ROBOTICS TRAINING ALGORITHMS FOR OPTIMIZING MOTOR LEARNING IN SPINAL CORD INJURED SUBJECTS

Thesis by

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Abstract

The circuitries within the spinal cord are remarkably robust and plastic. Even in the absence of supraspinal control, such circuitries are capable of generating functional movements and changing their level of excitability based on a specific combination of properceptive inputs going into the spinal cord. This has led to an increase in locomotor training, such as Body Weight Support Treadmill training (BWST) for spinal cord injured (SCI) patients. However, today, little is known about the underlying physiological mechanisms responsible for the locomotor recovery achieved with this type of rehabilitative training, and the optimal rehabilitative strategy is still unknown.

This thesis describes a mouse model to study the effect of rehabilitative training on SCI. Using this model, the effects of locomotor recovery on adult spinal mice following complete spinal cord transaction is examined. Results that indicate adult spinal mice can be robotically trained to step, and when combined with the administration of quipazine (a broad serotonin agonist), there is an interaction and retention effect. Results also demonstrate that the training paradigm can be optimized in using "Assisted-as-Needed" (AAN) training. To find the optimal AAN training parameters, a learning model is developed to test the effect of various parameters of the AAN training algorithm. Simulation results from our model show that learning is training-dependent. In addition, the model predicts that improved motor learning can improve post-SCI by making the AAN training more adaptable.

The primary contributions of this thesis are twofold, in biology and engineering. We develop a mouse model using novel robotic devices and controls that can be used to study SCI and other locomotor disorders in the future by taking advantage of the many

different strains of transgenic mice that are commercially available. We also further confirm that sensory integration responsible for motor control is distributed throughout the hierarchy of the neuromuscular system and can be achieved within the isolated spinal cord. Lastly, by developing a learning model, we can start looking into how variability plays a role in motor learning, the understanding of which will have profound implications in neurophysiology, machine learning and adaptive optimal controls research.

Table of Contents

Acknowledgement iii

Abstract v

Table of Contents vii

List of Figures x

List of Publications xi

CHAPTER 1: Prologue 1
1.1 Motivation 1
1.2 Objective 2
1.3 Historical Backgrounds 2
1.4 Thesis Overview 3
1.4 Chapter References: 6

CHAPTER 2: Plasticity of functional connectivity in the adult spinal cord 9
2.1 Summary 9
2.2 Introduction 9
2.3 Some Biochemical and Electrophysiological Changes Associated with Improved Motor Performance in Spinal Animals 12
2.4 General Control Demands: Hierarchically Designed Networks 16
2.5 Hierarchical Command Combined with "Smart" Sensory Control 21
2.6 Sensory Modulation of Motor Tasks 23
2.7 Implications of Synesthesia for Rehabilitation 26
2.8 Conclusion 28
2.9 Chapter References: 30

CHAPTER 3: Experimental Background 38
3.1 Robotic Design 38
3.1.1 Hardware Design 40
3.1.1 Software Design 42
3.2 Animal Protocol 44
3.2.1 Anesthetic and Surgical Procedures 44
3.2.2 Post-Surgical Care 45
3.2.3 Drug Administration 46
3.2.4 Training Procedure 46
3.2.5 Testing Procedure 48
3.2.6 Euthanization 48
3.3 Data Analysis 49
3.3.1 Number of Steps 50
3.3.2 Step Periodicity 51
3.3.3 Shape Consistency 52
3.4 Summary 53

CHAPTER 4: Spinal Cord-Transected Mice Learn to Step in Response to Quipazine Treatment and Robotic Training 58
4.1 Abstract 58
4.2 Introduction 59
4.3 Experimental Methods 61
4.4 Results 63
4.5 Conclusions and Discussions 71
4.5.1 Quipazine facilitated spinal processing of sensory information associated with weight-bearing stepping 71
4.5.2 Manual training did not improve stepping performance 75
4.5.3 The robotic system significantly enhanced training effectiveness and enabled quantitative locomotion analysis 76
4.6 Summary 76
4.7 Chapter References: 78

CHAPTER 5: Implications of Assist-As-Needed Robotic Step Training after a Complete Spinal Cord Injury on Intrinsic Strategies of Motor Learning 87
5.1 Abstract 87
5.2 Introduction 88
5.3 Materials and Methods 90
5.4 Results 94
5.5 Discussion 96
5.5.1 All step training algorithms improved stepping beyond the level that is achieved without any step training. 96
5.5.2 Permitting an intrinsic network solution facilitates stepping more effectively after a SCI than imposing an extrinsic motor solution. 97
5.5.3 An imposed interlimb coordination pattern facilitates learning to step. 99
5.5.4 Distinction between shape consistency and quality of stepping. 100
5.6 Summary 101
5.7 Chapter References 102
5.8 Chapter Appendix: Computation of the Band Vector Fields 105

CHAPTER 6: Computational Model of Motor Learning Based on Intrinsic Variability in Stepping 107
6.1 Abstract 107
6.2 Introduction 107
6.3 Summary of the learning/training procedure 113
6.3.1 First Learning Rule 114
6.3.2 The Second Learning Rule 116
6.3.3 Applying the Learning Model to a Biomechanical Model 118
6.4 Results 119
6.4.1 First Learning Rule Simulation Results 120
6.4.2 Second Learning Rule Simulation Results 121
6.4.3 Biomechanical Model Simulation Results 121
6.5 Discussions and Conclusions 124
6.5.1 Similar to the Animal Experiments, Learning Models Show that Motor Learning Is Dependent on the Amount of Variability the Training Allows. 124

6.5.2 Learning Models Allows Us to Find More Optimal Rehabilitative Training Paradigm. 126
6.5.3 Principle of Neuromuscular Control and Sensorimotor Integration Resemble that of Adaptive Control Theory. 127
6.6 Summary 128
6.7 Chapter References: 129

CHAPTER 7 – Concluding Remarks 132
7.1 Summary 132
7.2 Future Work 133

List of Figures

Figure 2. 1: Force and EMG recordings from muscles of cats 13
Figure 2. 2: Coupling in the generation of limb movements during walking. 17
Figure 2. 3: Mean waveforms and normalize burst integral from the hindlimb 20
Figure 2. 4: Schematic of several features of the sensorimotor control of movement. 25

Figure 3. 1: Schematic of current step training system. 41
Figure 3. 2: Stepping trajectories of nontrained and trained mice. 43
Figure 3. 3: Fast Fourier transforms analysis 49
Figure 3. 4: Principle component analysis. 51

Figure 4. 1: Experimental timeline 61
Figure 4. 2: Summary of experimental results. 69

