Lawrence and Kuypers (Lawrence and Kuypers 1968a; Lawrence and Kuypers 1968b). They showed the role of these two systems in monkeys using two-stage lesions such as those used in the present experiments. Bilateral PT lesions in monkeys reduced their abilities to reach and grasp food objects but this ability recovered after time. In contrast, DLF lesions (including the rubrospinal tract) or damage to red nucleus in PT-lesioned monkeys caused irreversible impairment in movement of hand and distal extremities. Later, a hypothesis was proposed that corticospinal system and rubrospinal system could interchangeably compensate for the other (Kennedy, 1990). In rats that were trained to maintain balance on a rotating bar, lesions of RST at cervical level C3 produced impairments on the side of the lesion, which recovered in a few days. A subsequent lesion of the opposite red nucleus in these rats

produced deficits, from which the rats recovered for the second time. Interestingly, lesions of red nucleus which were not preceded by RST lesions showed no recovery at all. Based on these findings in double lesion studies, it was suggested that the initial RST damage enabled the switching of control to CST system through rubro-olivary projections, so that even after subsequent lesions of red nucleus the rats could still recover, because the motor ability in these rats was now controlled through the intact CST (Kennedy and Humphrey, 1987; Kennedy 1990). Using a similar behavioural paradigm, it was shown that the rubro-olivary tract, along with other brainstem structures such as the inferior olivary nucleus and the ventrolateral thalamic nucleus, could be responsible for switching between the two systems (Fanardjian et al., 2000c). However closer comparisons with the present study have to be taken with caution, as maintenance of balance on a rotating bar is a different test than skilled pellet retrieval, and conceivably involves other pathways including vestibulospinal and reticulospinal pathways in addition to the corticospinal and rubrospinal systems.

Compensation could have also resulted by strengthening of corticorubral connectivity. The corticorubral projections originate from the corticorubral cells of the cerebral cortex and are mono-synaptically connected with the rubrospinal tract neurons of red nucleus (Fanardjian et al., 2000b). Normally, the corticorubral pathways are inhibited by the cerebral cortex and red nucleus (Fanardjian et al., 2000b) but could be activated after pyramidal tract lesions and thus could relay the cortical information to spinal cord via rubrospinal tract (Fanardjian et al., 2000a; Fanardjian et al., 2000b; Fanardjian et al., 2000c). Another form of plasticity could involve sprouting of corticofugal fibers to

connect to the red nucleus after pyramidal lesions (Z'Graggen et al., 1998; Z'Graggen et al., 2000). In both possibilities presented above, the cortical signals could be re-routed through the rubrospinal tract after damage to PT and thus could be involved in compensating for the loss of corticospinal input.

Spinal pathways contributing to residual behaviour in rats with combined PT and DLF damage

In this experiment, behavioural abilities were preserved even after combined damage to simultaneous PT and DLF damage, suggesting that some of the other spared pathways can mediate the residual reaching ability. From some of our results, it appears that ascending sensory axons (ASP) in the dorsal funiculus could play a role in mediating the residual sensorimotor behaviour after PT and DLF lesions. There were 3 rats in this study which had sustained damage to ASP in addition to PT and DLF pathways. One rat (V11, Fig. 7.1D) which had sustained the maximum damage to dorsal funiculus in addition to PT and DLF pathways could not perform any behaviour. The other two rats (KS16 and KS20 Fig. 7.1D) had minimally spared ASP axons and could perform ladder walking and pellet retrieval, albeit with severe deficits. In both these rats, there were more forelimb errors during ladder walking (Fig. 7.6A) and reduced reaching success (Fig. 7.8) compared to those rats with damage to both PT and DLF but no damage to ASP. The results from these 3 animals suggest that the ASP has an important role to play in maintenance of behavioural performance in rats with PT and DLF damage. This is consistent with our previous work showing that ASP axons do contribute to skilled forelimb usage. In particular, when lesions of the ASP are combined with either dorsal

CST lesions or with DLF lesions, more severe and persistent deficits are seen during locomotor tasks and skilled forelimb tasks (Chapters 4 and 6 this thesis). These findings together suggest that ASP axons might make a larger functional contribution to forelimb movement in PT or DLF-lesioned animals than in normal animals

Evidence for functional contributions from other spinal pathways arises from work in cats. After combined damage to corticospinal and rubrospinal tracts in the DLF, propriospinal axons or ventrally located reticulospinal axons have been shown to mediate residual reaching ability in cats (Alstermark et al., 1981; Alstermark et al., 1987; Pettersson et al., 2000; Matsuyama et al., 2004). When the DLF was damaged in cats at cervical level C2 (lesioning both CST and RST), reaching ability was reduced but not completely lost, suggesting that reaching was mediated through C3-C4 propriospinal neurons (Alstermark et al., 1981). In contrast, when the DLF lesions were placed at cervical level C5 (thus destroying CST, RST and additionally propriospinal axons) the reaching ability was lost completely but recovered over few weeks after lesions. Interestingly, when the ventral funiculus was damaged at cervical level C2 in C5-DLF lesioned cats, the reaching ability was completely abolished, suggesting that after the combined damage to CST and RST, ventrally located reticulospinal axons might have mediated the reaching ability in cats (Alstermark et al., 1981; Alstermark et al., 1987). In summary, it is apparent that there are several spinal pathways that contribute to functional compensation after PT and DLF damage.

7.6 Conclusions

Results from this study show that pathways running in the spinal DLF provide compensatory input to spinal circuitry to maintain skilled reaching abilities after lesions of the PT. Nevertheless, these same pathways do not appear to compensate for PT damage during either overground locomotion or skilled locomotion. Thus, this compensatory response is task-specific. In absence of both PT and DLF pathways, ascending sensory fibers in dorsal funiculus, along with other spinal pathways, appear to contribute to the maintenance of residual sensorimotor abilities in rats. These results highlight the fact that behavioural context determines the nature of compensation from spared pathways after spinal cord injuries.

Chapter 8. GENERAL DISCUSSION AND CONCLUSIONS

The studies presented in the thesis provide important information about the contribution of different pathways which travel in the dorsal half of the spinal cord. Of particular importance are the roles of corticospinal system, rubrospinal system and ascending pathways in dorsal funiculus and dorsolateral funiculus. This information is useful in understanding the roles of pathways in different sensorimotor tasks and can be used by researchers studying motor behaviour and those looking at recovery of function after spinal cord injuries. The present section discusses findings from the studies covered in the thesis, pertaining to our understanding about the corticospinal, rubrospinal and ascending sensory fiber systems, followed by conclusions from the studies.

8.1 Corticospinal system

In the studies presented in the thesis, we damaged the corticospinal system at three levels- (1) at the level of caudal medulla which effectively removes all corticospinal input in the spinal cord (pyramidal tract transection; Chapter 7), (2) at cervical dorsal funiculus, which destroys the dorsal corticospinal tract (Chapter 4) and (3) at thoracic dorsal funiculus, which destroys the dorsal corticospinal tract (Chapter 5).

One finding that has consistently emerged from these studies is that corticospinal tract does not play an apparent role in hindlimb function. Lesions of corticospinal tract at caudal medulla and cervical spinal cord caused impairments of forelimb function only (Chapter 4, 5 and 7), while the damage at thoracic level did not cause any long lasting hindlimb impairments, suggestive of minor influence of CST on hindlimb

function. The probable minor functional role of the corticospinal system in controlling hindlimb function is also somatotopically reflected in the rat sensorimotor cortex, where forelimbs have a much larger representation compared to the hindlimbs (Gioanni and Lamarche, 1985), suggesting a functional bias of the involvement of the CST in forelimb compared to hindlimb movements.

Another surprising finding is the similar magnitude of changes after damage to corticospinal tract at the level of the caudal medulla and at cervical dorsal funiculus. My initial reasoning was that damage to pyramidal tract would be more severe than damage to CST in the spinal cord, as pyramidal tract damage removes the ventral and lateral branches of CST, which are spared after dorsal CST lesions. This is important because it has been shown that the spared ventral and lateral CST branches are capable of compensating after dorsal CST lesions (Weidner et al., 2001). Instead in our studies, comparison of impairments caused by PT damage to those caused by dorsal CST damage at cervical level, suggests that both lesions cause comparable qualitative and quantitative forelimb impairments (Chapter 4 and Chapter 7). In fact, damage to the dorsal CST at the cervical level causes more severe sensorimotor impairments initially compared to PT lesions. The additional damage to ascending sensory fibers in the dorsal columns along with dorsal CST is probably responsible for the severity of forelimb deficits seen after cervical lesions. This finding provides an indirect suggestion that sensory fibers in the dorsal columns are capable of compensating after corticospinal damage at least for forelimb function during skilled movements (Chapter 7).

Our results along with earlier reports provide a clear picture that corticospinal tract is not important during locomotion, yet is important, but not indispensable, during execution of sensorimotor tasks. So the question is, what role does CST play in rats?

Based on the original suggestion of Kuypers, many authors have opined that functions of the different descending pathways are better understood in terms of their spinal terminations (Lemon, 2005). In rodents, particularly in rats, the CST has the highest termination density in laminae III-VI of the dorsal horn (Brosamle and Schwab, 2000). The exact role of dense dorsal horn termination pattern of CST is still unknown, but since dorsal horn is primarily involved in sensory information processing (Brown AG, 1982) it is likely that the CST is involved in descending modulation of sensory afferent input, particularly those generated by limb movements (proprioceptive inputs) (Lemon, 2005). Thus, it is suggested that the CST might be involved in gating or filtering the afferent input generated during the movements of limbs, by exerting a pre-synaptic inhibitory influence on the primary sensory afferent fibers (Wall and Lidierth, 1997; Lemon, 2005). Though the CST mediates skilled forelimb movements, the unfiltered proprioceptive information arising from the forelimbs after CST damage, might be partly responsible for the alterations in the forelimb movements during the skilled forelimb usage (Chapters 4 and 7).

8.2 Rubrospinal system

The results of our studies also relate to the function and plasticity of the rubrospinal system. In rats, both corticospinal and rubrospinal system have collaborative roles in

execution of skilled forelimb movements (Whishaw et al., 1990; Whishaw and Gorny, 1996; Whishaw et al., 1998; Whishaw et al., 1992). Expectedly, combined lesions to corticospinal and rubrospinal systems cause severe impairments in forelimb movements (Whishaw et al., 1999; Chapter 7). Earlier studies have suggested that the rubrospinal system has the potential for compensation after damage to corticospinal system. After damage to pyramidal tract, the corticofugal fibers sprout to make more connections with the red nucleus (Z'Graggen et al., 1999). In the spinal cord, rubrospinal tract fibers sprout to send more collaterals to the spinal gray matter (Raineteau et al., 2002; Raineteau et al., 2001). Both of these processes might underlie behavioural compensation during skilled forelimb movements after the loss of corticospinal input. Our studies provide behavioural evidence for this phenomenon. In Chapter 7, rats with staggered PT and DLF lesions performed worse than those with simultaneous PT and DLF lesions, suggesting that intact RST is capable of compensating for loss of CST innervation in rats. These findings lend evidence to re-routing of cortical signals through rubrospinal pathways which might underlie the functional recovery in rats with corticospinal damage.

Alternatively, compensation might also be mediated by mechanisms other than collateral sprouting, such as through already existent, but newly “awakened” pathways in response to PT damage (Kennedy, 1990; Fanardjian et al., 2000a, 2000b). Based on the studies looking at beneficial effects of preconditioning lesions to RST before damaging the red nucleus, a theory was proposed that corticospinal or rubrospinal system can provide compensation after damage to one another (Kennedy, 1990; Fanardjian et al., 2000a, 2000b). According to this theory, after damage to the corticospinal system the process of

controlling movements is “switched to” rubrospinal system via rubro-olivary tract along with other brainstem structures such as the inferior olivary nucleus and the ventrolateral thalamic nucleus, thus mitigating the effects of corticospinal damage (Kennedy, 1990; Fanardjian et al., 2000a, 2000b, 2000c). The results from Chapter 7 provide indirect evidence for this concept, in that RST appears to provide compensation after the loss of corticospinal innervation. Importantly, this compensation is task-specific, evident only during skilled forelimb movements.

In rats, there are differences in the neuronal activities of the rubrospinal and corticospinal systems during execution of different tasks. While neurons in both systems fire similarly during walking, they fire at different phases during execution of skilled forelimb movements (Hermer-Vazquez et al., 2004). Specifically, during skilled reaching, rubrospinal neurons are active only during the phase that involves postural shifts (such as during arm alignment and advancing arm to reach for food pellet) (Hermer-Vazquez et al., 2004). It might be this difference in activity pattern of these two systems that perhaps facilitates compensation to be possible and apparent during skilled movements. i.e. rubrospinal system might compensate for the loss of axial and proximal musculature control (caused due to corticospinal damage), through its control of postural movements. In contrast, the compensation is not apparent during locomotion as both systems have similar firing patterns and also, importantly, because CST has no major role during locomotion (Hermer-Vazquez et al., 2004; Metz et al, 1998; Muir and Whishaw, 1999; Chapter 4, 5 and 7).

8.3 Ascending sensory pathways

One of the important contributions of the studies presented in this thesis concerns the locomotor role of ascending sensory pathways travelling in the dorsal and dorsolateral funiculus. Of special interest are the roles of dorsal column sensory fibers (fasciculus gracilis and fasciculus cuneatus), including the contribution of ascending sensory fibers to compensation after DLF lesions.

It has been proposed that rats rely less on visual cues and more on tactile and proprioceptive information during normal exploratory behaviour. Bearing this in mind, it has been proposed that, during locomotion, forelimbs are initially used to explore the terrain during a soft contact phase (time interval between the initial and maximum contact of the paws) to permit active tactile sampling by low threshold mechanoceptors (Chapin and Woodward, 1982; Clarke, 1995). This is thought to provide critical information that is required for continuing with the remainder of stance and for on-going locomotion (Clarke, 1995). Interestingly, in the somatotopic map representation of forepaw sensory receptors, those from digits 2 and 3 which come to contact the ground before other digits, have greater neuronal representation in the ventrobasal thalamus, a relay nucleus which is functionally significant in active tactile sampling, with subsequent direct projections to the somatosensory cortex (Angel and Clarke, 1975;). This suggests that tactile sensory information from these 2 digits, and generally from forelimbs, has a major role in shaping locomotor behaviour. In support of this, we found changes specific to the forelimb forces during overground locomotion (Chapter-4). This suggests that it is

the loss of sensory information arising from forelimbs, travelling in sensory fibers of dorsal columns that cause gait disturbances.

The sparing of hindlimb deficits is more related to functional differences in the information transmission from the hindlimbs. The sensory pathways carrying the hindlimb information leave the fasciculus gracilis (in dorsal funiculus) before they reach the upper lumbar cord and are transmitted to brain via fibers in the dorsolateral fasciculus, including the dorsal spinocerebellar tract (Clark, 1972). Hence even after damage to DF pathways, proprioceptive information from hindlimbs still gets transmitted to brain via the DLF pathways, thus no hindlimb deficits are observed after thoracic DF lesions (Chapter-5). In contrast, damage to DLF pathways, even if at cervical levels, results in hindlimb impairments (Chapter 6 and Chapter 7). The forelimb impairment after DLF lesions (Chapter 6 and 7) might be a result of collateral damage to pathways such as the spinocervical tract (which are damaged by C2-C3 lesions, such as those performed in our experiments) which carry some forelimb sensory information and provide alternative passage to forelimb information besides sensory fibers in the dorsal funiculus (Baker and Giesler, 1984).

Normally, damage to the dorsal column sensory pathways have moderate effects on forelimb movement. These impairments recover with time, possibly due to existence of alternative pathways for transmission of forelimb information such as the spinocervical and spinothalamic tracts, both of which travel in the lateral funiculus (Baker and Giesler, 1984). But after damage to dorsolateral funicular pathways, our results suggest that there

is an overreliance on dorsal column pathways in the maintenance of residual behavioural ability, at least in the forelimbs and especially in tasks requiring skilled movement of the limbs. In both the cases it might be damage to DLF sensory pathways in particular that could make the DF pathways contribute more than in normal animals.

After the lesions of the DLF, the ASP in the DF becomes the only source of proprioceptive information arising from the forelimbs, and could be used more than normal. Thus after secondary damage to these pathways, the deficits observed are more severe than the damage to ASP alone. This is similar to observations in monkeys, where sensory functions survive lesions of dorsal columns but are abolished by lesions involving dorsal quadrant (DF+DLF) (Eidelberg and Woodbury, 1972; Vavrek, 1974). Thus in Chapter 6, when a lesion of DF is added on top of DLF, a substantial and enduring sensory loss is produced which is more severe than either ASP or DLF lesions

In general, this thesis provides unique information pertaining to the sensorimotor abilities of rats with bilateral injuries to dorsal spinal pathways.

8.4 Conclusions

- 1) In Chapter 4, I hypothesized that bilateral lesions of the ascending sensory pathways in the dorsal funiculus would produce persistent changes during overground locomotion and that these changes would be independent of concurrent damage to corticospinal tract. This hypothesis was supported by my results, which demonstrated a role for ascending sensory information, but not

descending CST input, during overground locomotion and emphasized the normal contribution of both ascending sensory axons and CST axons during skilled limb movements.

- 2) In Chapter 5, I hypothesized that rats with bilateral lesions of the cervical dorsal funiculus would display locomotor deficits involving all four limbs, while the rats with bilateral lesions of the thoracic dorsal funiculus would display deficits involving only the hindlimbs. This hypothesis was not supported by my results, suggesting that the pathways present in the dorsal funiculus exert different functional effects on limb control at different levels of the spinal cord.
- 3) In Chapter 6, I hypothesized that rats with staggered lesions to the dorsolateral and dorsal spinal funiculi would show more severe sensorimotor deficits than the rats with simultaneous lesions to dorsolateral and dorsal spinal funiculi. This hypothesis was not supported by my results, suggesting that DF pathways in rats do not compensate for loss of DLF pathways during the execution of locomotor tasks, though there is indirect evidence that DLF-lesioned rats might rely more on ascending sensory pathways in the DF during skilled forelimb movements. The plastic changes mediating recovery are therefore necessarily occurring in other regions of the CNS, and, importantly, need time to develop,
- 4) In Chapter 7, I hypothesized that rats with dorsolateral funiculus lesions which were performed six weeks after pyramidal tract lesions would exhibit more

deficits on several behavioural tasks compared to animals which received pyramidal tract and dorsolateral funiculus lesions simultaneously. This hypothesis was partly supported by my data, which demonstrated that pathways running in the spinal DLF provide compensatory input to spinal circuitry to maintain skilled reaching abilities after lesions of the PT. Nevertheless, these same pathways do not appear to compensate for PT damage during either overground locomotion or skilled locomotion. Thus, this compensatory response is task-specific. In absence of both PT and DLF pathways, ascending sensory fibers in dorsal funiculus, along with other spinal pathways, appear contribute to the maintenance of residual sensorimotor abilities in rats. These results highlight the fact that behavioural context determines the nature of compensation from spared pathways after spinal cord injuries.

LIST OF PUBLICATIONS

Muir GD, Webb AA, **Kanagal S**, Taylor L. Dorsolateral cervical spinal injury differentially affects forelimb and hindlimb action in rats. *European Journal of Neuroscience* 25, 1501-1510, 2007.

Kanagal SG, Muir GD. Bilateral dorsal funicular lesions alter sensorimotor behaviour in rats. *Experimental Neurology* 205(2): 513-24, 2007.

Kanagal SG, Muir GD. Differential effects of thoracic and cervical dorsal funicular lesions in rats. *Behavioural Brain Research*, 187: 379-386, 2008.

Kanagal SG, Muir GD. Locomotor and sensorimotor abilities in rats after pyramidal tract and dorsolateral funicular lesions in rats . *Experimental Neurology* 2008 (*under final revision*).