Figure 5. 1: AAN training paradigm I (Band). 91
Figure 5. 2: AAN training paradigm II (Window). 92
Figure 5. 3: The stepping trajectories of the ankle of a neonatal mouse. 94
Figure 5. 4: Average number of steps performed. 95
Figure 5. 5: Step rhythmicity as depicted by the plot of the inverse FWHM. 96
Figure 5. 6: Step shape consistency as measured by PCA. 97
Figure 5. 7: Convergent velocity field of a unit circle. 105

Figure 6. 1: Block diagrams of mechanical controllers. 110
Figure 6. 2: Schematic of a semi-active fixed-trajectory paradigm for step training. 112
Figure 6. 3: Idealized one dimensional learning model 114
Figure 6. 4: A simple biomechanical model of the mouse hindlimb. 118
Figure 6. 5: Numerical simulation of the first learning rule. 119
Figure 6. 6: Numberical simulation of the second learning rule 120
Figure 6. 7: The learning system tracking the hip angle. 122
Figure 6. 8: Application of the learning rule to the biomechanical model. 122
Figure 6. 9: The learning system tracking of a physiological trajectory. 123
Figure 6. 10: Actual ankle trajectory recordings. 125
Figure 6. 11: Tracking of a circular target using a variable window size 126

List of Publications

Below is a complete list of references of publications of the author that have been included in this thesis work

1. Cai, L.L., Fong A.J., Otoshi, C.O., Liang, Y.Q., Burdick, J.W., Roy, R.R., Edgerton, V.R. "Implications of Assist-As-Needed Robotic Step Training after a Complete Spinal Cord Injury on Intrinsic Strategies of Motor Learning" *J Neurosci* (in review)
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CHAPTER 1: Prologue

1.1 Motivation

> "*The frog instantly dies when the spinal cord is pierced; and previous to this it lived without head, without heart or any bowels or intestines or skin; and here therefore it would seem lies the foundation of movement and life.*" – Leonardo da Vinci.

Spinal cord injury (SCI) is one of the most traumatic conditions a person will have to live through, affecting every aspect of daily life, resulting in an enormous impact from psychological and social perspective (Bedbrook 1987). Spinal cord injury has an enormous economical impact as well. As of 2005, it is estimated that a person with a paraplegic spinal cord injury person will need to spend more than $250,000 during the initial year of injury and more than $25,000 each subsequent year (SCIIN 2005). The estimate is even higher for tetraplegia patients.

Currently, there are between 250,000 and 400,000 Americans suffering from spinal cord injury and an additional 11,000 Americans are struck with spinal cord injury each year (NSCIA 2006). Many of these injuries are caused by accidents such as motor vehicle accidents, falls and sport injuries. As such, the demographic group most likely to suffer a spinal cord injury is men (~80%) between 16 and 30 years old (NSCIA 2006). Therefore, depending on the intensity of the injury, many of these people have to live with disability, and most likely paralysis, for the greater part of their adult life. Thus, any research that can improve their mobility and motor functions will not only greatly improve their quality of life, it will have significant impacts on the general population as a whole.

1.2 Objective

As the name implies, bioengineering is an interdisciplinary field that combines biology and engineering. Because of this synergy between the physical and biological sciences, many advances have been made recently in the development and application of technology and the adaptation of new engineering discoveries to biology and medicine. This, however, should not be the only goal of bioengineering. A less explored route of bioengineering is to use engineering technologies to further investigate and contribute to the better understanding of basic biological, physiological and pathological processes. This has been the driving force behind this thesis work. Our objective is to develop robotic devices and control algorithms for spinal cord injury rehabilitation. In the meantime, using these devices, we want to examine the neural mechanism responsible for the plasticity observed in the isolated spinal cord, thus providing an insight into motor learning and neuromuscular control in general.

1.3 Historical Backgrounds

Spinal vertebral injury has attracted the interests of the medical and scientific community ever since the dawn of civilization, with documentations dating as far back as 2500 B.C.; however, progress in treating and curing spinal cord injury has been slow in coming (Hughes 1988). It took more then two centuries before the central role of the spinal cord was even recognized (Lifshutz and Colohan 2004). Guy de Chauliac (1300–1368), considered by many to be the father of modern surgery, had once written, “One should not labor to cure the paralysis of spinal cord injury,” a sentiment shared by many for centuries (Walker 1967; Lifshutz and Colohan 2004). However, within the last centuries,

there has been a renewed effort in the scientific community to study spinal cord injury. Researchers from many fields are tackling the problem of spinal cord repair with a number of different approaches. Major areas of research include: neural regeneration, sometimes called neuroengineering, where researchers try to reconnect the damage neural tissues through axon regeneration (Baitinger, Cheney et al. 1983; Herdegen, Skene et al. 1997); stem cell research, where stem cells are implanted in the injured spinal cord for regrowth (Gimenez y Ribotta, Gaviria et al. 2002; Luque and Gimenez y Ribotta 2004; Pencalet, Serguera et al. 2006); neural stimulation and epidural stimulation, where the isolated spinal cord is stimulated electrically to elicit locomotor activities (Dimitrijevic and Dimitrijevic 2002; Gerasimenko, Avelev et al. 2003; Minassian, Jilge et al. 2004); biochemistry and pharmacology (Rossignol and Barbeau 1993; Tillakaratne, Mouria et al. 2000); and rehabilitation (de Leon, Hodgson et al. 1998; Edgerton, Leon et al. 2001). Perhaps some of the greatest clinical advances in the care of patients with SCI during this century have been in physical therapy and rehabilitation (Lifshutz and Colohan 2004; Edgerton, Kim et al. 2006). This advancement resulted from our progressive understanding of spinal cord plasticity as well as neural control of locomotion post-SCI, which is reviewed in detail in the following chapter.

1.4 Thesis Overview

This thesis is comprised of peer-reviewed articles from archival journals in biological science and engineering. The author is either the lead author or co-author of these articles. The organization of this thesis is meant to highlight the interdisciplinary nature

of the topics at hand while providing a clear emphasis on the discipline-specific contributions of this work.

Chapter 2 will give an indepth review of spinal cord plasticity and neural control of locomotion post spinal cord injury (SCI), which is critical in understanding the significance of this thesis work. This review brings together perspectives from various disciplines to emphasize the importance of variability in neural plasticity even at the spinal cord level.

Chapter 3 consists of experimental background information including description of robotic design, surgical procedures, animal care and data analysis techniques that will be the basis of studies described in the subsequent chapters, mainly Chapter 5 and 6. The critical contributions of this chapter will be on the development of a mouse model to study SCI as well as other neuromuscular disease, and on the implantation of quantitative assessment of locomotor performance rather than relaying on frequently used qualitative methods such as the Basso, Beattie and Bresnahan (BBB) locomotor rating scale.