Kanagal SG, Muir GD. Sensorimotor behavioural changes in rats after dorsal spinal cord injuries. (*Manuscript ready for submission*)

REFERENCE LIST

- Adkins, R. J., Cegnar, M. R., Rafuse, D. D. 1971. Differential effects of lesions of the anterior and posterior sigmoid gyri in cats. *Brain Res.* 30, 411-414.
- Adogwa, A. O., Lakshminarasimhan, A. 1982. The morphology and cytoarchitecture of the red nucleus of the one-humped camel (*Camelus dromedarius*). *J.Hirnforsch.* 23, 627-633.
- Aicher, S. A., Milner, T. A., Pickel, V. M., Reis, D. J. 2000. Anatomical substrates for baroreflex sympathoinhibition in the rat. *Brain Res.Bull.* 51, 107-110.
- Akintunde, A., Buxton, D. F. 1992. Origins and collateralization of corticospinal, corticopontine, corticorubral and corticostriatal tracts: a multiple retrograde fluorescent tracing study. *Brain Res.* 586, 208-218.
- Al-Chaer, E. D., Lawand, N. B., Westlund, K. N., Willis, W. D. 1996. Pelvic visceral input into the nucleus gracilis is largely mediated by the postsynaptic dorsal column pathway. *J.Neurophysiol.* 76, 2675-2690.
- Allen, A. 1911. Surgery of experimental lesion of spinal cord equivalent to crush injury of fracture dislocation of spinal column: A preliminary report. *JAMA* 37, 878-880.
- Alstermark, B., Lundberg, A., Norrsell, U., Sybirska, E. 1981. Integration in descending motor pathways controlling the forelimb in the cat. 9. Differential behavioural defects after spinal cord lesions interrupting defined pathways from higher centres to motoneurones. *Exp.Brain Res.* 42, 299-318.
- Alstermark, B., Lundberg, A., Pettersson, L. G., Tantisira, B., Walkowska, M. 1987. Motor recovery after serial spinal cord lesions of defined descending pathways in cats. *Neurosci.Res.* 5, 68-73. Ballermann, M., McKenna, J., Whishaw, I. Q. 2001. A grasp-related deficit in tactile discrimination following dorsal column lesion in the rat. *Brain Res.Bull.* 54, 237-242.
- Alstermark, B., Isa, T., Lundberg, A., Pettersson, L. G., Tantisira, B. 1989. The effect of low pyramidal lesions on forelimb movements in the cat. *Neurosci.Res.* 7, 71-75.
- Alstermark, B., Ogawa, J., Isa, T. 2004. Lack of monosynaptic corticomotoneuronal EPSPs in rats: disynaptic EPSPs mediated via reticulospinal neurons and polysynaptic EPSPs via segmental interneurons. *J.Neurophysiol.* 91, 1832-1839.
- Anderson, K. D., Gunawan, A., Steward, O. 2005. Quantitative assessment of forelimb motor function after cervical spinal cord injury in rats: relationship to the corticospinal tract. *Exp.Neurol.* 194, 161-174.

- Angaut-Petit, D. 1975. The dorsal column system: I. Existence of long ascending postsynaptic fibres in the cat's fasciculus gracilis. *Exp.Brain Res.* 22, 457-470.
- Angel, A., Clarke, K. A. 1975. An analysis of the representation of the forelimb in the ventrobasal thalamic complex of the albino rat. *J.Physiol* 249, 399-423.
- Antal, M. 1984. Termination areas of corticobulbar and corticospinal fibres in the rat. *J.Hirnforsch.* 25, 647-659.
- Antal, M., Sholomenko, G. N., Moschovakis, A. K., Storm-Mathisen, J., Heizmann, C. W., Hunziker, W. 1992. The termination pattern and postsynaptic targets of rubrospinal fibers in the rat spinal cord: a light and electron microscopic study. *J.Comp Neurol.* 325, 22-37.
- Armstrong, D. M. 1986. Supraspinal contributions to the initiation and control of locomotion in the cat. *Prog.Neurobiol.* 26, 273-361.
- Armstrong, D. M. 1988. The supraspinal control of mammalian locomotion. *J.Physiol* 405, 1-37.
- Armstrong, D. M., Marple-Horvat, D. E. 1996. Role of the cerebellum and motor cortex in the regulation of visually controlled locomotion. *Can.J.Physiol Pharmacol.* 74, 443-455.
- Babalian, A., Liang, F., Rouiller, E.M. 1993. Cortical influences on cervical motoneurons in the rat: recordings of synaptic responses from motoneurons and compound action potential from corticospinal axons. *Neurosci.Res.* 16, 301-310.
- Baker, M. L., Giesler, G. J., Jr. 1984. Anatomical studies of the spinocervical tract of the rat. *Somatosens.Res.* 2, 1-18.
- Ballermann, M. and Fouad, K. 2006. Spontaneous locomotor recovery in spinal cord injured rats is accompanied by anatomical plasticity of reticulospinal fibers. *Eur J Neurosci.* 23(8):1988-1996
- Ballermann, M., Tse, A. D., Misiaszek, J. E., and Fouad, K. 2006. Adaptations in the walking pattern of spinal cord injured rats. *J Neurotrauma* 23(6):897-907
- Ballermann, M., McKenna, J., Whishaw, I. Q. 2001. A grasp-related deficit in tactile discrimination following dorsal column lesion in the rat. *Brain Res.Bull.* 54, 237-242.
- Ballion, B., Morin, D., Viala, D. 2001. Forelimb locomotor generators and quadrupedal locomotion in the neonatal rat. *Eur.J.Neurosci.* 14, 1727-1738.

- Barbeau, H., Julien, C., Rossignol, S. 1987. The effects of clonidine and yohimbine on locomotion and cutaneous reflexes in the adult chronic spinal cat. *Brain Res.* 437, 83-96.
- Barbeau, H., Rossignol, S. 1991. Initiation and modulation of the locomotor pattern in the adult chronic spinal cat by noradrenergic, serotonergic and dopaminergic drugs. *Brain Res.* 546, 250-260.
- Bareyre, F. M., Kerschensteiner, M., Raineteau, O., Mettenleiter, T. C., Weinmann, O., Schwab, M. E. 2004. The injured spinal cord spontaneously forms a new intraspinal circuit in adult rats. *Nat.Neurosci.* 7, 269-277.
- Basso, D. M., Beattie, M. S., Bresnahan, J. C. 1995. A sensitive and reliable locomotor rating scale for open field testing in rats. *J.Neurotrauma* 12, 1-21.
- Basso, D. M., Beattie, M. S., Bresnahan, J. C. 1996. Graded histological and locomotor outcomes after spinal cord contusion using the NYU weight-drop device versus transection. *Exp.Neurol.* 139, 244-256.
- Basso, D. M., Beattie, M. S., Bresnahan, J. C. 2002. Descending systems contributing to locomotor recovery after mild or moderate spinal cord injury in rats: experimental evidence and a review of literature. *Restor.Neurol.Neurosci.* 20, 189-218.
- Beloozerova, I. N., Sirota, M. G. 1993. The role of the motor cortex in the control of accuracy of locomotor movements in the cat. *J.Physiol* 461, 1-25.
- Bing, Z., Villanueva, L., Le, B. D. 1990. Ascending pathways in the spinal cord involved in the activation of subnucleus reticularis dorsalis neurons in the medulla of the rat. *J.Neurophysiol.* 63, 424-438.
- Blakemore, W. F., Patterson, R. C. 1978. Suppression of remyelination in the CNS by X-irradiation. *Acta Neuropathol.* 42, 105-113.
- Bloedel, J. R., Courville, J. 1981. Cerebellar afferent systems. In American Physiological Society, B. (Ed.), *The Nervous System sect 1 Vol II: Motor Control*, pp. 735-829.
- Bolton, D. A., Tse, A. D., Ballermann, M., Misiaszek, J. E., Fouad, K. 2006. Task specific adaptations in rat locomotion: runway versus horizontal ladder. *Behav.Brain Res.* 168, 272-279.
- Bosco, G., Poppele, R. E. 2001. Proprioception from a spinocerebellar perspective. *Physiol Rev.* 81, 539-568.
- Boseila, A. W., Hashem, S. M., Badawy, Y. H. 1975. Volumetric studies on the red nucleus of the rat at different ages. *Acta Anat.(Basel)* 91, 175-180.

- Bouyer, L. J., Rossignol, S. 1998. The contribution of cutaneous inputs to locomotion in the intact and the spinal cat. *Ann.N.Y.Acad.Sci.* 860, 508-512.
- Brooks, V. B. 1979. Motor programs revisited. Posture and movement Raven Press, New York, NY, pp. 13-49.
- Brosamle, C., Schwab, M. E. 1997. Cells of origin, course, and termination patterns of the ventral, uncrossed component of the mature rat corticospinal tract. *J.Comp Neurol.* 386, 293-303.
- Brown, L. T., Jr. 1971. Projections and termination of the corticospinal tract in rodents. *Exp.Brain Res.* 13, 432-450.
- Brown, L. T. 1974. Rubrospinal projections in the rat. *J.Comp Neurol.* 154, 169-187.
- Brown, A. G. 1982. The dorsal horn of the spinal cord. *Q.J.Exp.Physiol* 67, 193-212.
- Brown, T. G., Sherrington, C. S. 1912. The rule of reflex response in the limb reflexes of the mammal and its exceptions. *J.Physiol* 44, 125-130.
- Brus-Ramer, M., Carmel, J. B., Chakrabarty, S., Martin, J. H. 2007. Electrical stimulation of spared corticospinal axons augments connections with ipsilateral spinal motor circuits after injury. *J.Neurosci.* 27, 13793-13801.
- Bunge, R. P., Puckett, W. R., Becerra, J. L., Marcillo, A., Quencer, R. M. 1993. Observations on the pathology of human spinal cord injury. A review and classification of 22 new cases with details from a case of chronic cord compression with extensive focal demyelination. *Adv.Neurol.* 59, 75-89.
- Burstein, R., Dado, R. J., Cliffer, K. D., Giesler, G. J., Jr. 1991. Physiological characterization of spinohypothalamic tract neurons in the lumbar enlargement of rats. *J.Neurophysiol.* 66, 261-284.
- Burstein, R., Falkowsky, O., Borsook, D., Strassman, A. 1996. Distinct lateral and medial projections of the spinohypothalamic tract of the rat. *J.Comp Neurol.* 373, 549-574.
- Cameron, T., Prado, R., Watson, B. D., Gonzalez-Carvajal, M., Holets, V. R. 1990. Photochemically induced cystic lesion in the rat spinal cord. I. Behavioral and morphological analysis. *Exp.Neurol.* 109, 214-223.
- Casale, E. J., Light, A. R., Rustioni, A. 1988. Direct projection of the corticospinal tract to the superficial laminae of the spinal cord in the rat. *J.Comp Neurol.* 278, 275-286.
- Catsman-Berrevoets, C. E., Kuypers, H. G. 1981. A search for corticospinal collaterals to thalamus and mesencephalon by means of multiple retrograde fluorescent tracers

- in cat and rat. *Brain Res.* 218, 15-33.
- Cha, J., Heng, C., Reinkensmeyer, D. J., Roy, R. R., Edgerton, V. R., De Leon, R. D. 2007. Locomotor ability in spinal rats is dependent on the amount of activity imposed on the hindlimbs during treadmill training. *J.Neurotrauma* 24, 1000-1012.
- Chan, C. C., Khodarahmi, K., Liu, J., Sutherland, D., Oschipok, L. W., Steeves, J. D., Tetzlaff, W. 2005. Dose-dependent beneficial and detrimental effects of ROCK inhibitor Y27632 on axonal sprouting and functional recovery after rat spinal cord injury. *Exp.Neurol.* 196, 352-364.
- Chaouch, A., Menetrey, D., Binder, D., Besson, J. M. 1983. Neurons at the origin of the medial component of the bulbopontine spinoreticular tract in the rat: an anatomical study using horseradish peroxidase retrograde transport. *J.Comp Neurol.* 214, 309-320.
- Chapin, J. K., Woodward, D. J. 1982. Somatic sensory transmission to the cortex during movement: phasic modulation over the locomotor step cycle. *Exp.Neurol.* 78, 670-684.
- Cheema, S. S., Rustioni, A., Whitsel, B. L. 1984. Light and electron microscopic evidence for a direct corticospinal projection to superficial laminae of the dorsal horn in cats and monkeys. *J.Comp Neurol.* 225, 276-290.
- Chen, X. Y., Wolpaw, J. R. 2002. Probable corticospinal tract control of spinal cord plasticity in the rat. *J.Neurophysiol.* 87, 645-652.
- Chen, X. Y., Wolpaw, J. R., Jakeman, L. B., and Stokes, B. T. 1999. Operant conditioning of H-reflex increase in spinal cord-injured rats. *J Neurotrauma* 16(2):175-186
- Chung, K., Coggeshall, R. E. 1983. Propriospinal fibers in the rat. *J.Comp Neurol.* 217, 47-53.
- Chung, K., Langford, L. A., Coggeshall, R. E. 1987. Primary afferent and propriospinal fibers in the rat dorsal and dorsolateral funiculi. *J.Comp Neurol.* 263, 68-75.
- Clark, F. J. 1972. Central projection of sensory fibers from the cat knee joint. *J.Neurobiol.* 3, 101-110.
- Clark, F. M., Proudfoot, H. K. 1992. Anatomical evidence for genetic differences in the innervation of the rat spinal cord by noradrenergic locus coeruleus neurons. *Brain Res.* 591, 44-53.
- Clarke, K. A. 1995. Differential fore- and hindpaw force transmission in the walking rat. *Physiol Behav.* 58, 415-419.

- Clayton, H. M. 1996. Instrumentation and techniques in locomotion and lameness. *Vet.Clin.North Am.Equine Pract.* 12, 337-350.
- Cliffer, K. D., Burstein, R., Giesler, G. J., Jr. 1991. Distributions of spinothalamic, spinohypothalamic, and spinotelencephalic fibers revealed by anterograde transport of PHA-L in rats. *J.Neurosci.* 11, 852-868.
- Collazos-Castro JE, Lopez-Dolado E, and Nieto-Sampedro M. 2006. Locomotor deficits and adaptive mechanisms after thoracic spinal cord contusion in the adult rat. *J Neurotrauma* 23(1):1-17
- Commissiong, J. W. 1981. Evidence that the noradrenergic coeruleospinal projection decussates at the spinal level. *Brain Res.* 212, 145-151.
- Coumans, J. V., Lin, T. T., Dai, H. N., MacArthur, L., McAtee, M., Nash, C., Bregman, B. S. 2001. Axonal regeneration and functional recovery after complete spinal cord transection in rats by delayed treatment with transplants and neurotrophins. *J.Neurosci.* 21, 9334-9344.
- Courtine, G., Song, B., Roy, R. R., Zhong, H., Herrmann, J. E., Ao, Y., Qi, J., Edgerton, V. R., Sofroniew, M. V. 2008. Recovery of supraspinal control of stepping via indirect propriospinal relay connections after spinal cord injury. *Nat.Med.* 14, 69-74.
- Davidoff, R. A. 1989. The dorsal columns. *Neurology* 39, 1377-1385.
- Diener, P. S., Bregman, B. S. 1998. Fetal spinal cord transplants support the development of target reaching and coordinated postural adjustments after neonatal cervical spinal cord injury. *J.Neurosci.* 18, 763-778.
- Donatelle, J. M. 1977. Growth of the corticospinal tract and the development of placing reactions in the postnatal rat. *J.Comp Neurol.* 175, 207-231.
- Donoghue, J. P., Wise, S. P. 1982. The motor cortex of the rat: cytoarchitecture and microstimulation mapping. *J.Comp Neurol.* 212, 76-88.
- Drew, T. 1988. Motor cortical cell discharge during voluntary gait modification. *Brain Res.* 457, 181-187.
- Drew, T., Jiang, W., Kably, B., Lavoie, S. 1996. Role of the motor cortex in the control of visually triggered gait modifications. *Can.J.Physiol Pharmacol.* 74, 426-442.
- Edgerton, V. R., Tillakaratne, N. J., Bigbee, A. J., De Leon, R. D., Roy, R. R. 2004. Plasticity of the spinal neural circuitry after injury. *Annu.Rev.Neurosci.* 27, 145-167.

- Eidelberg, E., Woodbury, C. M. 1972. Apparent redundancy in the somatosensory system in monkeys. *Exp.Neurol.* 37, 573-581.
- Eidelberg, E., Yu, J. 1981. Effects of corticospinal lesions upon treadmill locomotion by cats. *Exp.Brain Res.* 43, 101-103.
- English, A. W. 1985. Interlimb coordination during stepping in the cat: the role of the dorsal spinocerebellar tract. *Exp.Neurol.* 87, 96-108.
- Fanardjian, V. V., Papoyan, E. V., Pogossian, V. I., Gevorkyan, O. V. 1999. Comparison of the effects of electrolytic and chemical destruction of the red nucleus on the compensatory capacity of rats with rubrospinal tract lesions. *Neural Plast.* 6, 123-131.
- Fanardjian, V. V., Gevorkyan, O. V., Mallina, R. K., Melik-Moussian, A. B., Meliksetyan, I. B. 2000. Enhanced behavioral recovery from sensorimotor cortex lesions after pyramidotomy in adult rats. *Neural Plast.* 7, 261-277.
- Fanardjian, V. V., Gevorkyan, O. V., Mallina, R. K., Melik-Musyan, A. B., Meliksetyan, I. B. 2000. Increased corticofugal plasticity after pyramidotomy in adult rats. *Dokl.Biol.Sci.* 375, 556-560.
- Fanardjian, V. V., Papoyan, E. V., Hovhannisyan, E. A., Melik-Moussian, A. B., Gevorkyan, O. V., Pogossian, V. I. 2000. The role of some brain structures in the switching of the descending influences in operantly conditioned rats. *Neuroscience* 98, 385-395.
- Farooque, M. 2000. Spinal cord compression injury in the mouse: presentation of a model including assessment of motor dysfunction. *Acta Neuropathol.* 100, 13-22.
- Finger, S., Walbran, B., Stein, D. G. 1973. Brain damage and behavioral recovery: serial lesion phenomena. *Brain Res.* 63, 1-18.
- Flumerfelt, B. A. 1978. Organization of the mammalian red nucleus and its interconnections with the cerebellum. *Experientia* 34, 1178-1179.
- Follis, F., Scremenin, O. U., Blisard, K. S., Scremenin, A. M., Pett, S. B., Scott, W. J., Kessler, R. M., Wernly, J. A. 1993. Selective vulnerability of white matter during spinal cord ischemia. *J.Cereb.Blood Flow Metab* 13, 170-178.
- Forssberg, H., Grillner, S., Halbertsma, J., Rossignol, S. 1980. The locomotion of the low spinal cat. II. Interlimb coordination. *Acta Physiol Scand.* 108, 283-295.
- Fox, J. E. 1970. Reticulospinal neurones in the rat. *Brain Res.* 23, 35-40.

- Gale, K., Kerasidis, H., Wrathall, J. R. 1985. Spinal cord contusion in the rat: behavioral analysis of functional neurologic impairment. *Exp.Neurol.* 88, 123-134.
- Gassel, M. M., Marchiafava, P. L., Pompeiano, O. 1965. Activity of the red nucleus during deep desynchronized sleep in unrestrained cats. *Arch.Ital.Biol.* 103, 369-396.
- Gemma, M., Perego, G. B., Pizzini, G., Tredici, G. 1987. Distribution of the cortico-spinal fibres in the cervical and lumbar enlargements of the rat spinal cord. *J.Hirnforsch.* 28, 457-462.
- Gensel, J. C., Tovar, C. A., Hamers, F. P., Deibert, R. J., Beattie, M. S., Bresnahan, J. C. 2006. Behavioral and histological characterization of unilateral cervical spinal cord contusion injury in rats. *J.Neurotrauma* 23, 36-54.
- Giesler, G. J., Jr., Spiel, H. R., Willis, W. D. 1981. Organization of spinothalamic tract axons within the rat spinal cord. *J.Comp Neurol.* 195, 243-252.
- Giesler, G. J., Jr., Nahin, R. L., Madsen, A. M. 1984. Postsynaptic dorsal column pathway of the rat. I. Anatomical studies. *J.Neurophysiol.* 51, 260-275.
- Giesler, G. J., Jr., Katter, J. T., Dado, R. J. 1994. Direct spinal pathways to the limbic system for nociceptive information. *Trends Neurosci.* 17, 244-250.
- Gioanni, Y., Lamarche, M. 1985. A reappraisal of rat motor cortex organization by intracortical microstimulation. *Brain Res.* 344, 49-61.
- Girgis, J., Merrett, D., Kirkland, S., Metz, G. A., Verge, V., Fouad, K. 2007. Reaching training in rats with spinal cord injury promotes plasticity and task specific recovery. *Brain* 130, 2993-3003.
- Goldberger, M. E., Bregman, B. S., Vierck, C. J., Jr., Brown, M. 1990. Criteria for assessing recovery of function after spinal cord injury: behavioral methods. *Exp.Neurol.* 107, 113-117.
- Gorska, T., Sybirska, E. 1980. Effects of pyramidal lesions on forelimb movements in the cat. *Acta Neurobiol.Exp.(Wars.)* 40, 843-859.
- Gorska, T., Majczynski, H., Bem, T., Zmyslowski, W. 1993. Hindlimb swing, stance and step relationships during unrestrained walking in cats with lateral funicular lesion. *Acta Neurobiol.Exp.(Wars.)* 53, 133-142.
- Graca, D. L., Blakemore, W. F. 1986. Delayed remyelination in rat spinal cord following ethidium bromide injection. *Neuropathol.Appl.Neurobiol.* 12, 593-605.
- Granum, S. L. 1986. The spinothalamic system of the rat. I. Locations of cells of origin. *J.Comp Neurol.* 247, 159-180.

- Gribnau, A. A., Dederen, P. J. 1989. Collateralization of the cervical corticospinal tract in the rat. *Neurosci.Lett.* 105, 47-51.
- Grillner S. 1981. Control of locomotion in bipeds, tetrapods and fish. In VB Brooks (Ed.), *Handbook of Physiology American Physiological Society*, Bethesda, MD, pp. 1179-1236.
- Grillner, S. 1985. Neurobiological bases of rhythmic motor acts in vertebrates. *Science* 228, 143-149.
- Gruner, J. A. 1992. A monitored contusion model of spinal cord injury in the rat. *J.Neurotrauma* 9, 123-126.
- Gulino, R., Dimartino, M., Casabona, A., Lombardo, S. A., Perciavalle, V. 2007. Synaptic plasticity modulates the spontaneous recovery of locomotion after spinal cord hemisection. *Neurosci.Res.* 57, 148-156.
- Hall, E. D. 2001. Pharmacological treatment of acute spinal cord injury: how do we build on past success? *J.Spinl Cord.Med.* 24, 142-146.
- Hao, J. X., Xu, X. J., Aldskogius, H., Seiger, A., Wiesenfeld-Hallin, Z. 1991. Allodynia-like effects in rat after ischaemic spinal cord injury photochemically induced by laser irradiation. *Pain* 45, 175-185.
- Hendriks, W. T., Eggers, R., Ruitenberg, M. J., Blits, B., Hamers, F. P., Verhaagen, J., Boe, G. J. 2006. Profound differences in spontaneous long-term functional recovery after defined spinal tract lesions in the rat. *J.Neurotrauma* 23, 18-35.
- Hermer-Vazquez, L., Hermer-Vazquez, R., Moxon, K. A., Kuo, K. H., Viau, V., Zhan, Y., Chapin, J. K. 2004. Distinct temporal activity patterns in the rat M1 and red nucleus during skilled versus unskilled limb movement. *Behav.Brain Res.* 150, 93-107.
- Hicks, S. P., D'Amato, C. J. 1975. Motor-sensory cortex-corticospinal system and developing locomotion and placing in rats. *Am.J.Anat.* 143, 1-42.
- Hiebert, G. W., Gorassini, M. A., Jiang, W., Prochazka, A., Pearson, K. G. 1994. Corrective responses to loss of ground support during walking. II. Comparison of intact and chronic spinal cats. *J.Neurophysiol.* 71, 611-622.
- Hongo, T., Jankowska, E., Lundberg, A. 1969. The rubrospinal tract. I. Effects on alpha-motoneurones innervating hindlimb muscles in cats. *Exp.Brain Res.* 7, 344-364.
- Houle, J. D., Jin, Y. 2001. Chronically injured supraspinal neurons exhibit only modest axonal dieback in response to a cervical hemisection lesion. *Exp.Neurol.* 169, 208-217.

- Huisman, A. M., Kuypers, H. G., Verburgh, C. A. 1981. Quantitative differences in collateralization of the descending spinal pathways from red nucleus and other brain stem cell groups in rat as demonstrated with the multiple fluorescent retrograde tracer technique. *Brain Res.* 209, 271-286.
- Hultborn, H., Conway, B. A., Gossard, J. P., Brownstone, R., Fedirchuk, B., Schomburg, E. D., Enriquez-Denton, M., Perreault, M. C. 1998. How do we approach the locomotor network in the mammalian spinal cord? *Ann.N.Y.Acad.Sci.* 860, 70-82.
- Ivancic, T. L., Pellis, S. M., Whishaw, I. Q. 1996. Skilled forelimb movements in prey catching and in reaching by rats (*Rattus norvegicus*) and opossums (*Monodelphis domestica*): relations to anatomical differences in motor systems. *Behav.Brain Res.* 79, 163-181.
- Jankowska, E., Lundberg, A. 1981. Interneurones in the spinal cord. *Trends in Neurosciences* 4, 230-233.
- Jeffery ND and Blakemore WF. Spinal cord injury in small animals 2. Current and future options for therapy. *Vet Rec* 1999; 145(7):183-190
- Jerath, I. 1964. The red nucleus in the mice, monkey and man. *Indian J.Physiol Pharmacol.* 8, 143-148.
- Jiang, W., Drew, T. 1996. Effects of bilateral lesions of the dorsolateral funiculi and dorsal columns at the level of the low thoracic spinal cord on the control of locomotion in the adult cat. I. Treadmill walking. *J.Neurophysiol.* 76, 849-866.
- Jones, S. L. 1991. Descending noradrenergic influences on pain. *Prog.Brain Res.* 88, 381-394.
- Jordan, L. M. 1998. Initiation of locomotion in mammals. *Ann.N.Y.Acad.Sci.* 860, 83-93.
- Kalaska, J. F., Drew, T. 1993. Motor cortex and visuomotor behavior. *Exerc.Sport Sci.Rev.* 21, 397-436.
- Kanagal, S. G., Muir, G. D. 2007. Bilateral dorsal funicular lesions alter sensorimotor behaviour in rats. *Exp.Neurol.* 205, 513-524.
- Kanagal, S. G., Muir, G. D. 2008. The differential effects of cervical and thoracic dorsal funiculus lesions in rats. *Behav.Brain Res.* 187, 379-386.
- Kanagal, S. G., Muir, G. D. 2008. Effects of combined dorsolateral and dorsal funicular lesions on sensorimotor behaviour in rats. (*Exp.Neurol-Accepted -under final review*)

- Kato, H., Kanellopoulos, G. K., Matsuo, S., Wu, Y. J., Jacquin, M. F., Hsu, C. Y., Kouchoukos, N. T., Choi, D. W. 1997. Neuronal apoptosis and necrosis following spinal cord ischemia in the rat. *Exp.Neurol.* 148, 464-474.
- Kato, M. 1990. Chronically isolated lumbar half spinal cord generates locomotor activities in the ipsilateral hindlimb of the cat. *Neurosci.Res.* 9, 22-34.
- Katter, J. T., Dado, R. J., Kostarczyk, E., Giesler, G. J., Jr. 1996a. Spinothalamic and spinohypothalamic tract neurons in the sacral spinal cord of rats. I. Locations of antidromically identified axons in the cervical cord and diencephalon. *J.Neurophysiol.* 75, 2581-2605.
- Katter, J. T., Dado, R. J., Kostarczyk, E., Giesler, G. J., Jr. 1996b. Spinothalamic and spinohypothalamic tract neurons in the sacral spinal cord of rats. II. Responses to cutaneous and visceral stimuli. *J.Neurophysiol.* 75, 2606-2628.
- Kemplay, S. K., Webster, K. E. 1986. A qualitative and quantitative analysis of the distributions of cells in the spinal cord and spinomedullary junction projecting to the thalamus of the rat. *Neuroscience* 17, 769-789.
- Kennedy, P. R., Gibson, A. R., Houk, J. C. 1986. Functional and anatomic differentiation between parvicellular and magnocellular regions of red nucleus in the monkey. *Brain Res.* 364, 124-136.
- Kennedy, P.R., Humphrey, D.R. 1987. The compensatory role of the parvocellular division of the red nucleus in operantly conditioned rats. *Neurosci. Res.* 5, 39-62.
- Kennedy, P. R. 1990. Corticospinal, rubrospinal and rubro-olivary projections: a unifying hypothesis. *Trends Neurosci.* 13, 474-479.
- Kerasidis, H., Wrathall, J. R., Gale, K. 1987. Behavioral assessment of functional deficit in rats with contusive spinal cord injury. *J.Neurosci.Methods* 20, 167-179.
- Kesslak JP and Keirstead HS. 2003. Assessment of behavior in animal models of spinal cord injury. *J Spinal Cord Med.* 26(4):323-328
- Keyvan-Fouladi, N., Raisman, G., Li, Y. 2003. Functional repair of the corticospinal tract by delayed transplantation of olfactory ensheathing cells in adult rats. *J.Neurosci.* 23, 9428-9434.
- Khan, M., Griebel, R. 1983. Acute spinal cord injury in the rat: comparison of three experimental techniques. *Can.J.Neurol.Sci.* 10, 161-165.
- Khumsap, S., Clayton, H. M., Lanovaz, J. L. 2001. Effect of walking velocity on hindlimb kinetics during stance in normal horses. *Equine Vet.J.Suppl* 21-26.

- Khumsap, S., Clayton, H. M., Lanovaz, J. L., Bouchey, M. 2002. Effect of walking velocity on forelimb kinematics and kinetics. Equine Vet.J.Suppl 325-329.
- Kjaerulff, O., Kiehn, O. 1996. Distribution of networks generating and coordinating locomotor activity in the neonatal rat spinal cord in vitro: a lesion study. J.Neurosci. 16, 5777-5794.
- Klapka, N., Hermanns, S., Straten, G., Masanneck, C., Duis, S., Hamers, F. P., Muller, D., Zuschratter, W., Muller, H. W. 2005. Suppression of fibrous scarring in spinal cord injury of rat promotes long-distance regeneration of corticospinal tract axons, rescue of primary motoneurons in somatosensory cortex and significant functional recovery. Eur.J.Neurosci. 22, 3047-3058.
- Kostarczyk, E., Zhang, X., Giesler, G. J., Jr. 1997. Spinohypothalamic tract neurons in the cervical enlargement of rats: locations of antidromically identified ascending axons and their collateral branches in the contralateral brain. J.Neurophysiol. 77, 435-451.
- Kremer, E., Lev-Tov, A. 1997. Localization of the spinal network associated with generation of hindlimb locomotion in the neonatal rat and organization of its transverse coupling system. J.Neurophysiol. 77, 1155-1170.
- Kuang, R. Z., Kalil, K. 1990. Branching patterns of corticospinal axon arbors in the rodent. J.Comp Neurol. 292, 585-598.
- Kuchler, M., Fouad, K., Weinmann, O., Schwab, M. E., Raineteau, O. 2002. Red nucleus projections to distinct motor neuron pools in the rat spinal cord. J.Comp Neurol. 448, 349-359.
- Kunkel-Bagden, E., Dai, H. N., Bregman, B. S. 1993. Methods to assess the development and recovery of locomotor function after spinal cord injury in rats. Exp.Neurol. 119, 153-164.
- Kwiat, G. C., Basbaum, A. I. 1992. The origin of brainstem noradrenergic and serotonergic projections to the spinal cord dorsal horn in the rat. Somatosens.Mot.Res. 9, 157-173.
- Kwon, B. K., Oxland, T. R., Tetzlaff, W. 2002. Animal models used in spinal cord regeneration research. Spine 27, 1504-1510.
- Kwon, B. K., Liu, J., Lam, C., Plunet, W., Oschipok, L. W., Hauswirth, W., Di, P. A., Blesch, A., Tetzlaff, W. 2007. Brain-derived neurotrophic factor gene transfer with adeno-associated viral and lentiviral vectors prevents rubrospinal neuronal atrophy and stimulates regeneration-associated gene expression after acute cervical spinal cord injury. Spine 32, 1164-1173.

- Lavoie, S., Drew, T. 2002. Discharge characteristics of neurons in the red nucleus during voluntary gait modifications: a comparison with the motor cortex. *J.Neurophysiol.* 88, 1791-1814.
- Lawrence, D. G., Kuypers, H. G. 1968a. The functional organization of the motor system in the monkey. I. The effects of bilateral pyramidal lesions. *Brain* 91, 1-14.
- Lawrence, D. G., Kuypers, H. G. 1968b. The functional organization of the motor system in the monkey. II. The effects of lesions of the descending brain-stem pathways. *Brain* 91, 15-36.
- Lemon, R. N., Griffiths, J. 2005. Comparing the function of the corticospinal system in different species: organizational differences for motor specialization? *Muscle Nerve* 32, 261-279.
- Lemon, R. N. 2008. Descending pathways in motor control. *Annu.Rev.Neurosci.* 31, 195-218.
- Leong, S. K., Shieh, J. Y., Wong, W. C. 1984. Localizing spinal-cord-projecting neurons in neonatal and immature albino rats. *J.Comp Neurol.* 228, 18-23.
- Li, X. G., Florence, S. L., Kaas, J. H. 1990. Areal distributions of cortical neurons projecting to different levels of the caudal brain stem and spinal cord in rats. *Somatosens.Mot.Res.* 7, 315-335.
- Li, Y., Field, P. M., Raisman, G. 1997. Repair of adult rat corticospinal tract by transplants of olfactory ensheathing cells. *Science* 277, 2000-2002.
- Liang, F.Y., Moret, V., Wiesendanger, M., Rouiller, E.M. 1991. Corticomotoneuronal connections in the rat: evidence from double-labeling of motoneurons and corticospinal axon arborizations. *J.Comp.Neurol.* 15, 356-366.
- Little, J. W., Harris, R. M., Sohlberg, R. C. 1988. Locomotor recovery following subtotal spinal cord lesions in a rat model. *Neurosci.Lett.* 87, 189-194.
- Loy, D. N., Magnuson, D. S., Zhang, Y. P., Onifer, S. M., Mills, M. D., Cao, Q. L., Darnall, J. B., Fajardo, L. C., Burke, D. A., Whittemore, S. R. 2002. Functional redundancy of ventral spinal locomotor pathways. *J.Neurosci.* 22, 315-323.
- Loy, D. N., Talbott, J. F., Onifer, S. M., Mills, M. D., Burke, D. A., Dennison, J. B., Fajardo, L. C., Magnuson, D. S., Whittemore, S. R. 2002. Both dorsal and ventral spinal cord pathways contribute to overground locomotion in the adult rat. *Exp.Neurool.* 177, 575-580.
- Magnuson, D. S., Green, D. M., Sengoku, T. 1998. Lumbar spinoreticular neurons in the rat: part of the central pattern generator for locomotion? *Ann.N.Y.Acad.Sci.* 860,

436-440.

- Maier, M. A., Illert, M., Kirkwood, P. A., Nielsen, J., Lemon, R. N. 1998. Does a C3-C4 propriospinal system transmit corticospinal excitation in the primate? An investigation in the macaque monkey. *J.Physiol* 511 (Pt 1), 191-212.
- Maier IC and Schwab ME. 2006. Sprouting, regeneration and circuit formation in the injured spinal cord: factors and activity. *Philos Trans R Soc Lond B Biol Sci.* 361(1473):1611-1634
- Marple-Horvat, D. E., Armstrong, D. M. 1999. Central regulation of motor cortex neuronal responses to forelimb nerve inputs during precision walking in the cat. *J.Physiol* 519 Pt 1, 279-299.
- Martin, G. F., Dom, R. 1970. The rubro-spinal tract of the opossum (*Didelphis virginiana*). *J.Comp Neurol.* 138, 19-30.
- Martin, G. F., Vertes, R. P., Waltzer, R. 1985. Spinal projections of the gigantocellular reticular formation in the rat. Evidence for projections from different areas to laminae I and II and lamina IX. *Exp.Brain Res.* 58, 154-162.
- Martin, J. H., Ghez, C. 1988. Red nucleus and motor cortex: parallel motor systems for the initiation and control of skilled movement. *Behav.Brain Res.* 28, 217-223.
- Massion, J. 1988. Red nucleus: past and future. *Behav.Brain Res.* 28, 1-8.
- Masson, R. L., Jr., Sparkes, M. L., Ritz, L. A. 1991. Descending projections to the rat sacrocaudal spinal cord. *J.Comp Neurol.* 307, 120-130.
- Matesz, C., Baeskai, T., Nagy, E., Halasi, G., Kulik, A. 2002. Efferent connections of the vestibular nuclei in the rat: a neuromorphological study using PHA-L. *Brain Res.Bull.* 57, 313-315.
- Matsushita M. Projections from the upper lumbar cord to the cerebellar nuclei in the rat, studied by anterograde axonal tracing. *J Comp Neurol* 1999; 412(4):633-648
- Matsushita, M. 1998. Ascending propriospinal afferents to area X (substantia grisea centralis) of the spinal cord in the rat. *Exp.Brain Res.* 119, 356-366.
- Matsuyama, K., Drew, T. 2000a. Vestibulospinal and reticulospinal neuronal activity during locomotion in the intact cat. I. Walking on a level surface. *J.Neurophysiol.* 84, 2237-2256.
- Matsuyama, K., Drew, T. 2000b. Vestibulospinal and reticulospinal neuronal activity during locomotion in the intact cat. II. Walking on an inclined plane. *J.Neurophysiol.* 84, 2257-2276.

- Matsuyama, K., Mori, F., Nakajima, K., Drew, T., Aoki, M., Mori, S. 2004. Locomotor role of the corticoreticular-reticulospinal-spinal interneuronal system. *Prog.Brain Res.* 143, 239-249.
- McCrea, D. A. 2001. Spinal circuitry of sensorimotor control of locomotion. *J.Physiol* 533, 41-50.
- McEwen ML and Springer JE. Quantification of locomotor recovery following spinal cord contusion in adult rats. *J Neurotrauma* 2006; 23(11):1632-1653
- McKenna, J. E., Whishaw, I. Q. 1999. Complete compensation in skilled reaching success with associated impairments in limb synergies, after dorsal column lesion in the rat. *J.Neurosci.* 19, 1885-1894.
- Menetrey, D., Chaouch, A., Binder, D., Besson, J. M. 1982. The origin of the spinomesencephalic tract in the rat: an anatomical study using the retrograde transport of horseradish peroxidase. *J.Comp Neurol.* 206, 193-207.
- Menetrey, D., de, P. J., Roudier, F. 1985. Propriospinal fibers reaching the lumbar enlargement in the rat. *Neurosci.Lett.* 58, 257-261.
- Metz, G. A., Dietz, V., Schwab, M. E., van de, M. H. 1998. The effects of unilateral pyramidal tract section on hindlimb motor performance in the rat. *Behav.Brain Res.* 96, 37-46.
- Metz, G. A., Merkler, D., Dietz, V., Schwab, M. E., Fouad, K. 2000. Efficient testing of motor function in spinal cord injured rats. *Brain Res.* 883, 165-177.
- Metz, G. A., Whishaw, I. Q. 2002. Cortical and subcortical lesions impair skilled walking in the ladder rung walking test: a new task to evaluate fore- and hindlimb stepping, placing, and co-ordination. *J.Neurosci.Methods* 115, 169-179.
- Miller, M. W. 1987. The origin of corticospinal projection neurons in rat. *Exp.Brain Res.* 67, 339-351.
- Muir GD, Katz SL, Gosline JM, and Steeves JD. 1998. Asymmetric bipedal locomotion-an adaptive response to incomplete spinal injury in the chick. *Exp Brain Res.* 122(3):275-282
- Muir, G. D., Whishaw, I. Q. 1999a. Complete locomotor recovery following corticospinal tract lesions: measurement of ground reaction forces during overground locomotion in rats. *Behav.Brain Res.* 103, 45-53.
- Muir, G. D., Whishaw, I. Q. 1999b. Ground reaction forces in locomoting hemi-parkinsonian rats: a definitive test for impairments and compensations. *Exp.Brain Res.* 126, 307-314.

- Muir, G. D., Webb, A. A. 2000. Mini-review: assessment of behavioural recovery following spinal cord injury in rats. *Eur.J.Neurosci.* 12, 3079-3086.
- Muir, G. D., Whishaw, I. Q. 2000. Red nucleus lesions impair overground locomotion in rats: a kinetic analysis. *Eur.J.Neurosci.* 12, 1113-1122.
- Muir, G. D., Webb, A. A., Kanagal, S., Taylor, L. 2007. Dorsolateral cervical spinal injury differentially affects forelimb and hindlimb action in rats. *Eur.J.Neurosci.* 25, 1501-1510.
- Nathan, P. W., Smith, M. C. 1982. The rubrospinal and central tegmental tracts in man. *Brain* 105, 223-269.
- Nathan, P. W., Smith, M., Deacon, P. 1996. Vestibulospinal, reticulospinal and descending propriospinal nerve fibres in man. *Brain* 119 (Pt 6), 1809-1833.
- Neafsey, E. J., Bold, E. L., Haas, G., Hurley-Gius, K. M., Quirk, G., Sievert, C. F., Terreberry, R. R. 1986. The organization of the rat motor cortex: a microstimulation mapping study. *Brain Res.* 396, 77-96.
- Newman, D. B. 1985a. Distinguishing rat brainstem reticulospinal nuclei by their neuronal morphology. I. Medullary nuclei. *J.Hirnforsch.* 26, 187-226.
- Newman, D. B. 1985b. Distinguishing rat brainstem reticulospinal nuclei by their neuronal morphology. II. Pontine and mesencephalic nuclei. *J.Hirnforsch.* 26, 385-418.
- Newman, D. B., Liu, R. P. 1987. Nuclear origins of brainstem reticulocortical systems in the rat. *Am.J.Anat.* 178, 279-299.
- Noga, B. R., Kriellaars, D. J., Jordan, L. M. 1991. The effect of selective brainstem or spinal cord lesions on treadmill locomotion evoked by stimulation of the mesencephalic or pontomedullary locomotor regions. *J.Neurosci.* 11, 1691-1700.
- Norrie, B. A., Nevett-Duchcherer, J. M., Gorassini, M. A. 2005. Reduced functional recovery by delaying motor training after spinal cord injury. *J.Neurophysiol.* 94, 255-264.
- Noyes, D. H. 1987. Electromechanical impactor for producing experimental spinal cord injury in animals. *Med.Biol.Eng Comput.* 25, 335-340.
- Nudo, R. J., Masterton, R. B. 1988. Descending pathways to the spinal cord: a comparative study of 22 mammals. *J.Comp Neurol.* 277, 53-79.
- Oka, H., Jinnai, K. 1978. Electrophysiological study of parvocellular red nucleus neurons. *Brain Res.* 149, 239-246.