Chapter 4 and 5 together consist of two animal studies, the first of which is used to examine the feasibility of our animal model. Using this model, we have examined: 1) Whether adult mice with a complete spinal cord transaction can be robotically trained to step; 2) the effect of pharmacological agents such as quipazine (a broad serotonin against) has on the locomotor recovery post-SCI; 3) the combining effect of robotic training and quipazine administration. In the second animal study, we develop various forms of robotic training to see if rehabilitative training will be more optimal if the training is done in an assist-as-needed manner, thereby challenging the injured subject to use the intrinsic neural circuitries that reside within the isolated spinal cord. The results

of this study have great implications on understanding the underlying mechanism behind locomotor recovery.

Chapter 6 consists of the theoretical contribution of this thesis, which examines how optimal robotic-facilitated rehabilitative training can be achieved, giving the intrinsic properties of neuromuscular control of locomotion. It provides a learning model that captures pheromones such as "learned helplessness" and indicates how motor learning can be best achieved.

Lastly, Chapter 7 consists of concluding remarks that will discuss relevance and contribution of this thesis. In addition, it will touch upon the future direction of this research.

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CHAPTER 2: Plasticity of functional connectivity in the adult spinal cord

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2.1 Summary

This chapter emphasizes several characteristics of the neural control of locomotion that provide opportunities for developing strategies to maximize the recovery of postural and locomotor function after a spinal cord injury (SCI). The major points of this chapter are: 1) the circuitry that controls standing and stepping is extremely malleable and reflects a continuously varying combination of neurons that are activated when executing stereotypical movements; 2) the connectivity between neurons is more accurately perceived as a functional rather than as an anatomical phenomenon; 3) the functional connectivity that controls standing and stepping reflects the physiological state of a given assembly of synapses, where the probability of these synaptic events is not deterministic; 4) rather, this probability can be modulated by other factors such as pharmacological agents, epidural stimulation, and/or motor training; and 5) the variability observed in the kinematics of consecutive steps reflects a fundamental feature of the neural control system.

2.2 Introduction

The title of this chapter may induce a myriad of perceptions, most of which will imply physiological mechanisms related to how the adaptation of neural events within the central nervous system (CNS) respond to a spinal cord injury (SCI). Clearly, after an injury of any part of the neuromuscular system, there are changes in the connectivity of

those sensorimotor circuits that generate a motor task. Changes also occur during the subsequent adaptive events that follow the injury. In this chapter, emphasis will be placed on the concept of functional rather than anatomical connectivity within the spinal cord. The term "functional connectivity" will be used to indicate that the likelihood of a given neuron being activated is dependent on its physiological state of "readiness" rather than merely on the existence of an anatomical connection. This emphasis is not to imply that changes in the actual number of synaptic connections cannot or do not occur in response to SCI. In fact, there is good evidence for the presence of such adaptations and that these changes can be associated with an improvement in motor performance capacity following a SCI (Bregman, Diener et al. 1997; Raineteau and Schwab 2001; Bregman, Coumans et al. 2002). Instead, this chapter will focus on the importance of rapid, and sometimes persistent, changes in functional connectivity between a given combinations of spatially and temporally linked sensory and motor circuits that are involved in the generation of posture and locomotion. A measure of functional connectivity, in the context of how we are using this term, is the probability of a specific set of neurons being activated for a given physiological state.

Many correlations have been drawn between anatomical connections and functional recovery post-SCI (Hase, Kawaguchi et al. 2002; Lee, Lin et al. 2004). However, the variability in normal stepping, even under well-controlled conditions, demonstrates the versatility and complexity in the activation of the associated spinal circuitry. We propose that as the physiological states change, the continuous adaptation in functional connectivity brings about routine variability in the activation patterns during repetitive movements, such as stepping. As a result, no two steps are generated by the

same combination and sequence of neuronal activation. As the limb trajectory varies from step to step, the precise pattern of activation of the involved motor pools also must vary (Figure 2.1). This variation is reflected in the electromyographic (EMG) signals from normal (Courtine, Roy et al. 2005) and complete spinal animals (Lovely, Gregor et al. 1990; Edgerton, Roy et al. 1992) stepping on a treadmill. Thus, even within the robust size principle of recruitment of motor neurons (Burke and Edgerton 1975; Henneman and Mendell 1981), there remains a significant level of variability in the exact combination and order of motor neurons activated within a given motor pool, and certainly across synergistic motor pools (Cope and Sokoloff 1999), to generate a specific movement.

The source of this variability in stepping is undoubtedly derived from both supraspinal as well as spinal neuronal networks. It also is reasonably obvious that the variability in limb kinematics will be greater following a SCI as recovery of stepping occurs either spontaneously or as a result of motor training. After a SCI, however, the variability in stepping is significantly reduced by motor training (de Leon, Hodgson et al. 1998). We have proposed that this variability reflects the intrinsic probability of a given assembly of neurons being activated at any given time (Edgerton, Roy et al. 2001). Thus, the underlying explanation for the presence of variability in the limb kinematics during stepping under normal conditions is that whether or not an assembly of neurons is activated is not deterministic at any given instant.

After a SCI, the probability that an appropriate combination of neurons is activated in the appropriate sequence is markedly altered. During the "reorganization" of the spinal circuitry following SCI, these probabilities can become lower or higher depending, to a large degree, on the frequency with which the sensorimotor circuits

experience the specific patterns of activity. For example, the repetitive performance of a motor task, such as stepping, over a period of weeks increases the probability of completing a successful step (Lovely, Gregor et al. 1986; Barbeau and Rossignol 1987; de Leon, Hodgson et al. 1998). It appears from the results of virtually all of the studies involving motor training after a SCI that the benefits of step training can be manifested as an increased probability of generating a successful step. At the systems level, a number of motor training-induced biochemical and electrophysiological changes in the spinal cord are associated with improved motor performance after a SCI (de Leon, Tamaki et al. 1999; Tillakaratne, Mouria et al. 2000; Tillakaratne, de Leon et al. 2002; Cote, Menard et al. 2003; Cote and Gossard 2004).