- Oka, Y., Satou, M., Ueda, K. 1986. Descending pathways to the spinal cord in the hime salmon (landlocked red salmon, *Oncorhynchus nerka*). *J.Comp Neurol.* 254, 91-103.
- Onifer, S. M., Zhang, Y. P., Burke, D. A., Brooks, D. L., Decker, J. A., McClure, N. J., Floyd, A. R., Hall, J., Proffitt, B. L., Shields, C. B., Magnuson, D. S. 2005. Adult rat forelimb dysfunction after dorsal cervical spinal cord injury. *Exp.Neurol.* 192, 25-38.
- Onifer, S. M., Rabchevsky, A. G., Scheff, S. W. 2007. Rat models of traumatic spinal cord injury to assess motor recovery. *ILAR.J.* 48, 385-395.
- Orlovsky, G. N. 1972. Activity of rubrospinal neurons during locomotion. *Brain Res.* 46, 99-112.
- Pearson, K. G. 1995. Proprioceptive regulation of locomotion. *Curr.Opin.Neurobiol.* 5, 786-791.
- Pearson, K. G. 2000. Plasticity of neuronal networks in the spinal cord: modifications in response to altered sensory input. *Prog Brain Res.* 128: 61-70
- Pearson, K. G. 2000. Neural adaptation in the generation of rhythmic behavior. *Annu.Rev.Physiol* 62, 723-753.
- Peterson, G. M. 1932. Mechanism of handedness in the rat. (9), 21-43. Comparative Psychology Monographs.
- Peterson, B. W., Maunz, R. A., Fukushima, K. 1978. Properties of a new vestibulospinal projection, the caudal vestibulospinal tract. *Exp.Brain Res.* 32, 287-292.
- Pettersson, L. G., Lundberg, A., Alstermark, B., Isa, T., Tantisira, B. 1997. Effect of spinal cord lesions on forelimb target-reaching and on visually guided switching of target-reaching in the cat. *Neurosci.Res.* 29, 241-256.
- Pettersson, L. G., Blagovechtchenski, E., Perfiliev, S., Krasnochokova, E., Lundberg, A. 2000. Recovery of food-taking in cats after lesions of the corticospinal (complete) and rubrospinal (complete and incomplete) tracts. *Neurosci.Res.* 38, 109-112.
- Pettersson, L. G., Alstermark, B., Blagovechtchenski, E., Isa, T., Sasaski, S. 2007. Skilled digit movements in feline and primate--recovery after selective spinal cord lesions. *Acta Physiol (Oxf)* 189, 141-154.
- Piantino, J., Burdick, J. A., Goldberg, D., Langer, R., Benowitz, L. I. 2006. An injectable, biodegradable hydrogel for trophic factor delivery enhances axonal rewiring and improves performance after spinal cord injury. *Exp.Neurol.* 201(2), 359-367.

- Piecharka, D. M., Kleim, J. A., Whishaw, I. Q. 2005. Limits on recovery in the corticospinal tract of the rat: partial lesions impair skilled reaching and the topographic representation of the forelimb in motor cortex. *Brain Res.Bull.* 66, 203-211.
- Pisa, M. 1988. Motor functions of the striatum in the rat: critical role of the lateral region in tongue and forelimb reaching. *Neuroscience* 24, 453-463.
- Pisa, M., Cyr, J. 1990. Regionally selective roles of the rat's striatum in modality-specific discrimination learning and forelimb reaching. *Behav.Brain Res.* 37, 281-292.
- Popovich, P. G., Guan, Z., McGaughy, V., Fisher, L., Hickey, W. F., Basso, D. M. 2002. The neuropathological and behavioral consequences of intraspinal microglial/macrophage activation. *J.Neuropathol.Exp.Neurol.* 61, 623-633.
- Poppele, R. E., Rankin, A., Eian, J. 2003. Dorsal spinocerebellar tract neurons respond to contralateral limb stepping. *Exp.Brain Res.* 149, 361-370.
- Porter, R., Lemon, R. N. 1993. Corticospinal function and voluntary movement Oxford; Clarendon Press; Oxford University Press, Oxford, New York.
- Poulton, N. P., Muir, G. D. 2005. Treadmill training ameliorates dopamine loss but not behavioral deficits in hemi-parkinsonian rats. *Exp.Neurol.* 193, 181-197.
- Prasada Rao, P. D., Jadhao, A. G., Sharma, S. C. 1987. Descending projection neurons to the spinal cord of the goldfish, *Carassius auratus*. *J.Comp Neurol.* 265, 96-108.
- Raineteau, O., Schwab, M. E. 2001. Plasticity of motor systems after incomplete spinal cord injury. *Nat.Rev.Neurosci.* 2, 263-273.
- Raineteau, O., Fouad, K., Bareyre, F. M., Schwab, M. E. 2002. Reorganization of descending motor tracts in the rat spinal cord. *Eur.J.Neurosci.* 16, 1761-1771.
- Ramon-Cueto, A., Cordero, M. I., Santos-Benito, F. F., Avila, J. 2000. Functional recovery of paraplegic rats and motor axon regeneration in their spinal cords by olfactory ensheathing glia. *Neuron* 25, 425-435.
- Rexed, B. 1952. The cytoarchitectonic organization of the spinal cord in the cat. *J.Comp Neurol.* 96, 414-495.
- Rivlin, A. S., Tator, C. H. 1977. Objective clinical assessment of motor function after experimental spinal cord injury in the rat. *J.Neurosurg.* 47, 577-581.
- Robbins, A., Pfaff, D. W., Schwartz-Giblin, S. 1992. Reticulospinal and reticuloreticular pathways for activating the lumbar back muscles in the rat. *Exp.Brain Res.* 92, 46-58.

- Rossignol, S., Chau, C., Brustein, E., Giroux, N., Bouyer, L., Barbeau, H., Reader, T. A. 1998. Pharmacological activation and modulation of the central pattern generator for locomotion in the cat. *Ann.N.Y.Acad.Sci.* 860, 346-359.
- Saling, M., Sitarova, T., Vejsada, R., Hnik, P. 1992. Reaching behavior in the rat: absence of forelimb peripheral input. *Physiol Behav.* 51, 1151-1156.
- Sanchez-Camacho, C., Marin, O., Smeets, W. J., Ten Donkelaar, H. J., Gonzalez, A. 2001. Descending supraspinal pathways in amphibians. II. Distribution and origin of the catecholaminergic innervation of the spinal cord. *J.Comp Neurol.* 434, 209-232.
- Sasaki, S. 1999. Direct connection of the nucleus reticularis gigantocellularis neurons with neck motoneurons in cats. *Exp.Brain Res.* 128, 527-530.
- Satoh, K. 1979. The origin of reticulospinal fibers in the rat: a HRP study. *J.Hirnforsch.* 20, 313-322.
- Schnell, L., Schneider, R., Kolbeck, R., Barde, Y. A., Schwab, M. E. 1994. Neurotrophin-3 enhances sprouting of corticospinal tract during development and after adult spinal cord lesion. *Nature* 367, 170-173.
- Schrimsher, G. W., Reier, P. J. 1993. Forelimb motor performance following dorsal column, dorsolateral funiculi, or ventrolateral funiculi lesions of the cervical spinal cord in the rat. *Exp.Neurol.* 120, 264-276.
- Schucht, P., Raineteau, O., Schwab, M. E., Fouad, K. 2002. Anatomical correlates of locomotor recovery following dorsal and ventral lesions of the rat spinal cord. *Exp.Neurol.* 176, 143-153.
- Shapovalov, A. I., Gurevitch, N. R. 1970. Monosynaptic and disynaptic reticulospinal actions on lumbar motoneurons of the rat. *Brain Res.* 21, 249-263.
- Shieh, J. Y., Leong, S. K., Wong, W. C. 1983. Origin of the rubrospinal tract in neonatal, developing, and mature rats. *J.Comp Neurol.* 214, 79-86.
- Shik, M. L., Severin, F. V., Orlovsky, G. N. 1969. Control of walking and running by means of electrical stimulation of the mesencephalon. *Electroencephalogr.Clin.Neurophysiol.* 26, 549.
- Shik, M. L., Orlovsky, G. N. 1976. Neurophysiology of locomotor automatism. *Physiol Rev.* 56, 465-501.
- Sinnamon, H. M. 1993. Preoptic and hypothalamic neurons and the initiation of locomotion in the anesthetized rat. *Prog.Neurobiol.* 41, 323-344.

- Sluka, K. A., Westlund, K. N. 1992. Spinal projections of the locus coeruleus and the nucleus subcoeruleus in the Harlan and the Sasco Sprague-Dawley rat. *Brain Res.* 579, 67-73.
- Snyder, R. L., Faull, R. L., Mehler, W. R. 1978. A comparative study of the neurons of origin of the spinocerebellar afferents in the rat, cat and squirrel monkey based on the retrograde transport of horseradish peroxidase. *J.Comp Neurol.* 181, 833-852.
- Soblosky, J. S., Colgin, L. L., Chorney-Lane, D., Davidson, J. F., Carey, M. E. 1997. Ladder beam and camera video recording system for evaluating forelimb and hindlimb deficits after sensorimotor cortex injury in rats. *J.Neurosci.Methods* 78, 75-83.
- Soblosky, J. S., Song, J. H., Dinh, D. H. 2001. Graded unilateral cervical spinal cord injury in the rat: evaluation of forelimb recovery and histological effects. *Behav.Brain Res.* 119, 1-13.
- Steeves JD and Jordan LM. Localization of a descending pathway in the spinal cord which is necessary for controlled treadmill locomotion. *Neurosci Lett* 1980; 20(3):283-288
- Steward, O., Zheng, B., Ho, C., Anderson, K., Tessier-Lavigne, M. 2004. The dorsolateral corticospinal tract in mice: an alternative route for corticospinal input to caudal segments following dorsal column lesions. *J.Comp Neurol.* 472, 463-477.
- Stokke, M. F., Nissen, U. V., Glover, J. C., Kiehn, O. 2002. Projection patterns of commissural interneurons in the lumbar spinal cord of the neonatal rat. *J.Comp Neurol.* 446, 349-359.
- Sybiriska, E., Gorska, T. 1980. Effects of red nucleus lesions on forelimb movements in the cat. *Acta Neurobiol.Exp.(Wars.)* 40, 821-841.
- Taira, Y., Marsala, M. 1996. Effect of proximal arterial perfusion pressure on function, spinal cord blood flow, and histopathologic changes after increasing intervals of aortic occlusion in the rat. *Stroke* 27, 1850-1858.
- Ten Donkelaar, H. J., Kusuma, A., de Boer-Van, H. R. 1980. Cells of origin of pathways descending to the spinal cord in some quadrupedal reptiles. *J.Comp Neurol.* 192, 827-851.
- Ten Donkelaar, H. J. 1988. Evolution of the red nucleus and rubrospinal tract. *Behav.Brain Res.* 28, 9-20.
- Terashima, T. 1995. Anatomy, development and lesion-induced plasticity of rodent corticospinal tract. *Neurosci.Res.* 22, 139-161.
- Terman, J. R., Wang, X. M., Martin, G. F. 1998. Origin, course, and laterality of spinocerebellar axons in the North American opossum, *Didelphis virginiana*.

Anat.Rec. 251, 528-547.

- Tracey, D. J., Walmsley, B., Forsythe, I. D. 1988. Spinocerebellar neurones in the guinea pig- a morphological study. Brain Res. 453, 129-135.
- Tracey, D. 2004. Ascending and descending pathways in the spinal cord. In George Paxinos (Ed.), The rat nervous system Elsevier Academic Press, Amsterdam; Boston, pp. 149-164.
- Valverde, F. 1966. The pyramidal tract in rodents. A study of its relations with the posterior column nuclei, dorsolateral reticular formation of the medulla oblongata, and cervical spinal cord. (Golgi and electron microscopic observations). Z.Zellforsch.Mikrosk.Anat. 71, 298-363.
- Van Meeteren, N. L., Eggers, R., Lankhorst, A. J., Gispen, W. H., Hamers, F. P. 2003. Locomotor recovery after spinal cord contusion injury in rats is improved by spontaneous exercise. J.Neurotrauma 20, 1029-1037.
- VandenBerg, P.M., Hogg, T.M., Kleim, J.A., Whishaw, I.Q. 2002. Long-Evans rats have a larger cortical topographic representation of movement than Fisher-344 rats: A microstimulation study of motor cortex in naïve and skilled reaching-trained rats. Brain Res.Bull. 59, 197-203.
- Vanicky, I., Urdzikova, L., Saganova, K., Cizkova, D., Galik, J. 2001. A simple and reproducible model of spinal cord injury induced by epidural balloon inflation in the rat. J.Neurotrauma 18, 1399-1407.
- Vavrek, R., Giris, J., Tetzlaff, W., Hiebert, G. W., Fouad, K. 2006. BDNF promotes connections of corticospinal neurons onto spared descending interneurons in spinal cord injured rats. Brain 129, 1534-1545.
- Verhaart, W. J. 1962. The pyramidal tract. Its structure and functions in man and animals. World Neurol. 3, 43-53.
- Viala, D., Vidal, C. 1978. Evidence for distinct spinal locomotion generators supplying respectively fore- and hindlimbs in the rabbit. Brain Res. 155, 182-186.
- Vierck, C. J., Jr. 1974. Tactile movement detection and discrimination following dorsal column lesions in monkeys. Exp.Brain Res. 20, 331-346.
- Villanueva, L., Bouhassira, D., Le, B. D. 1996. The medullary subnucleus reticularis dorsalis (SRD) as a key link in both the transmission and modulation of pain signals. Pain 67, 231-240.
- Waldron, H. A., Gwyn, D. G. 1969. Descending nerve tracts in the spinal cord of the rat. I. Fibers from the midbrain. J.Comp Neurol. 137, 143-153.

- Wall, P. D., Lidierth, M. 1997. Five sources of a dorsal root potential: their interactions and origins in the superficial dorsal horn. *J.Neurophysiol.* 78, 860-871.
- Wang, C. C., Willis, W. D., Westlund, K. N. 1999. Ascending projections from the area around the spinal cord central canal: A Phaseolus vulgaris leucoagglutinin study in rats. *J.Comp Neurol.* 415, 341-367.
- Watson, B. D., Prado, R., Dietrich, W. D., Ginsberg, M. D., Green, B. A. 1986. Photochemically induced spinal cord injury in the rat. *Brain Res.* 367, 296-300.
- Webb, A. A., Muir, G. D. 2002. Compensatory locomotor adjustments of rats with cervical or thoracic spinal cord hemisections. *J.Neurotrauma* 19, 239-256.
- Webb, A. A. 2003. Sensorimotor adjustments after unilateral spinal cord injury in adult rats. PhD Thesis, University of Saskatchewan.
- Webb, A. A., Muir, G. D. 2003a. Unilateral dorsal column and rubrospinal tract injuries affect overground locomotion in the unrestrained rat. *Eur.J.Neurosci.* 18, 412-422.
- Webb, A. A., Gowribai, K., Muir, G. D. 2003b. Fischer (F-344) rats have different morphology, sensorimotor and locomotor abilities compared to Lewis, Long-Evans, Sprague-Dawley and Wistar rats. *Behav.Brain Res.* 144, 143-156.
- Webb, A. A., Muir, G. D. 2004. Course of motor recovery following ventrolateral spinal cord injury in the rat. *Behav.Brain Res.* 155, 55-65.
- Webb, A. A., Muir, G. D. 2005. Sensorimotor behaviour following incomplete cervical spinal cord injury in the rat. *Behav.Brain Res.* 165, 147-159.
- Webster, D. M., Steeves, J. D. 1988. Origins of brainstem-spinal projections in the duck and goose. *J.Comp Neurol.* 273, 573-583.
- Webster, D. M., Rogers, L. J., Pettigrew, J. D., Steeves, J. D. 1990. Origins of descending spinal pathways in prehensile birds: do parrots have a homologue to the corticospinal tract of mammals? *Brain Behav.Evol.* 36, 216-226.
- Weidner, N., Ner, A., Salimi, N., Tuszynski, M. H. 2001. Spontaneous corticospinal axonal plasticity and functional recovery after adult central nervous system injury. *Proc.Natl.Acad.Sci.U.S.A* 98, 3513-3518.
- West, W. L., Yeomans, D. C., Proudfit, H. K. 1993. The function of noradrenergic neurons in mediating antinociception induced by electrical stimulation of the locus coeruleus in two different sources of Sprague-Dawley rats. *Brain Res.* 626, 127-135.

- Whishaw, I. Q., O'Connor, W. T., Dunnett, S. B. 1986. The contributions of motor cortex, nigrostriatal dopamine and caudate-putamen to skilled forelimb use in the rat. *Brain* 109 (5), 805-843.
- Whishaw, I. Q., Tomie, J. A. 1989. Olfaction directs skilled forelimb reaching in the rat. *Behav.Brain Res.* 32, 11-21.
- Whishaw, I. Q., Tomie, J. A., Ladowsky, R. L. 1990. Red nucleus lesions do not affect limb preference or use, but exacerbate the effects of motor cortex lesions on grasping in the rat. *Behav.Brain Res.* 40, 131-144.
- Whishaw, I. Q., Pellis, S. M., Gorny, B. P., Pellis, V. C. 1991. The impairments in reaching and the movements of compensation in rats with motor cortex lesions: an endpoint, videorecording, and movement notation analysis. *Behav.Brain Res.* 42, 77-91.
- Whishaw, I. Q., Dringenberg, H. C., Pellis, S. M. 1992. Spontaneous forelimb grasping in free feeding by rats: motor cortex aids limb and digit positioning. *Behav.Brain Res.* 48, 113-125.
- Whishaw, I. Q., Pellis, S. M., Gorny, B. P. 1992. Skilled reaching in rats and humans: evidence for parallel development or homology. *Behav.Brain Res.* 47, 59-70.
- Whishaw, I. Q., Pellis, S. M., Pellis, V. C. 1992. A behavioral study of the contributions of cells and fibers of passage in the red nucleus of the rat to postural righting, skilled movements, and learning. *Behav.Brain Res.* 52, 29-44.
- Whishaw, I. Q., Pellis, S. M., Gorny, B., Kolb, B., Tetzlaff, W. 1993. Proximal and distal impairments in rat forelimb use in reaching follow unilateral pyramidal tract lesions. *Behav.Brain Res.* 56, 59-76.
- Whishaw, I. Q., Gorny, B. 1996. Does the red nucleus provide the tonic support against which fractionated movements occur? A study on forepaw movements used in skilled reaching by the rat. *Behav.Brain Res.* 74, 79-90.
- Whishaw, I. Q., Gorny, B., Sarna, J. 1998. Paw and limb use in skilled and spontaneous reaching after pyramidal tract, red nucleus and combined lesions in the rat: behavioral and anatomical dissociations. *Behav.Brain Res.* 93, 167-183.
- Whishaw, I. Q., Metz, G. A. 2002. Absence of impairments or recovery mediated by the uncrossed pyramidal tract in the rat versus enduring deficits produced by the crossed pyramidal tract. *Behav.Brain Res.* 134, 323-336.
- Whishaw, I. Q. 2003. Did a change in sensory control of skilled movements stimulate the evolution of the primate frontal cortex? *Behav.Brain Res.* 146, 31-41.

- Whishaw, I.Q., Gorny, B., Foroud, A., Kleim, J.A. 2003. Long-Evans and Sprague-Dawley rats have similar skilled reaching success and limb representations in motor cortex but different movements: some cautionary insights into the selection of rat strains for neurobiological motor research. *Behav.Brain Res.* 145, 221-232.
- Willis, W. D., Al-Chaer, E. D., Quast, M. J., Westlund, K. N. 1999. A visceral pain pathway in the dorsal column of the spinal cord. *Proc.Natl.Acad.Sci.U.S.A* 96, 7675-7679.
- Willis, W. D., Coggeshall, R. E. 2004. in *Sensory mechanisms of the spinal cord*. Kluwer Academic/Plenum, New York.
- Willis, W. D., Westlund, K. N. 1997. Neuroanatomy of the pain system and of the pathways that modulate pain. *J.Clin.Neurophysiol.* 14, 2-31.
- Wolpaw JR and Carp JS. 2006. Plasticity from muscle to brain. *Prog Neurobiol.* 78(3-5):233-263
- Wolpaw, J. R. 1997. The complex structure of a simple memory. *Trends Neurosci.* 20, 588-594.
- Wolpaw, J. R., Tennissen, A. M. 2001. Activity-dependent spinal cord plasticity in health and disease. *Annu.Rev.Neurosci.* 24, 807-843.
- Xu, Q., Grant, G. 1994. Course of spinocerebellar axons in the ventral and lateral funiculi of the spinal cord with projections to the anterior lobe: an experimental anatomical study in the cat with retrograde tracing techniques. *J.Comp Neurol.* 345, 288-302.
- Yajima, K., Suzuki, K. 1979. Demyelination and remyelination in the rat central nervous system following ethidium bromide injection. *Lab Invest* 41, 385-392.
- Yamada, J., Shirao, K., Kitamura, T., Sato, H. 1991. Trajectory of spinocerebellar fibers passing through the inferior and superior cerebellar peduncles in the rat spinal cord: a study using horseradish peroxidase with pedunculotomy. *J.Comp Neurol.* 304, 147-160.
- Yang, H. W., Lemon, R. N. 2003. An electron microscopic examination of the corticospinal projection to the cervical spinal cord in the rat: lack of evidence for cortico-motoneuronal synapses. *Exp.Brain Res.* 149, 458-469.
- Yeomans, J. S., Li, L., Scott, B. W., Frankland, P. W. 2002. Tactile, acoustic and vestibular systems sum to elicit the startle reflex. *Neurosci.Biobehav.Rev.* 26, 1-11.
- Yezierski, R. P. 1988. Spinomesencephalic tract: projections from the lumbosacral spinal cord of the rat, cat, and monkey. *J.Comp Neurol.* 267, 131-146.