2.3 Some Biochemical and Electrophysiological Changes Associated with Improved Motor Performance in Spinal Animals

Prior research has identified a number of biochemical and physiological changes in the spinal cord after a complete thoracic spinal cord transection in response to training of a specific motor task. Many of these changes have been reviewed recently (Edgerton, Tillakaratne et al. 2004). Briefly, the biochemical changes generally reflect an up-regulation of both the glycinergic and GABAergic (Gamma-aminobutyric acid) neurotransmitter systems within the lumbosacral spinal cord. The biochemical indicators consist of an increased number of glycinergic receptors (Edgerton, Leon et al. 2001), an increased responsiveness to strychnine administration, an agent that blocks the glycinergic receptor (de Leon, Tamaki et al. 1999), an increased level of glutamic acid decarboxylase (GAD_{67}) (Tillakaratne, Mouria et al. 2000), and improved locomotion when blocking GABAergic inhibition with biccuculine (Robinson and Goldberger 1986;

Robinson and Goldberger 1986). As importantly, however, is the observation that the increased level of inhibition in the spinal cord after a SCI can be countered by motor training (Edgerton, Leon et al. 2001; Tillakaratne, de Leon et al. 2002; Edgerton, Tillakaratne et al. 2004).

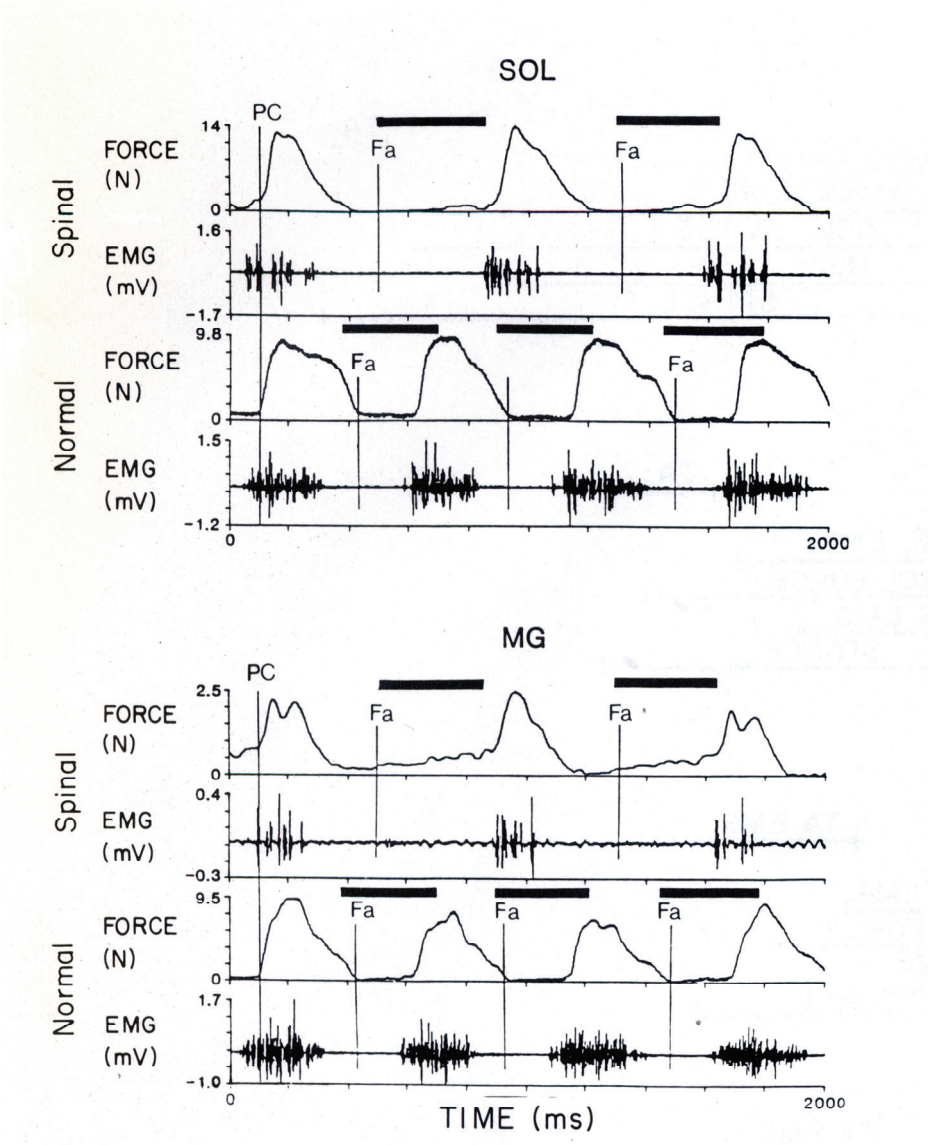

Figure 2.1 Force and EMG recordings from the soleus (SOL, top) and medial gastrocnemius (MG, bottom) muscles of a normal, intact cat and an adult spinal cat during stepping on a treadmill at 0.8 m/sec. Bold bars indicate the period of contralateral support. Compared to normal, the spinal cat exhibited a longer cycle period, a steeper decline in force beginning at mid-support, a delay in the onset of flexion at the ankle (Fa), lower peak EMG forces, and clonus in the EMG and force records of both muscles. PC, paw contact. (taken from Lovely et al. 1990)

It appears that repetition of a specific motor task reduces the level of persistent inhibition present in the neural networks that normally generate the motor task. These effects have been demonstrated in spinal animals that have been trained to step (de Leon, Hodgson et al. 1998) or stand (de Leon, Hodgson et al. 1998). However, it remains unclear as to how specific excitatory vs. inhibitory neural pathways are modulated by repetitive use. Repetitive use of the extensor musculature may down-regulate the GAD_{67} associated with extensor motor neurons, and simultaneously enhance the levels of GAD_{67} by increasing the inhibition of flexor motor neurons (Tillakaratne, de Leon et al. 2002). A limitation of these observations is that linking the level of excitation vs. inhibition of specific neural pathways to specific adaptations within the different neurotransmitter systems has not been possible to date. Furthermore, there has been relatively little identification of the receptor subtypes that may be associated with the observed level of behavioral performance. These data are further limited in that it is not certain whether the observed biochemical changes are simply correlated with changes in motor performance as opposed to there being a cause and effect relationship. Further studies are needed to address these issues.

Electrophysiological changes also have been observed in chronic spinal animals, and there is strong evidence that the efficacy of selected neuromotor pathways can be modified by repetitive training of a motor task. For example, there is improved coordination of motor pools controlling the hindlimb musculature following step training in spinal animals, as shown by EMG bursting patterns (Lovely, Gregor et al. 1990). Likewise, step training greatly improves the transmission in polysynaptic excitatory group I load pathways (Cote, Menard et al. 2003) that convey locomotor drive to extensor

motor neurons, and thus could contribute to improved recovery of weight-bearing during stepping in spinal animals. The mean amplitude of excitatory postsynaptic potentials has similarly been reported to increase in response to activation of skin sensory receptors located under the paw of chronic spinal cats that have been trained to step (Cote and Gossard 2004). Recent observations also indicate that improved stepping following training in complete spinal rats correlates with the peak amplitude of the segmental excitatory post-synaptic potential and action potential afterhyperpolarization depth of those motor neurons recruited during locomotor activity (Petruska, Ichiyama et al. 2004).

Based on the results of these electrophysiological studies, it appears that in chronic spinal animals, a number of spinal neural pathways can respond specifically to step training. Accordingly, it is likely that after repetitive exposure of the spinal cord to a given motor task, significant alterations may occur in the transmission of many, if not all, sensorimotor pathways caudal to the lesion. Such task-dependent functional plasticity of the spinal motor infrastructure could, in turn, contribute to the observed decrease in the intrinsic variability in performing a motor task (de Leon, Hodgson et al. 1998), i.e., there would be an increase in the probability of activating specific motor pathways, and therefore specific functional sets of neurons, to accomplish the required task (Edgerton, Roy et al. 2001). These results suggest an improved efficacy of the interneurons that are responsible for coordinating motor pools, e.g. Ia interneurons that provide reciprocal inhibition between antagonistic motor pools. Direct measurements of decreases followed by increases in the excitability of synapses associated with Ia interneurons in response to spinal cord transection have been observed, but not the training-related changes (Valero-Cabre, Fores et al. 2004; Valero-Cabre, Tsironis et al. 2004). On the other hand,

significant increases in the excitability of lumbar monosynaptic reflexes have been reported (Thompson, Parmer et al. 1998).

Thus, it seems reasonable to hypothesize that the observed biochemical and electrophysiological adaptations of spinal animals associated with step training are not due to an induction of a specific set of synaptic events required for the acquisition of stepping ability. Rather, these adaptations result from the increased probability of the neural circuitries within the spinal cord of generating a successful step.

2.4 General Control Demands: Hierarchically Designed Networks

Several observations demonstrate that supraspinal control can be, and probably often is, relatively nonspecific. Supraspinal input can instruct the spinal cord to walk by providing a relatively nonspecific tonic input, leaving the detailed decisions of which neuronal systems have to be activated at the spinal level. Stepping can be induced in decerebrated animals via tonic stimulation of the mesencephalic locomotor region (Shik, Severin et al. 1966). Fictive locomotion can be generated via stimulation of the dorsal roots of the spinal cord (Sjostrom and Zangger 1976). Locomotion can be induced pharmacologically in complete spinal animals (Rossignol and Barbeau 1993; de Leon, Tamaki et al. 1999; Antri, Orsal et al. 2002; Orsal, Barthe et al. 2002), and chronic complete, low thoracic spinal animals can be trained to step even without any pharmacological enhancement (Lovely, Gregor et al. 1986; Barbeau and Rossignol 1987; de Leon, Hodgson et al. 1998). Another illustration of the nonspecific nature of the signals (in this case from the periphery) that generate walking is the locomotor-like movements that can be elicited in humans in a recumbent position by applying non-specific tonic vibration to the relaxed leg musculature (Gurfinkel, Levik et al. 1998).

These widely different manipulations represent an impressive array of different techniques that change the physiological state of the spinal cord, all having a remarkably similar effect, i.e. they induce or improve stepping ability.

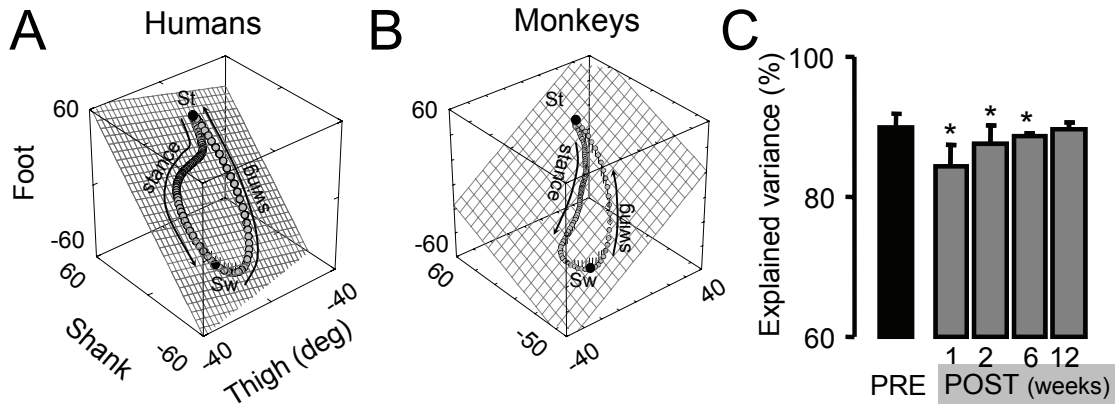

Figure 2.2: Coupling in the generation of limb movements during walking in humans and monkeys. (**A**) When plotted in a 3-D space, the angular oscillation of thigh, shank, and foot segment with respect to the direction of gravity, i.e. elevation angles, covaries close to a plane, both during human (**A**) and monkey (**B**) locomotion. The gait loop evolves in the counterclockwise direction. The onset of stance (St) and swing (Sw) are indicated. (**C**) The degree of coupling among limb movements is evaluated by applying a principal component (PC) analysis on the elevation angles of hindlimb segments (thigh, shank, foot). Mean (SD) values of the variance explained by the first PC during treadmill locomotion performed pre-lesion (PRE) and 1, 2, 6, and 12 weeks after a unilateral lesion to the thoracic dorsolateral column (POST) is shown for 3 monkeys. *, significant difference between pre- and post-lesion values. The high variance accounted for by the first PC reflects the high degree of coupling in the neuronal systems that generate the oscillation of the limbs during stepping both in intact and spinal cord-injured animals. (adapted from Courtine et al., 2005a)

The generality of the supraspinal, and even spinal, commands also is apparent from the strong interrelationships among the kinematics of multiple joints within and across limbs during locomotion in intact, as well as in complete and incomplete SCI animals (Figure 2.2). This stereotypical output implies a close link between the neuronal systems controlling each of these joints and all of the musculature associated with their dynamics. Such a high intrinsic coupling in the generation of limb oscillation also simplifies the details required by the brain to generate a complex motor task, such as stepping at a range of speeds (Bianchi, Angelini et al. 1998), grades (de la Torre and

Goldsmith 1990), and directions (Courtine and Schieppati 2004). Furthermore, this stereotypical output supports the concept of automaticity in the control of locomotion (Orlovsky and Feldman 1972). Briefly, automaticity is the ability to generate a range of motor tasks, such as stepping and standing, in response to highly predictable ensembles of sensory stimuli from the periphery and motor commands from the brain. Considerable automaticity remains within the spinal cord in the absence of supraspinal input.