- Yezierski, R. P., Mendez, C. M. 1991. Spinal distribution and collateral projections of rat spinomesencephalic tract cells. *Neuroscience* 44, 113-130.
- Ying, Z., Roy, R. R., Edgerton, V. R., Gomez-Pinilla, F. 2005. Exercise restores levels of neurotrophins and synaptic plasticity following spinal cord injury. *Exp.Neurol.* 193, 411-419.
- You, S. W., Chen, B. Y., Liu, H. L., Lang, B., Xia, J. L., Jiao, X. Y., Ju, G. 2003. Spontaneous recovery of locomotion induced by remaining fibers after spinal cord transection in adult rats. *Restor.Neurol.Neurosci.* 21, 39-45.
- Zemlan, F. P., Kow, L. M., Pfaff, D. W. 1983. Effect of interruption of bulbospinal pathways on lordosis, posture, and locomotion. *Exp.Neurol.* 81, 177-194.
- Zemlan, F. P., Leonard, C. M., Kow, L. M., Pfaff, D. W. 1978. Ascending tracts of the lateral columns of the rat spinal cord: a study using the silver impregnation and horseradish peroxidase techniques. *Exp.Neurol.* 62, 298-334.
- Z'Graggen, W. J., Fouad, K., Raineteau, O., Metz, G. A., Schwab, M. E., Kartje, G. L. 2000. Compensatory sprouting and impulse rerouting after unilateral pyramidal tract lesion in neonatal rats. *J.Neurosci.* 20, 6561-6569.
- Zhang, X., Gokin, A. P., Giesler, G. J., Jr. 2002. Responses of spinohypothalamic tract neurons in the thoracic spinal cord of rats to somatic stimuli and to graded distention of the bile duct. *Somatosens.Mot.Res.* 19, 5-17.

APPENDIX

Statistical analyses results

Chapter 4

I) Skilled reaching- Single pellet reaching (Section 4.4.2.1)

Statistical test used: Two-way repeated measures ANOVA

Post-hoc analyses: Bonferroni t-test

Level of significance: $p < 0.05$

Data variable: % successful pellet retrievals

Source of Variation	DF	SS	MS	F	P
Lesion	1	15376.385	15376.385	38.852	<0.001
Rats(Lesion)	11	4353.423	395.766		
Time	3	4204.796	1401.599	10.051	<0.001
Lesion x Time	3	79.796	26.599	0.191	0.902
Residual	33	4601.935	139.453		
Total	51	28592.308	560.633		

There was a statistically significant difference in Time ($P = <0.001$).

There was a statistically significant difference between Lesions ($P = <0.001$).

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Time within ASP**

Comparison	Diff of Means	t	P	P<0.05
w8 vs. w2	22.857	3.798	0.004	Yes
w8 vs. w4	15.000	2.492	0.165	No
w8 vs. w6	8.571	1.424	1.000	Do Not Test
w8 vs. Presurg	2.143	0.356	1.000	Do Not Test
Presurg vs. w2	20.714	3.442	0.013	Yes
Presurg vs. w4	12.857	2.136	0.382	Do Not Test
Presurg vs. w6	6.429	1.068	1.000	Do Not Test
w6 vs. w2	14.286	2.374	0.220	No
w6 vs. w4	6.429	1.068	1.000	Do Not Test
w4 vs. w2	7.857	1.306	1.000	Do Not Test

Comparisons for factor: **Time within ASP+CST**

Comparison	Diff of Means	t	P	P<0.05
Presurg vs. w2	58.333	8.974	<0.001	Yes
Presurg vs. w4	53.333	8.205	<0.001	Yes
Presurg vs. w6	40.000	6.154	<0.001	Yes
Presurg vs. w8	34.167	5.256	<0.001	Yes
w8 vs. w2	24.167	3.718	0.006	Yes
w8 vs. w4	19.167	2.949	0.051	No
w8 vs. w6	5.833	0.897	1.000	Do Not Test
w6 vs. w2	18.333	2.820	0.072	No
w6 vs. w4	13.333	2.051	0.462	Do Not Test
w4 vs. w2	5.000	0.769	1.000	Do Not Test

Comparisons for factor: **Lesion within Presurg**

Comparison	Diff of Means	t	P	P<0.05
ASP+CST vs. ASP	2.500	0.348	0.730	No
Comparisons for factor: Lesion within w2				
Comparison	Diff of Means	t	P	P<0.05
ASP vs. ASP+CST	35.119	4.883	<0.001	Yes
Comparisons for factor: Lesion within w4				
Comparison	Diff of Means	t	P	P<0.05
ASP vs. ASP+CST	37.976	5.280	<0.001	Yes
Comparisons for factor: Lesion within w6				
Comparison	Diff of Means	t	P	P<0.05
ASP vs. ASP+CST	31.071	4.320	<0.001	Yes
Comparisons for factor: Lesion within w8				
Comparison	Diff of Means	t	P	P<0.05
ASP vs. ASP+CST	33.810	4.701	<0.001	Yes

II) Skilled locomotion- Ladder walking (Section 4.4.2.2)

Statistical test used: Two-way repeated measures ANOVA

Post-hoc analyses: Bonferroni t-test

Level of significance: $p < 0.05$

Data variable: % forelimb correct steps

Source of Variation	DF	SS	MS	F	P
Lesion	1	18555.199	18555.199	172.788	<0.001
Rats(Lesion)	11	1181.261	107.387		
Time	3	15780.186	5260.062	140.439	<0.001
Lesion x Time	3	6106.645	2035.548	54.347	<0.001
Residual	33	1235.994	37.454		
Total	51	41484.657	813.425		

The effect of different levels of Lesion depends on what level of Time is present. There was a statistically significant interaction between Lesion and Time. ($P = <0.001$)

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: Lesion				
Comparison	Diff of Means	t	P	P<0.050
ASP vs. ASP+CST	29.676	11.777	<0.001	Yes

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
Presurg vs. w2	56.238	24.210	<0.001	Yes
Presurg vs. w4	35.072	15.098	<0.001	Yes
Presurg vs. w6	16.198	6.973	<0.001	Yes
Presurg vs. w8	12.210	5.256	<0.001	Yes
w8 vs. w2	44.028	18.953	<0.001	Yes
w8 vs. w4	22.862	9.842	<0.001	Yes
w8 vs. w6	3.988	1.717	0.930	No
w6 vs. w2	40.039	17.237	<0.001	Yes
w6 vs. w4	18.873	8.125	<0.001	Yes

w4 vs. w2	21.166	9.112	<0.001	Yes
-----------	--------	-------	--------	-----

Comparisons for factor: **Time within ASP**

Comparison	Diff of Means	t	P	P<0.05
Presurg vs. w2	18.609	5.896	<0.001	Yes
Presurg vs. w4	13.379	4.239	0.001	Yes
Presurg vs. w8	3.320	1.052	1.000	No
Presurg vs. w6	2.250	0.713	1.000	Do Not Test
w6 vs. w2	16.359	5.183	<0.001	Yes
w6 vs. w4	11.129	3.526	0.010	Yes
w6 vs. w8	1.070	0.339	1.000	Do Not Test
w8 vs. w2	15.289	4.844	<0.001	Yes
w8 vs. w4	10.059	3.187	0.026	Yes
w4 vs. w2	5.230	1.657	1.000	No

Comparisons for factor: **Time within ASP+CST**

Comparison	Diff of Means	t	P	P<0.05
Presurg vs. w2	93.867	27.534	<0.001	Yes
Presurg vs. w4	56.765	16.651	<0.001	Yes
Presurg vs. w6	30.147	8.843	<0.001	Yes
Presurg vs. w8	21.100	6.189	<0.001	Yes
w8 vs. w2	72.767	21.345	<0.001	Yes
w8 vs. w4	35.665	10.462	<0.001	Yes
w8 vs. w6	9.047	2.654	0.110	No
w6 vs. w2	63.720	18.691	<0.001	Yes
w6 vs. w4	26.618	7.808	<0.001	Yes
w4 vs. w2	37.102	10.883	<0.001	Yes

Comparisons for factor: **Lesion within Presurg**

Comparison	Diff of Means	t	P	P<0.05
ASP+CST vs. ASP	3.188	0.824	0.415	No

Comparisons for factor: **Lesion within w2**

Comparison	Diff of Means	t	P	P<0.05
ASP vs. ASP+CST	72.070	18.619	<0.001	Yes

Comparisons for factor: **Lesion within w4**

Comparison	Diff of Means	t	P	P<0.05
ASP vs. ASP+CST	40.198	10.385	<0.001	Yes

Comparisons for factor: **Lesion within w6**

Comparison	Diff of Means	t	P	P<0.05
ASP vs. ASP+CST	24.709	6.383	<0.001	Yes

Comparisons for factor: **Lesion within w8**

Comparison	Diff of Means	t	P	P<0.05
ASP vs. ASP+CST	14.592	3.770	<0.001	Yes

III) Ground reaction forces (Section 4.4.2.3.1)

Statistical test used: Two-way repeated measures ANOVA

Post-hoc analyses: Bonferroni t-test

Level of significance: $p < 0.05$

Data variable: Right forelimb peak vertical forces

Source of Variation	DF	SS	MS	F	P
Lesion	1	0.0242	0.0242	0.274	0.611
Rats(Lesion)	11	0.974	0.0885		
Time	4	0.0955	0.0239	3.344	0.018
Lesion x Time	4	0.0312	0.00781	1.093	0.372
Residual	44	0.314	0.00714		
Total	64	1.433	0.0224		

There was a statistically significant difference in time ($P = 0.018$).

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Lesion**

Comparison	Diff of Means	t	P	P<0.050
ASP vs. ASP+CST	0.111	1.984	0.073	No

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
Presurg vs. w2	0.138	3.796	0.004	Yes
Presurg vs. w4	0.127	3.500	0.011	Yes
Presurg vs. w6	0.125	3.440	0.013	Yes
Presurg vs. w8	0.0930	2.556	0.014	Yes
w8 vs. w2	0.0451	1.240	1.000	No
w8 vs. w4	0.0343	0.944	1.000	Do Not Test
w8 vs. w6	0.0322	0.884	1.000	Do Not Test
w6 vs. w2	0.0129	0.356	1.000	Do Not Test
w6 vs. w4	0.00216	0.0594	1.000	Do Not Test
w4 vs. w2	0.0108	0.296	1.000	Do Not Test

Comparisons for factor: **Time within ASP**

Comparison	Diff of Means	t	P	P<0.05
Presurg vs. w2	0.106	2.147	0.374	No
Presurg vs. w4	0.0785	1.588	1.000	Do Not Test
Presurg vs. w6	0.0781	1.580	1.000	Do Not Test
Presurg vs. w8	0.0230	0.465	1.000	Do Not Test
w8 vs. w2	0.0831	1.682	0.997	Do Not Test
w8 vs. w4	0.0555	1.123	1.000	Do Not Test
w8 vs. w6	0.0551	1.115	1.000	Do Not Test
w6 vs. w2	0.0280	0.567	1.000	Do Not Test
w6 vs. w4	0.000414	0.00838	1.000	Do Not Test
w4 vs. w2	0.0276	0.558	1.000	Do Not Test

Comparisons for factor: **Time within ASP+CST**

Comparison	Diff of Means	t	P	P<0.05
Presurg vs. w4	0.176	3.299	0.019	Yes
Presurg vs. w6	0.172	3.226	0.024	Yes
Presurg vs. w2	0.170	3.186	0.027	Yes
Presurg vs. w8	0.163	3.053	0.038	Yes
w8 vs. w4	0.0131	0.246	1.000	No
w8 vs. w6	0.00922	0.173	1.000	Do Not Test
w8 vs. w2	0.00708	0.133	1.000	Do Not Test
w2 vs. w4	0.00605	0.113	1.000	Do Not Test

w2 vs. w6	0.00214	0.0401	1.000	Do Not Test
w6 vs. w4	0.00391	0.0732	1.000	Do Not Test

Data variable: Left forelimb peak vertical forces

Source of Variation	DF	SS	MS	F	P
Lesion	1	0.0486	0.0486	0.920	0.358
Rats(Lesion)	11	0.582	0.0529		
Time	4	0.159	0.0397	4.914	0.002
Lesion x Time	4	0.0320	0.00801	0.991	0.422
Residual	44	0.356	0.00808		
Total	64	1.167	0.0182		

There was a statistically significant difference in time (P = 0.002).
All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Lesion**

Comparison	Diff of Means	t	P	P<0.050
ASP vs. ASP+CST	0.0679	1.423	0.183	No

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
Presurg vs. w2	0.173	4.691	<0.001	Yes
Presurg vs. w4	0.140	3.791	0.005	Yes
Presurg vs. w6	0.126	3.421	0.014	Yes
Presurg vs. w8	0.0953	2.584	0.131	No
w8 vs. w2	0.0777	2.107	0.409	No
w8 vs. w4	0.0445	1.206	1.000	Do Not Test
w8 vs. w6	0.0309	0.837	1.000	Do Not Test
w6 vs. w2	0.0468	1.270	1.000	Do Not Test
w6 vs. w4	0.0136	0.370	1.000	Do Not Test
w4 vs. w2	0.0332	0.900	1.000	Do Not Test

Comparisons for factor: **Time within ASP**

Comparison	Diff of Means	t	P	P<0.05
Presurg vs. w2	0.125	2.491	0.166	No
Presurg vs. w4	0.0751	1.498	1.000	Do Not Test
Presurg vs. w6	0.0698	1.393	1.000	Do Not Test
Presurg vs. w8	0.0295	0.589	1.000	Do Not Test
w8 vs. w2	0.0953	1.901	0.638	Do Not Test
w8 vs. w4	0.0455	0.909	1.000	Do Not Test
w8 vs. w6	0.0403	0.804	1.000	Do Not Test
w6 vs. w2	0.0550	1.097	1.000	Do Not Test
w6 vs. w4	0.00524	0.105	1.000	Do Not Test
w4 vs. w2	0.0497	0.993	1.000	Do Not Test

Comparisons for factor: **Time within ASP+CST**

Comparison	Diff of Means	t	P	P<0.05
Presurg vs. w2	0.221	4.087	0.002	Yes
Presurg vs. w4	0.205	3.779	0.005	Yes
Presurg vs. w6	0.183	3.372	0.016	Yes
Presurg vs. w8	0.161	2.976	0.047	Yes
w8 vs. w2	0.0601	1.111	1.000	No
w8 vs. w4	0.0434	0.803	1.000	Do Not Test
w8 vs. w6	0.0214	0.396	1.000	Do Not Test

w6 vs. w2	0.0387	0.715	1.000	Do Not Test
w6 vs. w4	0.0220	0.407	1.000	Do Not Test
w4 vs. w2	0.0167	0.308	1.000	Do Not Test

Data variable: Right forelimb propulsion forces

Source of Variation	DF	SS	MS	F	P
Lesion	1	0.000159	0.000159	0.0504	0.826
Rats(Lesion)	11	0.0346	0.00315		
Time	4	0.0339	0.00848	19.642	<0.001
Lesion x Time	4	0.000468	0.000117	0.271	0.895
Residual	44	0.0190	0.000432		
Total	64	0.0883	0.00138		

There was a statistically significant difference in Time ($P = <0.001$).

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Lesion**

Comparison	Diff of Means	t	P	P<0.050
ASP+CST vs. ASP	0.00314	0.225	0.826	No

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
week2 vs. presurg	0.0614	7.512	<0.001	Yes
week2 vs. week6	0.0129	1.576	1.000	No
week2 vs. week4	0.00936	1.146	1.000	Do Not Test
week2 vs. week8	0.000	0.000	1.000	Do Not Test
week8 vs. presurg	0.0614	7.512	<0.001	Yes
week8 vs. week6	0.0129	1.576	1.000	Do Not Test
week8 vs. week4	0.00936	1.146	1.000	Do Not Test
week4 vs. presurg	0.0520	6.367	<0.001	Yes
week4 vs. week6	0.00352	0.431	1.000	Do Not Test
week6 vs. presurg	0.0485	5.936	<0.001	Yes

Comparisons for factor: **Time within ASP**

Comparison	Diff of Means	t	P	P<0.05
week2 vs. presurg	0.0588	5.290	<0.001	Yes
week2 vs. week6	0.0139	1.248	1.000	No
week2 vs. week4	0.00293	0.264	1.000	Do Not Test
week2 vs. week8	2.776E-017	2.499E-015	1.000	Do Not Test
week8 vs. presurg	0.0588	5.290	<0.001	Yes
week8 vs. week6	0.0139	1.248	1.000	Do Not Test
week8 vs. week4	0.00293	0.264	1.000	Do Not Test
week4 vs. presurg	0.0558	5.026	<0.001	Yes
week4 vs. week6	0.0109	0.984	1.000	Do Not Test
week6 vs. presurg	0.0449	4.042	0.002	Yes

Comparisons for factor: **Time within ASP+CST**

Comparison	Diff of Means	t	P	P<0.05
week2 vs. presurg	0.0641	5.340	<0.001	Yes
week2 vs. week4	0.0158	1.317	1.000	No
week2 vs. week6	0.0119	0.993	1.000	Do Not Test
week2 vs. week8	2.776E-017	2.313E-015	1.000	Do Not Test
week8 vs. presurg	0.0641	5.340	<0.001	Yes
week8 vs. week4	0.0158	1.317	1.000	Do Not Test

week8 vs. week6	0.0119	0.993	1.000	Do Not Test
week6 vs. presurg	0.0522	4.347	<0.001	Yes
week6 vs. week4	0.00388	0.324	1.000	Do Not Test
week4 vs. presurg	0.0483	4.023	0.002	Yes

Comparisons for factor: **Lesion within presurg**

Comparison	Diff of Means	t	P	P<0.05
ASP+CST vs. ASP	0.00107	0.0618	0.951	No

Comparisons for factor: **Lesion within week2**

Comparison	Diff of Means	t	P	P<0.05
ASP+CST vs. ASP	0.00638	0.367	0.716	No

Comparisons for factor: **Lesion within week4**

Comparison	Diff of Means	t	P	P<0.05
ASP vs. ASP+CST	0.00649	0.373	0.712	No

Comparisons for factor: **Lesion within week6**

Comparison	Diff of Means	t	P	P<0.05
ASP+CST vs. ASP	0.00832	0.479	0.636	No

Comparisons for factor: **Lesion within week8**

Comparison	Diff of Means	t	P	P<0.05
ASP+CST vs. ASP	0.00638	0.367	0.716	No

Data variable: Left forelimb propulsion forces

Source of Variation	DF	SS	MS	F	P
Lesion	1	0.00141	0.00141	0.397	0.542
Rats(Lesion)	11	0.0391	0.00356		
Time	4	0.0250	0.00625	29.163	<0.001
Lesion x Time	4	0.00309	0.000774	3.610	0.012
Residual	44	0.00943	0.000214		
Total	64	0.0796	0.00124		

There was a statistically significant difference in time (P = <0.001).