One source of such automaticity is found in the organization of the spinal circuits generating the motor patterns for walking. Stimulation of such neural circuits, often referred to as central pattern generators (CPGs), produces rhythmic alternating flexor and extensor activity in several vertebrate models, e.g., lamprey eels, neonatal rats, or adult cats (Arshavsky, Deliagina et al. 1997; Grillner 2002; MacKay-Lyons 2002) that mimics locomotion. The structural organization of these neural circuitries in mammals, however, are unknown, but even within this most automated action from CPGs, automatic adjustments can be made by varying levels of hierarchical control. For example, extensor or flexor muscle activity can be altered independently during fictive locomotion without affecting the ongoing locomotor rhythm (Lafreniere-Roula & McCrea, 2005). This observation suggests that the spinal-generated rhythmical input drives multiple pattern formation modules, and that the activity of these modules can be modified by other sources, e.g. sensory or supraspinal inputs. Such hierarchical control would introduce both stereotypical and ongoing adjustments (variability) in the motor output to adjust locomotor kinematics. This hierarchal control can range from volitional circuits to extreme automaticity, i.e., CPGs. Although there is still no direct evidence for the existence of locomotor spinal circuits that display CPG properties in humans,

Dimitrijevic et al. report that non-patterned electrical stimulation of the posterior structures of the lumbar spinal cord in subjects with complete, long-term SCI can induce rhythmic, alternating stance and swing phases of the lower limbs (Dimitrijevic, Gerasimenko et al. 1998).

This predictability of a stereotypic stepping pattern may seem contradictory to the concept noted above that variability in stepping reflects an important feature of the neural control system. However, it should be recalled that spinal interneurons receive input from supraspinal, as well as from spinal, sources, and from the periphery. Nevertheless, given the apparent hierarchical organization of the neuronal systems that generate motor patterns and interpret sensory information as implied above, there are multiple combinations and levels of neuronal control systems that underlie the variability observed during stepping while simultaneously maintaining a very high probability of success from step to step (Figure 2.3).

It is worth noting that recovery of locomotion kinematics after an incomplete SCI in the monkey to levels observed pre-lesion (Figure 2.3) does not imply re-establishment of pre-lesion muscle synergies, but instead reflects novel activation patterns of interneurons and motor pools that may be possible as a result of the hierarchal features noted above (Courtine, Roy et al. 2005). It also has been found that new motor patterns underlie the learning of foot kinematics that are similar to those of non-disabled individuals during treadmill stepping following training in humans with an incomplete or complete SCI (Wernig, Muller et al. 1995; Grasso, Ivanenko et al. 2004). Such findings provide evidence that successful stepping, as defined kinematically, can be achieved

through activation of a variety of spinal motor neurons, and that there is no fixed locomotor circuitry for the generation of stepping in mammals, including primates.

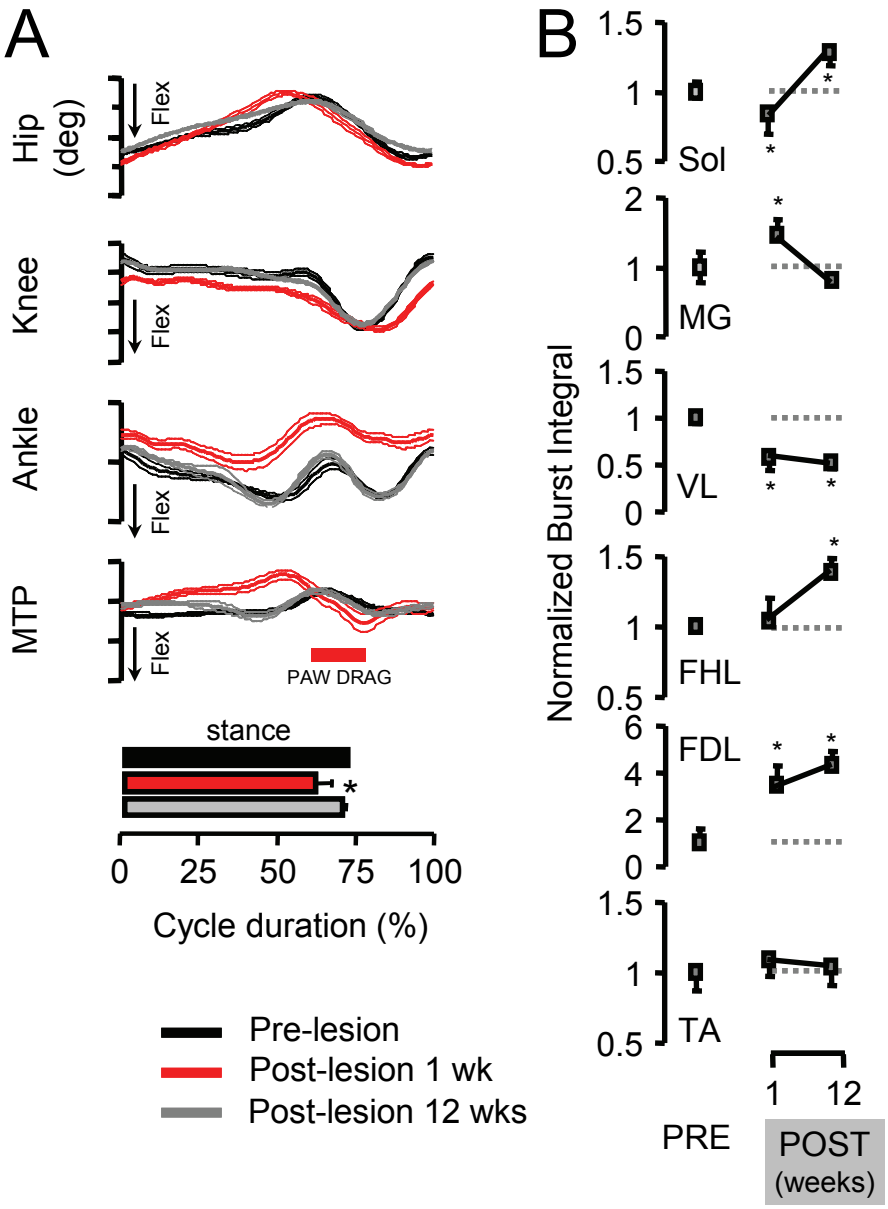

Figure 2.3: (**A**) Mean (SD) waveforms of each joint angle for the hindlimb ipsilateral to the lesion side recorded during treadmill locomotion (0.45 m/s) before (Pre-lesion) and 1 and 12 wks after (Post-lesion) a unilateral interruption of the lateral corticospinal tract in the thoracic spinal cord of adult Rhesus monkeys (n = 2)**.** The horizontal bars at the bottom indicate the mean (SD) value of the stance phase duration. (**B**) Mean (SD) values of EMG burst integrals for all recorded muscles. Sol, soleus; MG, medial gastrocnemius; VL, vastus lateralis; FHL, flexor hallucis longus; EDL, extensor digitorum longus; TA, tibialis anterior. Values are normalized to the Pre-lesion baseline (dashed lines) computed as the mean value of muscle activity during Pre-lesion locomotion. *, significant difference between pre- and post-lesion values. (Adapted from Courtine et al. 2005b)