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Lesion**

Comparison	Diff of Means	t	P	P<0.050
ASP vs. ASP+CST	0.00466	0.345	0.737	No

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
w8 vs. Presurg	0.0567	8.702	<0.001	Yes
w8 vs. w6	0.0179	2.756	0.085	No
w8 vs. w2	0.0157	2.415	0.200	Do Not Test
w8 vs. w4	0.0156	2.388	0.213	Do Not Test
w4 vs. Presurg	0.0411	6.314	<0.001	Yes
w4 vs. w6	0.00239	0.368	1.000	Do Not Test
w4 vs. w2	0.000175	0.0269	1.000	Do Not Test
w2 vs. Presurg	0.0409	6.287	<0.001	Yes
w2 vs. w6	0.00222	0.341	1.000	Do Not Test
w6 vs. Presurg	0.0387	5.946	<0.001	Yes

Comparisons for factor: **Time within ASP**

Comparison	Diff of Means	t	P	P<0.05
w8 vs. Presurg	0.0751	8.484	<0.001	Yes
w8 vs. w2	0.0275	3.104	0.033	Yes
w8 vs. w6	0.0253	2.864	0.064	No
w8 vs. w4	0.0182	2.053	0.460	Do Not Test
w4 vs. Presurg	0.0569	6.431	<0.001	Yes
w4 vs. w2	0.00930	1.051	1.000	No
w4 vs. w6	0.00717	0.810	1.000	Do Not Test
w6 vs. Presurg	0.0497	5.620	<0.001	Yes
w6 vs. w2	0.00213	0.241	1.000	Do Not Test
w2 vs. Presurg	0.0476	5.380	<0.001	Yes

Comparisons for factor: **Time within ASP+CST**

Comparison	Diff of Means	t	P	P<0.05
w8 vs. Presurg	0.0383	4.004	0.002	Yes
w8 vs. w4	0.0129	1.353	1.000	No
w8 vs. w6	0.0106	1.104	1.000	Do Not Test
w8 vs. w2	0.00398	0.417	1.000	Do Not Test
w2 vs. Presurg	0.0343	3.587	0.008	Yes
w2 vs. w4	0.00895	0.936	1.000	Do Not Test
w2 vs. w6	0.00657	0.687	1.000	Do Not Test
w6 vs. Presurg	0.0277	2.900	0.058	No
w6 vs. w4	0.00238	0.249	1.000	Do Not Test
w4 vs. Presurg	0.0253	2.651	0.111	Do Not Test

Data variable: Right hindlimb braking forces

Source of Variation	DF	SS	MS	F	P
Lesion	1	0.000523	0.000523	0.207	0.658
Rats(Lesion)	11	0.0278	0.00253		
Time	4	0.0124	0.00310	9.733	<0.001
Lesion x Time	4	0.00102	0.000255	0.801	0.531
Residual	44	0.0140	0.000318		
Total	64	0.0559	0.000874		

There was a statistically significant difference in time ($P = <0.001$).

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Lesion**

Comparison	Diff of Means	t	P	P<0.050
ASP vs. ASP+CST	0.00612	0.476	0.643	No

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
Presurg vs. w2	0.0381	5.149	<0.001	Yes
Presurg vs. w8	0.0361	4.885	<0.001	Yes
Presurg vs. w4	0.0350	4.730	<0.001	Yes
Presurg vs. w6	0.0266	3.597	0.008	Yes
w6 vs. w2	0.0115	1.552	1.000	No
w6 vs. w8	0.00952	1.288	1.000	Do Not Test
w6 vs. w4	0.00838	1.133	1.000	Do Not Test
w4 vs. w2	0.00310	0.419	1.000	Do Not Test
w4 vs. w8	0.00115	0.155	1.000	Do Not Test
w8 vs. w2	0.00195	0.264	1.000	Do Not Test

Comparisons for factor: **Time within ASP**

Comparison	Diff of Means	t	P	P<0.05
Presurg vs. w2	0.0408	4.059	0.002	Yes
Presurg vs. w8	0.0399	3.971	0.003	Yes
Presurg vs. w4	0.0303	3.021	0.042	Yes
Presurg vs. w6	0.0211	2.098	0.417	No
w6 vs. w2	0.0197	1.961	0.562	No
w6 vs. w8	0.0188	1.873	0.677	Do Not Test
w6 vs. w4	0.00927	0.923	1.000	Do Not Test
w4 vs. w2	0.0104	1.038	1.000	Do Not Test
w4 vs. w8	0.00954	0.950	1.000	Do Not Test
w8 vs. w2	0.000886	0.0882	1.000	Do Not Test

Comparisons for factor: **Time within ASP+CST**

Comparison	Diff of Means	t	P	P<0.05
Presurg vs. w4	0.0396	3.650	0.007	Yes
Presurg vs. w2	0.0354	3.260	0.022	Yes
Presurg vs. w8	0.0323	2.982	0.047	Yes
Presurg vs. w6	0.0321	2.960	0.049	Yes
w6 vs. w4	0.00748	0.690	1.000	No
w6 vs. w2	0.00325	0.300	1.000	Do Not Test
w6 vs. w8	0.000233	0.0215	1.000	Do Not Test
w8 vs. w4	0.00725	0.668	1.000	Do Not Test
w8 vs. w2	0.00302	0.278	1.000	Do Not Test
w2 vs. w4	0.00423	0.390	1.000	Do Not Test

Data variable: Left hindlimb braking forces

Source of Variation	DF	SS	MS	F	P
Lesion	1	0.00108	0.00108	0.588	0.459
Rats(Lesion)	11	0.0202	0.00183		
Time	4	0.0113	0.00284	9.497	<0.001
Lesion x Time	4	0.00251	0.000628	2.104	0.096
Residual	44	0.0131	0.000299		
Total	64	0.0488	0.000762		

There was a statistically significant difference in time ($P = <0.001$).

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Lesion**

Comparison	Diff of Means	t	P	P<0.050
ASP+CST vs. ASP	0.00749	0.792	0.445	No

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
Presurg vs. w4	0.0353	4.288	<0.001	Yes
Presurg vs. w8	0.0336	4.084	0.002	Yes
Presurg vs. w2	0.0314	3.816	0.004	Yes
Presurg vs. w6	0.0271	3.300	0.019	Yes
w6 vs. w4	0.00812	0.987	1.000	No
w6 vs. w8	0.00645	0.784	1.000	Do Not Test
w6 vs. w2	0.00424	0.515	1.000	Do Not Test
w2 vs. w4	0.00388	0.472	1.000	Do Not Test
w2 vs. w8	0.00221	0.268	1.000	Do Not Test
w8 vs. w4	0.00167	0.203	1.000	Do Not Test

Comparisons for factor: **Time within ASP**

Comparison	Diff of Means	t	P	P<0.05
Presurg vs. w8	0.0463	4.148	0.002	Yes
Presurg vs. w2	0.0424	3.798	0.004	Yes
Presurg vs. w4	0.0380	3.399	0.014	Yes
Presurg vs. w6	0.0255	2.281	0.274	No
w6 vs. w8	0.0209	1.867	0.686	No
w6 vs. w2	0.0169	1.517	1.000	Do Not Test
w6 vs. w4	0.0125	1.118	1.000	Do Not Test
w4 vs. w8	0.00837	0.749	1.000	Do Not Test
w4 vs. w2	0.00446	0.399	1.000	Do Not Test
w2 vs. w8	0.00391	0.350	1.000	Do Not Test

Data variable: Right forelimb stance (Section 4.4.2.3.1)

Source of Variation	DF	SS	MS	F	P
Lesion	1	0.00110	0.00110	0.250	0.627
Rats(Lesion)	11	0.0484	0.00440		
Time	4	0.0258	0.00645	11.693	<0.001
Lesion x Time	4	0.00222	0.000556	1.009	0.413
Residual	44	0.0243	0.000551		
Total	64	0.101	0.00159		

There was a statistically significant difference in time ($P = <0.001$).

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
w6 vs. Presurg	0.0532	5.755	<0.001	Yes
w6 vs. w8	0.00867	0.939	1.000	No
w6 vs. w2	0.00504	0.545	1.000	Do Not Test
w6 vs. w4	0.00143	0.154	1.000	Do Not Test
w4 vs. Presurg	0.0517	5.600	<0.001	Yes
w4 vs. w8	0.00725	0.785	1.000	Do Not Test
w4 vs. w2	0.00361	0.391	1.000	Do Not Test
w2 vs. Presurg	0.0481	5.209	<0.001	Yes
w2 vs. w8	0.00364	0.394	1.000	Do Not Test
w8 vs. Presurg	0.0445	4.816	<0.001	Yes

Data variable: Left forelimb stance

Source of Variation	DF	SS	MS	F	P
Lesion	1	0.000000318	0.000000318	0.0000768	0.993
Rats(Lesion)	11	0.0456	0.00415		
Time	4	0.0249	0.00623	10.505	<0.001
Lesion x Time	4	0.00112	0.000280	0.473	0.755
Residual	44	0.0261	0.000593		
Total	64	0.0975	0.00152		

There was a statistically significant difference in time ($P = <0.001$).

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
w6 vs. Presurg	0.0532	5.755	<0.001	Yes
w6 vs. w8	0.00867	0.939	1.000	No
w6 vs. w2	0.00504	0.545	1.000	Do Not Test
w6 vs. w4	0.00143	0.154	1.000	Do Not Test
w4 vs. Presurg	0.0517	5.600	<0.001	Yes
w4 vs. w8	0.00725	0.785	1.000	Do Not Test
w4 vs. w2	0.00361	0.391	1.000	Do Not Test
w2 vs. Presurg	0.0481	5.209	<0.001	Yes
w2 vs. w8	0.00364	0.394	1.000	Do Not Test
w8 vs. Presurg	0.0445	4.816	<0.001	Yes

w2 vs. Presurg	0.0513	5.355	<0.001	Yes
w2 vs. w8	0.00637	0.665	1.000	No
w2 vs. w4	0.00218	0.228	1.000	Do Not Test
w2 vs. w6	0.00143	0.149	1.000	Do Not Test
w6 vs. Presurg	0.0499	5.206	<0.001	Yes
w6 vs. w8	0.00494	0.516	1.000	Do Not Test
w6 vs. w4	0.000758	0.0791	1.000	Do Not Test
w4 vs. Presurg	0.0491	5.127	<0.001	Yes
w4 vs. w8	0.00418	0.437	1.000	Do Not Test
w8 vs. Presurg	0.0449	4.690	<0.001	Yes

Chapter 5

I) Ground reaction forces (Section 5.4.2.1.1)

Statistical test used: Two-way repeated measures ANOVA

Post-hoc analyses: Bonferroni t-test

Level of significance: $p < 0.05$

Data variable: Right forelimb vertical forces

Source of Variation	DF	SS	MS	F	P
Lesion	1	0.118	0.118	5.290	0.047
Col 17(Lesion)	9	0.200	0.0223		
Time	2	0.0219	0.0109	2.104	0.151
Lesion x Time	2	0.116	0.0582	11.205	<0.001
Residual	18	0.0935	0.00519		
Total	32	0.559	0.0175		

There was a statistically significant interaction between Lesion and Time. ($P = <0.001$)

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Lesion**

Comparison	Diff of Means	t	P	P<0.050
Thoracic DF vs. Cervical DF	0.120	2.300	0.047	Yes

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
Presurg vs. W6	0.0553	1.792	0.270	No
Presurg vs. W2	0.0544	1.761	0.285	Do Not Test
W2 vs. W6	0.000930	0.0301	1.000	Do Not Test

Comparisons for factor: **Time within Cervical DF**

Comparison	Diff of Means	t	P	P<0.05
Presurg vs. W2	0.199	4.794	<0.001	Yes
Presurg vs. W6	0.142	3.422	0.009	Yes
W6 vs. W2	0.0571	1.372	0.560	No

Comparisons for factor: **Time within Thoracic DF**

Comparison	Diff of Means	t	P	P<0.05
W2 vs. Presurg	0.0908	1.991	0.186	No
W2 vs. W6	0.0590	1.294	0.637	Do Not Test
W6 vs. Presurg	0.0318	0.698	1.000	Do Not Test

Comparisons for factor: Lesion within Presurg					
Comparison	Diff of Means	t	P	P<0.05	
Cervical DF vs. Thoracic DF	0.0348	0.552	0.588	No	
Comparisons for factor: Lesion within W2					
Comparison	Diff of Means	t	P	P<0.05	
Thoracic DF vs. Cervical DF	0.255	4.043	<0.001	Yes	
Comparisons for factor: Lesion within W6					
Comparison	Diff of Means	t	P	P<0.05	
Thoracic DF vs. Cervical DF	0.139	2.206	0.041	Yes	

Data variable: Left forelimb vertical forces

Source of Variation	DF	SS	MS	F	P
Lesion	1	0.144	0.144	9.199	0.014
Col 17(Lesion)	9	0.140	0.0156		
Time	2	0.0495	0.0247	2.094	0.152
Lesion x Time	2	0.119	0.0597	5.047	0.018
Residual	18	0.213	0.0118		
Total	32	0.679	0.0212		

There was a statistically significant interaction between Lesion and Time. (P = 0.018)
All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Lesion**

Comparison	Diff of Means	t	P	P<0.050	
Thoracic DF vs. Cervical DF	0.132	3.033	0.014	Yes	

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050	
Presurg vs. W6	0.0881	1.893	0.224	No	
Presurg vs. W2	0.0754	1.620	0.368	Do Not Test	
W2 vs. W6	0.0127	0.273	1.000	Do Not Test	

Comparisons for factor: **Time within Cervical DF**

Comparison	Diff of Means	t	P	P<0.05	
Presurg vs. W2	0.221	3.524	0.007	Yes	
Presurg vs. W6	0.183	2.908	0.028	Yes	
W6 vs. W2	0.0387	0.616	1.000	No	

Comparisons for factor: **Time within Thoracic DF**

Comparison	Diff of Means	t	P	P<0.05	
W2 vs. Presurg	0.0704	1.024	0.959	No	
W2 vs. W6	0.0641	0.932	1.000	Do Not Test	
W6 vs. Presurg	0.00630	0.0916	1.000	Do Not Test	

Comparisons for factor: **Lesion within Presurg**

Comparison	Diff of Means	t	P	P<0.05	
Cervical DF vs. Thoracic DF	0.0277	0.399	0.693	No	

Comparisons for factor: **Lesion within W2**

Comparison	Diff of Means	t	P	P<0.05	
Thoracic DF vs. Cervical DF	0.264	3.811	<0.001	Yes	

Comparisons for factor: **Lesion within W6**

Comparison	Diff of Means	t	P	P<0.05	

Thoracic DF vs. Cervical DF 0.161 2.327 0.028 Yes

Data variable: Right forelimb propulsion

Source of Variation	DF	SS	MS	F	P
Lesion	1	0.00397	0.00397	9.683	0.012
Col 17(Lesion)	9	0.00369	0.000410		
Time	2	0.000999	0.000499	1.987	0.166
Lesion x Time	2	0.00385	0.00192	7.659	0.004
Residual	18	0.00452	0.000251		
Total	32	0.0173	0.000541		

There was a statistically significant interaction between Lesion and Time. (P = 0.004)
All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Lesion**

Comparison	Diff of Means	t	P	P<0.050
Cervical DF vs. Thoracic DF	0.0220	3.112	0.012	Yes

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
W2 vs. Presurg	0.0126	1.851	0.242	No
W2 vs. W6	0.0106	1.567	0.404	Do Not Test
W6 vs. Presurg	0.00193	0.284	1.000	Do Not Test

Comparisons for factor: **Time within Cervical DF**

Comparison	Diff of Means	t	P	P<0.05
W2 vs. Presurg	0.0360	3.930	0.003	Yes
W2 vs. W6	0.0115	1.251	0.681	No
W6 vs. Presurg	0.0245	2.679	0.046	Yes

Comparisons for factor: **Time within Thoracic DF**

Comparison	Diff of Means	t	P	P<0.05
Presurg vs. W6	0.0207	2.061	0.162	No
Presurg vs. W2	0.0108	1.081	0.882	Do Not Test
W2 vs. W6	0.00982	0.979	1.000	Do Not Test

Comparisons for factor: **Lesion within Presurg**

Comparison	Diff of Means	t	P	P<0.05
Thoracic DF vs. Cervical DF	0.00863	0.817	0.421	No

Comparisons for factor: **Lesion within W2**

Comparison	Diff of Means	t	P	P<0.05
Cervical DF vs. Thoracic DF	0.0382	3.615	0.001	Yes

Comparisons for factor: **Lesion within W6**

Comparison	Diff of Means	t	P	P<0.05
Cervical DF vs. Thoracic DF	0.0365	3.460	0.002	Yes

Data variable: Right hindlimb vertical forces

Source of Variation	DF	SS	MS	F	P
Lesion	1	0.0242	0.0242	2.246	0.168
Col 17(Lesion)	9	0.0968	0.0108		
Time	2	0.0847	0.0423	7.378	0.005
Lesion x Time	2	0.00165	0.000827	0.144	0.867

Residual	18	0.103	0.00574
Total	32	0.314	0.00980

There was a statistically significant difference in time ($P = 0.005$)
 All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Lesion**

Comparison	Diff of Means	t	P	P<0.050
Thoracic DF vs. Cervical DF	0.0543	1.499	0.168	No

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
Presurg vs. W6	0.112	3.456	0.008	Yes
Presurg vs. W2	0.103	3.180	0.016	Yes
W2 vs. W6	0.00897	0.276	1.000	No

Comparisons for factor: **Time within Cervical DF**

Comparison	Diff of Means	t	P	P<0.05
Presurg vs. W6	0.127	2.908	0.028	Yes
Presurg vs. W2	0.118	2.703	0.044	Yes
W2 vs. W6	0.00895	0.205	1.000	No

Comparisons for factor: **Time within Thoracic DF**

Comparison	Diff of Means	t	P	P<0.05
Presurg vs. W6	0.0970	2.025	0.174	No
Presurg vs. W2	0.0881	1.838	0.248	Do Not Test
W2 vs. W6	0.00898	0.187	1.000	Do Not Test

Comparisons for factor: **Lesion within Presurg**

Comparison	Diff of Means	t	P	P<0.05
Thoracic DF vs. Cervical DF	0.0342	0.657	0.517	No

Comparisons for factor: **Lesion within W2**

Comparison	Diff of Means	t	P	P<0.05
Thoracic DF vs. Cervical DF	0.0644	1.236	0.228	No

Comparisons for factor: **Lesion within W6**

Comparison	Diff of Means	t	P	P<0.05
Thoracic DF vs. Cervical DF	0.0644	1.235	0.229	No

Data variable: Left hindlimb vertical forces

Source of Variation	DF	SS	MS	F	P
Lesion	1	0.0295	0.0295	4.499	0.063
Col 17(Lesion)	9	0.0590	0.00656		
Time	2	0.0961	0.0480	6.588	0.007
Lesion x Time	2	0.0105	0.00525	0.720	0.500
Residual	18	0.131	0.00729		
Total	32	0.333	0.0104		

There was a statistically significant difference in time ($P = 0.007$)
 All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Lesion**

Comparison	Diff of Means	t	P	P<0.050
Thoracic DF vs. Cervical DF	0.0601	2.121	0.063	No

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
Presurg vs. W6	0.126	3.446	0.009	Yes
Presurg vs. W2	0.0991	2.712	0.043	Yes
W2 vs. W6	0.0268	0.734	1.000	No

Comparisons for factor: **Time within Cervical DF**

Comparison	Diff of Means	t	P	P<0.05
Presurg vs. W6	0.170	3.444	0.009	Yes
Presurg vs. W2	0.123	2.503	0.066	No
W2 vs. W6	0.0464	0.940	1.000	No

Comparisons for factor: **Time within Thoracic DF**

Comparison	Diff of Means	t	P	P<0.05
Presurg vs. W6	0.0822	1.522	0.436	No
Presurg vs. W2	0.0749	1.387	0.548	Do Not Test
W2 vs. W6	0.00730	0.135	1.000	Do Not Test

Comparisons for factor: **Lesion within Presurg**

Comparison	Diff of Means	t	P	P<0.05
Thoracic DF vs. Cervical DF	0.0147	0.289	0.775	No

Comparisons for factor: **Lesion within W2**

Comparison	Diff of Means	t	P	P<0.05
Thoracic DF vs. Cervical DF	0.0632	1.244	0.224	No

Comparisons for factor: **Lesion within W6**

Comparison	Diff of Means	t	P	P<0.05
Thoracic DF vs. Cervical DF	0.102	2.012	0.054	No

Data variable: Right hindlimb braking forces

Source of Variation	DF	SS	MS	F	P
Lesion	1	0.00139	0.00139	1.677	0.228
Col 17(Lesion)	9	0.00745	0.000828		
Time	2	0.00305	0.00152	10.001	0.001
Lesion x Time	2	0.00135	0.000673	4.418	0.027
Residual	18	0.00274	0.000152		
Total	32	0.0164	0.000512		

There was a statistically significant interaction between Lesion and Time. (P = 0.027)
All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Lesion**

Comparison	Diff of Means	t	P	P<0.050
Thoracic DF vs. Cervical DF	0.0130	1.295	0.228	No

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
Presurg vs. W6	0.0205	3.887	0.003	Yes

Presurg vs. W2	0.0204	3.859	0.003	Yes
W2 vs. W6	0.000151	0.0286	1.000	No

Comparisons for factor: **Time within Cervical DF**

Comparison	Diff of Means	t	P	P<0.05
Presurg vs. W2	0.0354	4.964	<0.001	Yes
Presurg vs. W6	0.0321	4.508	<0.001	Yes
W6 vs. W2	0.00325	0.456	1.000	No

Comparisons for factor: **Time within Thoracic DF**

Comparison	Diff of Means	t	P	P<0.05
Presurg vs. W6	0.00896	1.148	0.798	No
Presurg vs. W2	0.00541	0.693	1.000	Do Not Test
W2 vs. W6	0.00355	0.455	1.000	Do Not Test

Comparisons for factor: **Lesion within Presurg**

Comparison	Diff of Means	t	P	P<0.05
Cervical DF vs. Thoracic DF	0.00468	0.398	0.696	No