Besides being able to accommodate the control that can be exerted by the brain on specific interneuronal assemblies or motor pools, even more direct neural connections must be present to control specific muscle units or combinations of units (Figure 2.4). It is generally accepted, at least in primates, that there are direct projections from the corticospinal tract to spinal motor neurons (cortico-motoneuronal connections), although a small portion of the corticospinal projections actually represent a direct target to motor neurons (Lacroix, Havton et al. 2004). The cortical projection to the spinal cord can be an important source of modulation in the production of skilled locomotor movements, such as stepping over an obstacle or the precise positioning of the paw during walking (Lawrence and Kuypers 1968; Georgopoulos and Grillner 1989; Drew, Jiang et al. 2002; Courtine, Roy et al. 2005; Courtine, Roy et al. 2005). Theoretically, corticospinal input to subsets of interneurons that control specific combinations of muscle units in specific motor pools (Drew et al., 2002 Fetz, E. E., S. I. Perlmutter, Current Op Neuro, 2000; Lemon et al., Prog Brain Res. 2004) could allow for precise voluntary activation and frequency control of groups of motor units (Kuypers 1978).

2.5 Hierarchical Command Combined with "Smart" Sensory Control

The progression from a supraspinal motor command to the detailed control of hundreds of thousands of muscle fibers reflects a phenomenal rostral-to-caudal anatomical and physiological divergence. Whereas convergence of inputs from cortical cell populations onto motor neuron assemblies exists, there is also a remarkable divergence in how the supraspinal drive can affect various interneuronal circuits (Figure 2.4). Similarly, projections of the signals from specific sensory receptors in the periphery

to the spinal cord networks and brain are highly divergent. For example, a single muscle spindle alerts thousands of neurons within the spinal cord (and even more in the brain) that a signal related to the physical environment of that mechanoreceptor has been generated (Scott and Mendell 1976). It is inevitable that all of the sensory information from the periphery projects to networks of neurons within the spinal cord. At the same time, it is apparent that the signals from multiple receptors merge and project at some level to common, as well as unique, combinations of neurons within the spinal cord. Every unique combination of neurons can, in turn, readily recognize complex and very specific sensory patterns that can trigger the appropriate motor responses for a given pattern of sensory information. In other words, a given pattern of sensory information provides very specific and recognizable information to the neurons that eventually generate the appropriate motor response to that given ensemble of sensory information. To what degree this extensive divergent and convergent information from the periphery is processed and integrated within the spinal networks prior to relaying this information to the brain is unknown, but it is readily apparent that these complex patterns of sensory input provide a continuous stream of critical information for the ongoing control of specific motor responses such as stepping and standing.

These observations are not meant to imply that the neural circuits within the spinal cord segments have a lesser role than the brain circuits in controlling stepping. For example, the ability of the spinal circuitry to generate rhythmic motor patterns that mimic locomotion without any sensory input, i.e., fictive locomotion, is clear (Grillner 2003). This fictive central pattern generation, however, cannot make any adjustments to changes in its environment and, therefore, there are no mechanisms to change stepping frequency,

to modulate the appropriate level of load bearing, or to adjust to any, even slight, perturbation. In fact, not only does the afferent input provide the spinal locomotor circuits with information related to unexpected events, but the ongoing sensory flux also contributes substantially to the activation of motor neurons, even under normal walking conditions (Pearson 2004). On the other hand, this central pattern generation, combined with its massive online sensory information processing capability, can effectively generate motor tasks such as stepping and standing without input from the brain (Edgerton, Tillakaratne et al. 2004).

2.6 Sensory Modulation of Motor Tasks

What is the evidence that the sensory information derived from the limbs during posture and locomotion represent a critical and primary influence on motor control in complete spinal animals? Several experiments demonstrate that sensory information can define most details of all postural and locomotor movements. Administration of strychnine, which blocks glycinergic inhibition, at a dose that did not generate spontaneous rhythmic motion of the hindlimbs, facilitated consistent, full weight-bearing treadmill stepping of the hindlimbs in chronic complete spinal cats that otherwise could not step (de Leon, Tamaki et al. 1999). In addition, the rate of stepping was modulated to accommodate the speed of the treadmill belt. These results demonstrate that the spinal cord was not induced to generate rhythmic activity and stepping by strychnine itself, but that strychnine changed the physiological state of the spinal neural circuits so that the sensory information could be processed and transformed with sufficient accuracy to control locomotion over a range of speeds and levels of loading. Similar observations

have been made after the administration of quipazine, a serotonergic agonist, to mice having a complete SCI (Fong, Edgerton et al. 2003; Fong, Cai et al. 2005). Combined, these results clearly demonstrate that the sensory input associated with standing and stepping generates successful and remarkably adaptive control of posture and locomotion in the absence of supraspinal input. Under these conditions, this adaptive control cannot be solely attributed to central pattern generation, i.e., repetitive cycles of flexion and extension. A very important additional feature of the neural circuitry that generates these patterns is its ability to interpret the sensory input in a manner that becomes meaningful to the success of the hindlimbs in responding to its environment.

Other observations support the importance of the interaction between CPG and sensory processing. Results similar to those described for strychnine above were observed when the dorsum of the lumbosacral spinal cord of complete mid-thoracic spinal rats (Ichiyama, Gerasimenko et al. 2005) and cats (Gerasimenko, Avelev et al. 2003; Gerasimenko, Lavrov et al. 2005) was stimulated via epidural electrodes. In this case, a tonic general stimulation of modest intensity applied to the dorsum of the spinal cord did not induce any rhythmic, step-like motion. When the hindlimbs were placed on a moving treadmill belt, however, the animals stepped at a rate consistent with the speed of the treadmill belt. Previous experiments also have demonstrated that complete spinal cats receiving tonic electrical stimulation of the dorsum of the lumbar spinal cord can step backwards when their hindlimbs are placed on a treadmill belt moving forward (Gerasimenko, Avelev et al. 2003). These data demonstrate that detailed complex signals that drive motor pools in a highly coordinated fashion can be derived from very general patterns of stimuli to the lumbosacral spinal cord. Furthermore, these experiments clearly

indicate that the sensory information provided to the spinal cord essentially defines the type of motor task that will be performed, as well as the characteristics of the motor pattern associated with the task.