Comparisons for factor: **Lesion within W2**

Comparison	Diff of Means	t	P	P<0.05
Thoracic DF vs. Cervical DF	0.0253	2.149	0.048	Yes

Comparisons for factor: **Lesion within W6**

Comparison	Diff of Means	t	P	P<0.05
Thoracic DF vs. Cervical DF	0.0185 1.571	0.136	No	

Data variable: Left hindlimb braking forces

Source of Variation	DF	SS	MS	F	P
Lesion	1	0.00158	0.00158	2.864	0.125
Col 17(Lesion)	9	0.00497	0.000553		
Time	2	0.00100	0.000502	2.743	0.091
Lesion x Time	2	0.00142	0.000709	3.871	0.040
Residual	18	0.00330	0.000183		
Total	32	0.0125	0.000391		

There was a statistically significant interaction between Lesion and Time. (P = 0.040)

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Lesion**

Comparison	Diff of Means	t	P	P<0.050
Thoracic DF vs. Cervical DF	0.0139	1.692	0.125	No

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
Presurg vs. W6	0.0129	2.218	0.119	No
Presurg vs. W2	0.0102	1.761	0.286	Do Not Test
W2 vs. W6	0.00265	0.457	1.000	Do Not Test

Comparisons for factor: **Time within Cervical DF**

Comparison	Diff of Means	t	P	P<0.05
Presurg vs. W6	0.0288	3.684	0.005	Yes
Presurg vs. W2	0.0203	3.600	0.004	Yes

W2 vs. W6	0.00847	1.084	0.879	No
-----------	---------	-------	-------	----

Comparisons for factor: **Time within Thoracic DF**

Comparison	Diff of Means	t	P	P<0.05
W6 vs. W2	0.00317	0.371	1.000	No
W6 vs. Presurg	0.00308	0.360	1.000	Do Not Test
Presurg vs. W2	0.0000920	0.0107	1.000	Do Not Test

Comparisons for factor: **Lesion within Presurg**

Comparison	Diff of Means	t	P	P<0.05
Cervical DF vs. Thoracic DF	0.00345	0.326	0.748	No

Comparisons for factor: **Lesion within W2**

Comparison	Diff of Means	t	P	P<0.05
Thoracic DF vs. Cervical DF	0.0168	1.582	0.129	No

Comparisons for factor: **Lesion within W6**

Comparison	Diff of Means	t	P	P<0.05
Thoracic DF vs. Cervical DF	0.0284	2.681	0.014	Yes

II) Skilled locomotion-Ladder walking (Section 5.4.2.2)

Statistical test used: Two-way repeated measures ANOVA

Post-hoc analyses: Bonferroni t-test

Level of significance: $p < 0.05$

Data variable: Forelimb correct steps

Source of Variation	DF	SS	MS	F	P
Lesion	1	11201.784	11201.784	368.174	<0.001
Rat#(Lesion)	9	273.827	30.425		
Time	2	14535.657	7267.828	378.782	<0.001
Lesion x Time	2	10673.918	5336.959	278.150	<0.001
Residual	18	345.373	19.187		
Total	32	39523.098	1235.097		

There was a statistically significant difference in Time. ($P = <0.001$)

There was a statistically significant difference in Lesion. ($P = <0.001$)

There was a statistically significant interaction between Lesion and Time. ($P = <0.001$)

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Lesion**

Comparison	Diff of Means	t	P	P<0.050
Thoracic DF vs. Cervical DF	37.001	19.188	<0.001	Yes

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
Presurg vs. W2	50.703	27.034	<0.001	Yes
Presurg vs. W6	16.951	9.038	<0.001	Yes
W6 vs. W2	33.752	17.996	<0.001	Yes

Comparisons for factor: **Time within Cervical DF**

Comparison	Diff of Means	t	P	P<0.05

Presurg vs. W2	93.867	37.116	<0.001	Yes
Presurg vs. W6	30.147	11.920	<0.001	Yes
W6 vs. W2	63.720	25.196	<0.001	Yes

Comparisons for factor: **Time within Thoracic DF**

Comparison	Diff of Means	t	P	P<0.05
Presurg vs. W2	7.539	2.721	0.042	Yes
Presurg vs. W6	3.755	1.355	0.576	No
W6 vs. W2	3.784	1.366	0.566	No

Comparisons for factor: **Lesion within Presurg**

Comparison	Diff of Means	t	P	P<0.05
Cervical DF vs. Thoracic DF	0.572	0.197	0.845	No

Comparisons for factor: **Lesion within W2**

Comparison	Diff of Means	t	P	P<0.05
Thoracic DF vs. Cervical DF	85.756	29.573	<0.001	Yes

Comparisons for factor: **Lesion within W6**

Comparison	Diff of Means	t	P	P<0.05
Thoracic DF vs. Cervical DF	25.820	8.904	<0.001	Yes

Data variable: Hindlimb correct steps

Source of Variation	DF	SS	MS	F	P
Lesion	1	97.259	97.259	14.963	0.004
Rat#(Lesion)	9	58.500	6.500		
Time	2	248.083	124.041	22.478	<0.001
Lesion x Time	2	97.433	48.716	8.828	0.002
Residual	18	99.328	5.518		
Total	32	575.019	17.969		

There was a statistically significant difference in Time. (P < 0.05)

There was a statistically significant difference in Lesion. (P < 0.05)

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Lesion**

Comparison	Diff of Means	t	P	P<0.050
Cervical DF vs. Thoracic DF	3.448	3.868	0.004	Yes

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
Presurg vs. W2	6.345	6.308	<0.001	Yes
Presurg vs. W6	1.193	1.186	0.753	No
W6 vs. W2	5.152	5.122	<0.001	Yes

Comparisons for factor: **Time within Cervical DF**

Comparison	Diff of Means	t	P	P<0.05
Presurg vs. W2	2.450	1.806	0.263	No
Presurg vs. W6	0.667	0.492	1.000	Do Not Test
W6 vs. W2	1.783	1.315	0.615	Do Not Test

Comparisons for factor: **Time within Thoracic DF**

Comparison	Diff of Means	t	P	P<0.05
Presurg vs. W2	10.240	6.892	<0.001	Yes
Presurg vs. W6	1.720	1.158	0.786	No

W6 vs. W2	8.520	5.735	<0.001	Yes
-----------	-------	-------	--------	-----

Comparisons for factor: **Lesion within Presurg**

Comparison	Diff of Means	t	P	P<0.05
Cervical DF vs. Thoracic DF	0.500	0.342	0.735	No

Comparisons for factor: **Lesion within W2**

Comparison	Diff of Means	t	P	P<0.05
Cervical DF vs. Thoracic DF	8.290	5.662	<0.001	Yes

Comparisons for factor: **Lesion within W6**

Comparison	Diff of Means	t	P	P<0.05
Cervical DF vs. Thoracic DF	1.553	1.061	0.298	No

Chapter 6

I) Ground reaction forces (Section 6.4.2.1.1)

Statistical test used: Two-way repeated measures ANOVA

Post-hoc analyses: Bonferroni t-test

Level of significance: $p < 0.05$

Data variable: Right forelimb vertical forces

Source of Variation	DF	SS	MS	F	P
Lesion	1	0.00461	0.00461	0.397	0.531
Time	4	0.494	0.123	10.619	<0.001
Lesion x Time	4	0.0644	0.0161	1.385	0.250
Residual	60	0.697	0.0116		
Total	69	1.260	0.0183		

There was a statistically significant difference in time ($P = <0.001$)

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Lesion**

Comparison	Diff of Means	t	P	P<0.050
DLF+ASP vs. DLF+DF	0.0439	(+inf)	<0.001	Yes

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
presurg vs. Week8	0.364	(+inf)	<0.001	Yes
presurg vs. Week2	0.194	(+inf)	<0.001	Yes
presurg vs. Week6	0.179	(+inf)	<0.001	Yes
presurg vs. Week10	0.151	(+inf)	<0.001	Yes
Week10 vs. Week8	0.213	(+inf)	<0.001	Yes
Week10 vs. Week2	0.0437	(+inf)	<0.001	Yes
Week10 vs. Week6	0.0284	(+inf)	<0.001	Yes
Week6 vs. Week8	0.185	(+inf)	<0.001	Yes
Week6 vs. Week2	0.0154	(+inf)	<0.001	Yes
Week2 vs. Week8	0.170	(+inf)	<0.001	Yes

Data variable: Right hindlimb vertical forces

Source of Variation	DF	SS	MS	F	P
Lesion	1	0.0325	0.0325	4.156	0.046
Time	4	0.253	0.0632	8.076	<0.001
Lesion x Time	4	0.0907	0.0227	2.899	0.029
Residual	60	0.469	0.00782		
Total	69	0.845	0.0123		

There was a statistically significant difference in time ($P = <0.05$)
 All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
presurg vs. Week6	0.211	(+inf)	<0.001	Yes
presurg vs. Week8	0.203	(+inf)	<0.001	Yes
presurg vs. Week10	0.189	(+inf)	<0.001	Yes
presurg vs. Week2	0.105	(+inf)	<0.001	Yes
Week2 vs. Week6	0.106	(+inf)	<0.001	Yes
Week2 vs. Week8	0.0979	(+inf)	<0.001	Yes
Week2 vs. Week10	0.0839	(+inf)	<0.001	Yes
Week10 vs. Week6	0.0222	(+inf)	<0.001	Yes
Week10 vs. Week8	0.0140	(+inf)	<0.001	Yes
Week8 vs. Week6	0.00820	(+inf)	<0.001	Yes

Data variable: Left forelimb vertical forces

Source of Variation	DF	SS	MS	F	P
Lesion	1	0.00000307	0.00000307	0.000461	0.983
Time	4	0.349	0.0873	13.108	<0.001
Lesion x Time	4	0.0902	0.0226	3.387	0.015
Residual	60	0.400	0.00666		
Total	69	0.839	0.0122		

There was a statistically significant difference in time ($P = <0.05$)
 All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
presurg vs. Week10	0.291	(+inf)	<0.001	Yes
presurg vs. Week8	0.285	(+inf)	<0.001	Yes
presurg vs. Week2	0.225	(+inf)	<0.001	Yes
presurg vs. Week6	0.118	(+inf)	<0.001	Yes
Week6 vs. Week10	0.172	(+inf)	<0.001	Yes
Week6 vs. Week8	0.167	(+inf)	<0.001	Yes
Week6 vs. Week2	0.106	(+inf)	<0.001	Yes
Week2 vs. Week10	0.0659	(+inf)	<0.001	Yes
Week2 vs. Week8	0.0605	(+inf)	<0.001	Yes
Week8 vs. Week10	0.00535	(+inf)	<0.001	Yes

Data variable: Left hindlimb vertical forces

Source of Variation	DF	SS	MS	F	P
Lesion	1	0.0767	0.0767	11.760	0.001
Time	4	0.0832	0.0208	3.192	0.019
Lesion x Time	4	0.0214	0.00534	0.819	0.518

Residual	60	0.391	0.00652
Total	69	0.573	0.00830

There was a statistically significant difference in time ($P = <0.05$)
 All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
Week10 vs. Week2	0.149	(+inf)	<0.001	Yes
Week10 vs. Week6	0.137	(+inf)	<0.001	Yes
Week10 vs. Week8	0.0165	(+inf)	<0.001	Yes
Week10 vs. presurg	0.00851	(+inf)	<0.001	Yes
presurg vs. Week2	0.140	(+inf)	<0.001	Yes
presurg vs. Week6	0.128	(+inf)	<0.001	Yes
presurg vs. Week8	0.00798	(+inf)	<0.001	Yes
Week8 vs. Week2	0.132	(+inf)	<0.001	Yes
Week8 vs. Week6	0.120	(+inf)	<0.001	Yes
Week6 vs. Week2	0.0119	(+inf)	<0.001	Yes

II) Skilled locomotion-ladder walking (Section 6.4.2.2)

Statistical test used: Two-way repeated measures ANOVA

Post-hoc analyses: Bonferroni t-test

Level of significance: $p < 0.05$

Data variable: Forelimb correct steps

Source of Variation	DF	SS	MS	F	P
Lesion	1	63.242	63.242	0.708	0.416
Rat#(Lesion)	12	1071.488	89.291		
Time	4	26421.641	6605.410	97.854	<0.001
Lesion x Time	4	191.741	47.935	0.710	0.589
Residual	48	3240.135	67.503		
Total	69	30988.246	449.105		

There was a statistically significant difference in time ($P = <0.001$).

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Lesion**

Comparison	Diff of Means	t	P	P<0.050
DLF+ASP vs. DLF+DF	1.901	0.842	0.416	No

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
ps vs. W8	55.754	17.954	<0.001	Yes
ps vs. W10	49.447	15.923	<0.001	Yes
ps vs. W2	32.428	10.443	<0.001	Yes
ps vs. W6	29.715	9.569	<0.001	Yes
W6 vs. W8	26.039	8.385	<0.001	Yes
W6 vs. W10	19.732	6.354	<0.001	Yes
W6 vs. W2	2.714	0.874	1.000	No
W2 vs. W8	23.326	7.511	<0.001	Yes
W2 vs. W10	17.019	5.480	<0.001	Yes
W10 vs. W8	6.307	2.031	0.478	No

Comparisons for factor: **Time within DLF+DF**

Comparison	Diff of Means	t	P	P<0.05
ps vs. W8	54.281	12.360	<0.001	Yes
ps vs. W10	50.719	11.549	<0.001	Yes
ps vs. W2	29.018	6.608	<0.001	Yes
ps vs. W6	27.426	6.245	<0.001	Yes
W6 vs. W8	26.854	6.115	<0.001	Yes
W6 vs. W10	23.293	5.304	<0.001	Yes
W6 vs. W2	1.591	0.362	1.000	No
W2 vs. W8	25.263	5.752	<0.001	Yes
W2 vs. W10	21.701	4.942	<0.001	Yes
W10 vs. W8	3.561	0.811	1.000	No

Comparisons for factor: **Time within DLF+ASP**

Comparison	Diff of Means	t	P	P<0.05
ps vs. W8	57.227	13.031	<0.001	Yes
ps vs. W10	48.174	10.970	<0.001	Yes
ps vs. W2	35.839	8.161	<0.001	Yes
ps vs. W6	32.003	7.287	<0.001	Yes
W6 vs. W8	25.224	5.744	<0.001	Yes
W6 vs. W10	16.171	3.682	0.006	Yes
W6 vs. W2	3.836	0.873	1.000	No
W2 vs. W8	21.389	4.870	<0.001	Yes
W2 vs. W10	12.336	2.809	0.072	No
W10 vs. W8	9.053	2.061	0.447	No

Data variable: Hindlimb correct steps

Source of Variation	DF	SS	MS	F	P
Lesion	1	310.635	310.635	5.183	0.042
Rat#(Lesion)	12	719.235	59.936		
Time	4	34547.416	8636.854	226.463	<0.001
Lesion x Time	4	188.973	47.243	1.239	0.307
Residual	48	1830.628	38.138		
Total	69	37596.888	544.882		

There was a statistically significant difference among the different levels of lesion (P = 0.042).

There was a statistically significant difference in time (P = <0.001)

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Lesion**

Comparison	Diff of Means	t	P	P<0.050
DLF+ASP vs. DLF+DF	4.213	2.277	0.042	Yes

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
ps vs. W8	62.684	26.855	<0.001	Yes
ps vs. W10	56.066	24.020	<0.001	Yes
ps vs. W2	49.884	21.371	<0.001	Yes
ps vs. W6	46.619	19.973	<0.001	Yes
W6 vs. W8	16.065	6.883	<0.001	Yes
W6 vs. W10	9.447	4.047	0.002	Yes
W6 vs. W2	3.264	1.398	1.000	No
W2 vs. W8	12.801	5.484	<0.001	Yes

W2 vs. W10	6.183	2.649	0.109	No
W10 vs. W8	6.618	2.835	0.067	No

Comparisons for factor: **Time within DLF+DF**

Comparison	Diff of Means	t	P	P<0.05
ps vs. W8	61.120	18.516	<0.001	Yes
ps vs. W10	59.270	17.955	<0.001	Yes
ps vs. W2	51.000	15.450	<0.001	Yes
ps vs. W6	48.650	14.738	<0.001	Yes
W6 vs. W8	12.470	3.778	0.004	Yes
W6 vs. W10	10.620	3.217	0.023	Yes
W6 vs. W2	2.350	0.712	1.000	No
W2 vs. W8	10.120	3.066	0.036	Yes
W2 vs. W10	8.270	2.505	0.157	No
W10 vs. W8	1.850	0.560	1.000	No

Comparisons for factor: **Time within DLF+ASP**

Comparison	Diff of Means	t	P	P<0.05
ps vs. W8	64.249	19.463	<0.001	Yes
ps vs. W10	52.863	16.014	<0.001	Yes
ps vs. W2	48.767	14.773	<0.001	Yes
ps vs. W6	44.589	13.508	<0.001	Yes
W6 vs. W8	19.660	5.956	<0.001	Yes
W6 vs. W10	8.274	2.507	0.156	No
W6 vs. W2	4.179	1.266	1.000	Do Not Test
W2 vs. W8	15.481	4.690	<0.001	Yes
W2 vs. W10	4.096	1.241	1.000	Do Not Test
W10 vs. W8	11.386	3.449	0.012	Yes

Comparisons for factor: **Lesion within ps**

Comparison	Diff of Means	t	P	P<0.05
DLF+ASP vs. DLF+DF	2.299	0.660	0.512	No

Comparisons for factor: **Lesion within W2**

Comparison	Diff of Means	t	P	P<0.05
DLF+ASP vs. DLF+DF	4.531	1.300	0.199	No

Comparisons for factor: **Lesion within W6**

Comparison	Diff of Means	t	P	P<0.05
DLF+ASP vs. DLF+DF	6.360	1.825	0.073	No

Comparisons for factor: **Lesion within W8**

Comparison	Diff of Means	t	P	P<0.05
DLF+DF vs. DLF+ASP	0.830	0.238	0.813	No

Comparisons for factor: **Lesion within W10**

Comparison	Diff of Means	t	P	P<0.05
DLF+ASP vs. DLF+DF	8.706	2.498	0.015	Yes

III) Skilled forepaw usage-single pellet reaching (Section 6.4.2.3)

Statistical test used: Two-way repeated measures ANOVA

Post-hoc analyses: Bonferroni t-test

Level of significance: $p < 0.05$

Data variable: % successful reaches

Source of Variation	DF	SS	MS	F	P
Lesion	1	29.927	29.927	0.297	0.641
TIME	3	2285.777	571.444	5.662	<0.001
Lesion x TIME	3	150.882	50.294	1.151	0.338
Residual	2	201.853	100.927		
Total	7	2855.970	407.996		

There was a statistically significant difference in time ($P = <0.001$)
 All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Lesion**

Comparison	Diff of Means	t	P	P<0.050
DLF+DF vs. DLF+ASP	16.207	(+inf)	<0.001	Yes

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
presurg vs. Week8	69.677	(+inf)	<0.001	Yes
presurg vs. Week10	56.347	(+inf)	<0.001	Yes
presurg vs. Week2	53.075	(+inf)	<0.001	Yes
presurg vs. Week6	40.485	(+inf)	<0.001	Yes
Week6 vs. Week8	29.192	(+inf)	<0.001	Yes
Week6 vs. Week10	15.862	(+inf)	<0.001	Yes
Week6 vs. Week2	12.590	(+inf)	<0.001	Yes
Week2 vs. Week8	16.602	(+inf)	<0.001	Yes
Week2 vs. Week10	3.272	(+inf)	<0.001	Yes
Week10 vs. Week8	13.330	(+inf)	<0.001	Yes

Chapter 7

I) Ground reaction forces (Section 7.4.3.1.1)

Data variable: Right forelimb vertical forces

a) In PT followed by DLF rats

Statistical test used: One-way repeated measures ANOVA

Post-hoc analyses: Bonferroni t-test

Level of significance: $p<0.05$

Source of Variation	DF	SS	MS	F	P
Between Subjects	4	0.176	0.0441		
Between Treatments	4	0.449	0.112	8.492	<0.001
Residual	16	0.211	0.0132		
Total	24	0.837			

There was a statistical difference for factor Time

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
w2 vs. w10	0.314	4.324	0.005	Yes
w2 vs. w8	0.314	4.324	0.005	Yes

w2 vs. w6	0.0799	1.098	1.000	No
w2 vs. Presurgery	0.0584	0.803	1.000	Do Not Test
Presurgery vs. w10	0.256	3.521	0.028	Yes
Presurgery vs. w8	0.256	3.521	0.028	Yes
Presurgery vs. w6	0.0215	0.295	1.000	Do Not Test
w6 vs. w10	0.234	3.225	0.053	No
w6 vs. w8	0.234	3.225	0.053	Do Not Test
w8 vs. w10	0.000	0.000	1.000	Do Not Test

b) Comparison between DLF and PT-DLF rats

Statistical test used: Two-way repeated measures ANOVA

Post-hoc analyses: Bonferroni t-test

Level of significance: $p < 0.05$

Source of Variation	DF	SS	MS	F	P
Lesion	1	0.0239	0.0239	1.754	0.215
Rat#(Lesion)	10	0.136	0.0136		
Time	3	0.288	0.0959	10.887	<0.001
Lesion x Time	3	0.0476	0.0159	1.804	0.168
Residual	30	0.264	0.00881		
Total	47	0.759	0.0162		

There were no differences between DLF and PT-DLF groups.

There was a statistical difference in PT-DLF group for factor **Time**

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
presurg vs. Week8	0.300	5.726	<0.001	Yes
presurg vs. Week10	0.223	4.258	0.003	Yes
presurg vs. Week2	0.175	3.336	0.028	Yes
presurg vs. Week6	0.150	2.873	0.024	Yes
Week6 vs. Week8	0.149	2.853	0.088	No
Week6 vs. Week10	0.0726	1.385	1.000	Do Not Test
Week6 vs. Week2	0.0243	0.464	1.000	Do Not Test
Week2 vs. Week8	0.125	2.390	0.251	Do Not Test
Week2 vs. Week10	0.0483	0.922	1.000	Do Not Test
Week10 vs. Week8	0.0769	1.468	1.000	Do Not Test

Data variable: Left forelimb vertical forces

a) In PT followed by DLF rats

Statistical test used: One-way repeated measures ANOVA

Post-hoc analyses: Bonferroni t-test

Level of significance: $p < 0.05$

Source of Variation	DF	SS	MS	F	P
Between Subjects	4	0.144	0.0360		
Between Treatments	4	0.545	0.136	19.970	<0.001
Residual	16	0.109	0.00683		
Total	24	0.798			

There was a statistical difference for factor Time

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
w2 vs. w8	0.336	6.437	<0.001	Yes
w2 vs. w10	0.332	6.360	<0.001	Yes
w2 vs. Presurgery	0.0550	1.052	1.000	No
w2 vs. w6	0.0516	0.987	1.000	Do Not Test
w6 vs. w8	0.285	5.450	<0.001	Yes
w6 vs. w10	0.281	5.373	<0.001	Yes
w6 vs. Presurgery	0.00341	0.0652	1.000	Do Not Test
Presurgery vs. w8	0.281	5.384	<0.001	Yes
Presurgery vs. w10	0.277	5.308	<0.001	Yes
w10 vs. w8	0.00400	0.0765	1.000	No

b) Comparison between DLF and PT-DLF rats

Statistical test used: Two-way repeated measures ANOVA

Post-hoc analyses: Bonferroni t-test

Level of significance: $p<0.05$

Source of Variation	DF	SS	MS	F	P
Lesion	1	0.0239	0.0239	1.754	0.215
Rat#(Lesion)	10	0.136	0.0136		
Time	3	0.288	0.0959	10.887	<0.001
Lesion x Time	3	0.0476	0.0159	1.804	0.168
Residual	30	0.264	0.00881		
Total	47	0.759	0.0162		

There were no differences between DLF and PT-DLF groups.

There was a statistical difference in PT-DLF group for factor **Time**

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
presurg vs. Week8	0.226	5.830	<0.001	Yes
presurg vs. Week10	0.153	3.938	0.006	Yes
presurg vs. Week2	0.128	3.302	0.030	Yes
presurg vs. Week6	0.118	3.043	0.025	Yes
Week6 vs. Week8	0.108	2.787	0.102	No
Week6 vs. Week10	0.0348	0.895	1.000	Do Not Test
Week6 vs. Week2	0.0101	0.259	1.000	Do Not Test
Week2 vs. Week8	0.0982	2.528	0.185	Do Not Test
Week2 vs. Week10	0.0247	0.636	1.000	Do Not Test
Week10 vs. Week8	0.0735	1.892	0.706	Do Not Test

Data variable: Right hindlimb vertical forces

a) In PT followed by DLF rats

Statistical test used: One-way repeated measures ANOVA

Post-hoc analyses: Bonferroni t-test

Level of significance: $p<0.05$

Source of Variation	DF	SS	MS	F	P
Between Subjects	4	0.0615	0.0154		
Between Treatments	4	0.383	0.0958	8.253	<0.001

Residual	16	0.186	0.0116
Total	24	0.631	

There was a statistical difference for factor Time
 All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
Presurgery vs. w8	0.282	4.141	0.008	Yes
Presurgery vs. w10	0.276	4.049	0.009	Yes
Presurgery vs. w6	0.0456	0.670	1.000	No
Presurgery vs. w2	0.0396	0.581	1.000	Do Not Test
w2 vs. w8	0.243	3.560	0.026	Yes
w2 vs. w10	0.236	3.468	0.032	Yes
w2 vs. w6	0.00604	0.0887	1.000	Do Not Test
w6 vs. w8	0.237	3.472	0.031	Yes
w6 vs. w10	0.230	3.380	0.038	Yes
w10 vs. w8	0.00628	0.0921	1.000	No

b) Comparison between DLF and PT-DLF rats

Statistical test used: Two-way repeated measures ANOVA

Post-hoc analyses: Bonferroni t-test

Level of significance: $p < 0.05$

Source of Variation	DF	SS	MS	F	P
Lesion	1	0.111	0.111	3.110	0.108
Rat#(Lesion)	10	0.358	0.0358		
Time	3	0.0572	0.0191	2.377	<0.001
Lesion x Time	3	0.0101	0.00337	0.421	0.739
Residual	30	0.240	0.00802		
Total	47	0.777	0.0165		

There was a statistically significant difference in time ($P = <0.001$)

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **lesion**

Comparison	Diff of Means	t	P	P<0.050
PT-DLF vs. DLF	0.0336	1.263	0.235	No

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
presurg vs. W2	0.197	4.651	<0.001	Yes
presurg vs. W8	0.113	2.674	0.044	Yes
W8 vs. W2	0.0838	1.978	0.186	No

Comparisons for factor: **Time within PT-DLF**

Comparison	Diff of Means	t	P	P<0.05
presurg vs. W2	0.232	3.872	0.003	Yes
presurg vs. W8	0.198	3.301	0.011	Yes
W8 vs. W2	0.0342	0.571	1.000	No

Comparisons for factor: **Time within DLF**

Comparison	Diff of Means	t	P	P<0.05
presurg vs. W2	0.162	2.706	0.041	Yes
presurg vs. W8	0.0288	0.480	1.000	No

W8 vs. W2	0.133	2.226	0.113	No
-----------	-------	-------	-------	----

Comparisons for factor: **lesion within presurg**

Comparison	Diff of Means	t	P	P<0.05
PT-DLF vs. DLF	0.113	2.033	0.051	No

Comparisons for factor: **lesion within W2**

Comparison	Diff of Means	t	P	P<0.05
PT-DLF vs. DLF	0.0434	0.779	0.443	No

Comparisons for factor: **lesion within W8**

Comparison	Diff of Means	t	P	P<0.05
DLF vs. PT-DLF	0.0558	1.002	0.325	No

Data variable: Left hindlimb vertical forces

a) In PT followed by DLF rats

Statistical test used: One-way repeated measures ANOVA

Post-hoc analyses: Bonferroni t-test

Level of significance: $p<0.05$

Source of Variation	DF	SS	MS	F	P
Between Subjects	4	0.144	0.0360		
Between Treatments	4	0.545	0.136	19.970	<0.001
Residual	16	0.109	0.00683		
Total	24	0.798			

There was a statistical difference for factor Time

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
w6 vs. w10	0.196	3.898	0.013	Yes
w6 vs. w8	0.186	3.707	0.019	Yes
w6 vs. w2	0.0501	0.998	1.000	No
w6 vs. Presurgery	0.0215	0.429	1.000	Do Not Test
Presurgery vs. w10	0.174	3.469	0.032	Yes
Presurgery vs. w8	0.165	3.278	0.047	Yes
Presurgery vs. w2	0.0286	0.569	1.000	Do Not Test
w2 vs. w10	0.146	2.901	0.104	No
w2 vs. w8	0.136	2.709	0.155	Do Not Test
w8 vs. w10	0.00960	0.191	1.000	Do Not Test

b) Comparison between DLF and PT-DLF rats

Statistical test used: Two-way repeated measures ANOVA

Post-hoc analyses: Bonferroni t-test

Level of significance: $p<0.05$

Source of Variation	DF	SS	MS	F	P
Lesion	1	0.116	0.116	7.050	0.024
Rat#(Lesion)	10	0.165	0.0165		
Time	3	0.0137	0.00457	0.804	<0.001

Lesion x Time	3	0.0274	0.00915	1.610	0.208
Residual	30	0.170	0.00568		
Total	47	0.493	0.0105		

There was a statistically significant difference in time ($P = <0.001$)
All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **lesion**

Comparison	Diff of Means	t	P	P<0.050
PT-DLF vs. DLF	0.0431	1.311	0.219	No

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
presurg vs. W2	0.228	6.504	<0.001	Yes
presurg vs. W8	0.142	4.051	0.002	Yes
W8 vs. W2	0.0861	2.453	0.070	No

Comparisons for factor: **Time within PT-DLF**

Comparison	Diff of Means	t	P	P<0.05
presurg vs. W2	0.302	6.085	<0.001	Yes
presurg vs. W8	0.221	4.458	<0.001	Yes
W8 vs. W2	0.0808	1.628	0.358	No

Comparisons for factor: **Time within DLF**

Comparison	Diff of Means	t	P	P<0.05
presurg vs. W2	0.155	3.112	0.016	Yes
presurg vs. W8	0.0631	1.271	0.015	Yes
W8 vs. W2	0.0915	1.841	0.241	No

II) Skilled locomotion (Section 7.4.3.2)

Statistical test used: Two-way repeated measures ANOVA

Post-hoc analyses: Bonferroni t-test

Level of significance: $p < 0.05$

Data variable: Forelimb correct steps

Source of Variation	DF	SS	MS	F	P
Lesion	2	5310.471	2655.236	24.612	<0.001
Col 46(Lesion)	14	1510.399	107.886		
TIME	2	119.930	59.965	0.801	0.459
Lesion x TIME	4	1273.396	318.349	4.252	<0.001
Residual	28	2096.310	74.868		
Total	50	10280.158	205.603		

There was a statistically significant interaction between Lesion and Time. ($P = <0.001$)

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Lesion**

Comparison	Diff of Means	t	P	P<0.050
DLF vs. PT+DLF	11.386	3.908	0.005	Yes
DLF vs. PT	6.907	2.261	0.121	No
PT vs. PT+DLF	4.479	1.466	0.494	No

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
ps vs. W6	33.824	12.485	<0.001	Yes
ps vs. W8	33.030	12.192	<0.001	Yes
ps vs. W2	32.397	11.959	<0.001	Yes
W2 vs. W6	1.427	0.527	1.000	No
W2 vs. W8	0.633	0.234	1.000	Do Not Test
W8 vs. W6	0.794	0.293	1.000	Do Not Test

Comparisons for factor: **Time within DLF**

Comparison	Diff of Means	t	P	P<0.05
ps vs. W6	28.292	6.227	<0.001	Yes
ps vs. W2	26.695	5.876	<0.001	Yes
ps vs. W8	14.927	3.285	0.012	Yes
W8 vs. W6	13.365	2.942	0.032	Yes
W8 vs. W2	11.768	2.590	0.079	No
W2 vs. W6	1.597	0.351	1.000	No

Comparisons for factor: **Time within PT**

Comparison	Diff of Means	t	P	P<0.05
ps vs. W8	43.548	8.750	<0.001	Yes
ps vs. W2	33.640	6.759	<0.001	Yes
ps vs. W6	30.088	6.045	<0.001	Yes
W6 vs. W8	13.460	2.704	0.059	No
W6 vs. W2	3.552	0.714	1.000	Do Not Test
W2 vs. W8	9.908	1.991	0.318	Do Not Test

Comparisons for factor: **Time within PT+DLF**

Comparison	Diff of Means	t	P	P<0.05
ps vs. W6	43.093	9.485	<0.001	Yes
ps vs. W8	40.615	8.940	<0.001	Yes
ps vs. W2	36.857	8.112	<0.001	Yes
W2 vs. W6	6.237	1.373	1.000	No
W2 vs. W8	3.758	0.827	1.000	Do Not Test
W8 vs. W6	2.478	0.545	1.000	Do Not Test

Comparisons for factor: **Lesion within ps**

Comparison	Diff of Means	t	P	P<0.05
PT vs. DLF	2.433	0.474	1.000	No
PT vs. PT+DLF	1.157	0.225	1.000	Do Not Test
PT+DLF vs. DLF	1.277	0.261	1.000	Do Not Test

Comparisons for factor: **Lesion within W2**

Comparison	Diff of Means	t	P	P<0.05
DLF vs. PT+DLF	8.885	1.815	0.226	No
DLF vs. PT	4.512	0.879	1.000	Do Not Test
PT vs. PT+DLF	4.373	0.852	1.000	Do Not Test

Comparisons for factor: **Lesion within W6**

Comparison	Diff of Means	t	P	P<0.05
PT vs. PT+DLF	14.162	2.758	0.024	Yes
PT vs. DLF	0.637	0.124	1.000	No
DLF vs. PT+DLF	13.525	2.763	0.024	Yes

Comparisons for factor: **Lesion within W8**

Comparison	Diff of Means	t	P	P<0.05
DLF vs. PT	26.188	5.100	<0.001	Yes
DLF vs. PT+DLF	24.412	4.986	<0.001	Yes

PT+DLF vs. PT	1.776	0.346	1.000	No
---------------	-------	-------	-------	----

Data variable: Hindlimb correct steps

Source of Variation	DF	SS	MS	F	P
Lesion	2	10142.931	5071.466	40.173	<0.001
Col 46(Lesion)	14	1767.391	126.242		
TIME	2	2182.401	1091.200	28.625	<0.001
Lesion x TIME	4	10816.883	2704.221	70.938	<0.001
Residual	28	1067.390	38.121		
Total	50	25276.578	505.532		

There was a statistically significant interaction between Lesion and Time. (P = <0.001)
All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Lesion**

Comparison	Diff of Means	t	P	P<0.050
PT vs. PT+DLF	30.349	12.200	<0.001	Yes
PT vs. DLF	13.271	5.335	<0.001	Yes
DLF vs. PT+DLF	17.078	7.200	<0.001	Yes

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
ps vs. W8	50.427	20.885	<0.001	Yes
ps vs. W2	41.220	17.072	<0.001	Yes
ps vs. W6	37.983	15.732	<0.001	Yes
W6 vs. W8	12.443	5.154	<0.001	Yes
W6 vs. W2	3.237	1.341	1.000	No
W2 vs. W8	9.206	3.813	0.003	Yes

Comparisons for factor: **Time within DLF**

Comparison	Diff of Means	t	P	P<0.05
ps vs. W6	49.725	12.280	<0.001	Yes
ps vs. W2	47.450	11.719	<0.001	Yes
ps vs. W8	38.183	9.430	<0.001	Yes
W8 vs. W6	11.542	2.850	0.040	Yes
W8 vs. W2	9.267	2.289	0.163	No
W2 vs. W6	2.275	0.562	1.000	No

Comparisons for factor: **Time within PT**

Comparison	Diff of Means	t	P	P<0.05
ps vs. W8	64.680	14.582	<0.001	Yes
ps vs. W2	13.494	3.042	0.024	Yes
ps vs. W6	3.180	0.717	1.000	No
W6 vs. W8	61.500	13.865	<0.001	Yes
W6 vs. W2	10.314	2.325	0.150	No
W2 vs. W8	51.186	11.540	<0.001	Yes

Comparisons for factor: **Time within PT+DLF**

Comparison	Diff of Means	t	P	P<0.05
ps vs. W2	62.717	15.489	<0.001	Yes
ps vs. W6	61.045	15.076	<0.001	Yes
ps vs. W8	48.417	11.957	<0.001	Yes
W8 vs. W2	14.300	3.532	0.006	Yes
W8 vs. W6	12.628	3.119	0.020	Yes
W6 vs. W2	1.672	0.413	1.000	No

Comparisons for factor: **Lesion within ps**

Comparison	Diff of Means	t	P	P<0.05
DLF vs. PT+DLF	7.873	1.860	0.205	No
DLF vs. PT	0.230	0.0518	1.000	Do Not Test
PT vs. PT+DLF	7.643	1.721	0.272	Do Not Test

Comparisons for factor: **Lesion within W2**

Comparison	Diff of Means	t	P	P<0.05
PT vs. PT+DLF	56.866	12.807	<0.001	Yes
PT vs. DLF	33.726	7.596	<0.001	Yes
DLF vs. PT+DLF	23.140	5.466	<0.001	Yes

Comparisons for factor: **Lesion within W6**

Comparison	Diff of Means	t	P	P<0.05
PT vs. PT+DLF	65.508	14.754	<0.001	Yes
PT vs. DLF	46.315	10.431	<0.001	Yes
DLF vs. PT+DLF	19.193	4.534	<0.001	Yes

Comparisons for factor: **Lesion within W8**

Comparison	Diff of Means	t	P	P<0.05
DLF vs. PT	26.727	6.019	<0.001	Yes
DLF vs. PT+DLF	18.107	4.277	<0.001	Yes
PT+DLF vs. PT	8.620	1.941	0.172	No

III) Skilled reaching (Section 8.4.3.3)

Statistical test used: Two-way repeated measures ANOVA

Post-hoc analyses: Bonferroni t-test

Level of significance: $p < 0.05$

Data variable: % successful reaches

Source of Variation	DF	SS	MS	F	P
Lesion	2	6782.823	3391.411	12.249	<0.001
Col 24(Lesion)	14	3876.335	276.881		
TIME	2	2155.894	1077.947	21.602	<0.001
Lesion x TIME	4	7957.246	1989.311	39.865	<0.001
Residual	28	1397.232	49.901		
Total	50	21668.146	433.363		

There was a statistical significant effect of lesion ($P = <0.001$)

There was a statistical significant effect of Time ($P = <0.001$)

There was a statistically significant interaction between Lesion and Time. ($P = <0.001$)

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Lesion**

Comparison	Diff of Means	t	P	P<0.050
DLF vs. PT	21.078	4.037	0.004	Yes
DLF vs. PT+DLF	19.240	3.865	0.005	Yes
PT+DLF vs. PT	1.838	0.352	1.000	No

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
ps vs. W8	35.845	14.608	<0.001	Yes
ps vs. W2	27.562	11.232	<0.001	Yes
ps vs. W6	23.916	9.746	<0.001	Yes
W6 vs. W8	11.929	4.862	<0.001	Yes
W6 vs. W2	3.646	1.486	0.869	No
W2 vs. W8	8.283	3.376	0.010	Yes

Comparisons for factor: **Time within DLF**

Comparison	Diff of Means	t	P	P<0.05
ps vs. W6	21.363	5.191	<0.001	Yes
ps vs. W8	18.037	4.383	<0.001	Yes
ps vs. W2	17.765	4.317	<0.001	Yes
W2 vs. W6	3.598	0.874	1.000	No
W2 vs. W8	0.272	0.0660	1.000	Do Not Test
W8 vs. W6	3.327	0.808	1.000	Do Not Test

Comparisons for factor: **Time within PT**

Comparison	Diff of Means	t	P	P<0.05
ps vs. W8	65.266	14.478	<0.001	Yes
ps vs. W2	28.214	6.259	<0.001	Yes
ps vs. W6	24.202	5.369	<0.001	Yes
W6 vs. W8	41.064	9.109	<0.001	Yes
W6 vs. W2	4.012	0.890	1.000	No
W2 vs. W8	37.052	8.219	<0.001	Yes

Comparisons for factor: **Time within PT+DLF**

Comparison	Diff of Means	t	P	P<0.05
ps vs. W2	36.707	8.920	<0.001	Yes
ps vs. W6	26.182	6.362	<0.001	Yes
ps vs. W8	24.232	5.888	<0.001	Yes
W8 vs. W2	12.475	3.032	0.025	Yes
W8 vs. W6	1.950	0.474	1.000	No
W6 vs. W2	10.525	2.558	0.085	No

Comparisons for factor: **Lesion within ps**

Comparison	Diff of Means	t	P	P<0.05
DLF vs. PT+DLF	11.752	1.919	0.194	No
DLF vs. PT	5.949	0.926	1.000	Do Not Test
PT vs. PT+DLF	5.803	0.904	1.000	Do Not Test

Comparisons for factor: **Lesion within W2**

Comparison	Diff of Means	t	P	P<0.05
DLF vs. PT+DLF	30.693	5.013	<0.001	Yes
DLF vs. PT	16.398	2.554	0.048	Yes
PT vs. PT+DLF	14.295	2.226	0.101	No

Comparisons for factor: **Lesion within W6**

Comparison	Diff of Means	t	P	P<0.05
DLF vs. PT+DLF	16.570	2.706	0.034	Yes
DLF vs. PT	8.788	1.369	0.544	No
PT vs. PT+DLF	7.782	1.212	0.705	No

Comparisons for factor: **Lesion within W8**

Comparison	Diff of Means	t	P	P<0.05
DLF vs. PT	53.178	8.282	<0.001	Yes
DLF vs. PT+DLF	17.947	2.931	0.019	Yes
PT+DLF vs. PT	35.232	5.487	<0.001	Yes