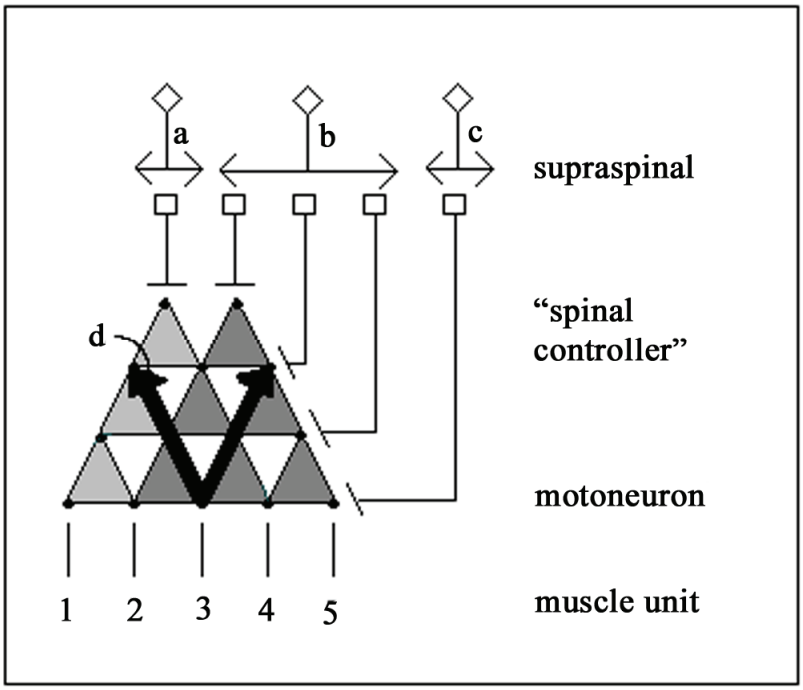

Figure 2.4: Cartoon depicting several features of the sensorimotor control of movement. The cartoon illustrates the possibility of a supraspinal control center with neurons projecting to control level neurons ("spinal controllers" of movements of differing complexities) that would project to a group of synergistic motor pools, muscles and muscle units. In cases illustrated by the projection of neuron **a** or neuron **b**, specific control of a small group of motor units might be unnecessary in executing a generalized motor program to control stepping. The numbers 1-5 denote five muscle units. The dots embedded in the triangles represent individual neurons. Activation of neuron **a** would result in muscle units 1-4 being recruited. Neuron **b** would recruit muscle units 2-5, whereas neuron **c** would recruit only muscle unit 5. On the other hand, there can be even more selective control of motor units as illustrated with neuron **c**. At least for some muscle groups in some species, there may be direct supraspinal connections to some motor pools as well as the more generalized command neurons that exert more general control signals among motor pools. Two sets of divergent triangles are illustrated to point out the flexibility in modulating the set of muscles may be recruited for a given movement. One also can view the upright triangles in the reverse direction (see arrows projecting upward, labeled as **d**), symbolizing a single sensory receptor projecting rostrally and diverging markedly, thus illustrating a single sensory receptor that could provide excitatory or inhibitory input to a large number of neurons within the spinal cord. This sensory information, in turn, may further diverge or even converge to specific supraspinal locations. The diverging circuits that enable different levels of control of multiple muscles also provide a means of detailed conscious control of fine movements, while also providing mechanisms for executing more general and predictable tasks, even when they are considerably complex.

2.7 Implications of Synesthesia for Rehabilitation

Synesthesia is the merging of different modes of sensation received by the nervous system. Each mode of sensation, e.g., hearing, seeing, or touching, is generally thought to be very closely linked with specific types of sensory receptors providing information to areas of the brain that have the capability to process sound, light or mechanical perturbation, respectively. There are many examples of how sensory modes can be merged or exchanged with respect to a sensor generating a predictable perception. For example, Cytowic (Cytowic 2002) described a subject who was born blind, but later regained vision. After his vision was restored, this individual had difficulty seeing an object without touching it with his hands. For example, when he saw a gorilla at a zoo, he could not understand its posture and movements until he had felt a statue of a gorilla. There also are impressive examples of utilizing this synesthetic capability to rehabilitate individuals that had their vestibular system destroyed by medication. Individuals that have extreme difficulty in standing and walking as a result of a pharmacologically induced loss of vestibular function can rapidly regain excellent control by substituting the vestibular information with the output from an accelerometer placed on the head. In these cases, the electrical output from the accelerometer was passed via a wire leading to the surface of the tongue (Tyler, Danilov et al. 2003). In some way, the subject's tongue was able to "calibrate" the accelerometer output with visual and, presumably, head, neck, trunk and lower limb proprioceptive signals, functionally merging the information so that virtually normal posture and locomotion could be sustained. Furthermore, it is interesting that once the accelerometer device was removed, the renewed control of posture and movement was maintained for days or even weeks. Essentially the brain of this patient

was able to substitute electrical signals derived from an accelerometer and “plug” this information into the circuitry that coordinates the musculature of the head, neck, trunk and lower limbs that performs postural and locomotor tasks.

With respect to the topics of the present chapter, the concept of synesthesia may be important in several ways when developing strategies to recover sensorimotor function. Perhaps the most important point from these observations on synesthesia is the degree to which the brain can reorganize its function, even in individuals without any detectable neural dysfunction. This raises the question as to what extent we can learn to substitute one sensory mode for another in facilitating recovery of function after a SCI. Following a severe, functionally incomplete SCI, for example, to what extent can the brain reorganize itself to utilize the small number of fibers preserved that can functionally project signals to the spinal cord below the lesion? In other words, can a residual source of control from the brain be modified to control a function that is different from its normal action? A second important point that can be derived from these examples of synesthesia is that it appears that two modes of sensory information can be substituted, or at least merged, to improve sensorimotor function.

Another example of functional sensorimotor reorganization after an injury is the perception of the phantom limb, with a subject sensing the presence, and even the touch, of an arm that has been amputated (Kuiken, Dumanian et al. 2004). Subjects that have had an arm amputated can learn to control prosthetic devices using the EMG signals derived from intact muscles of the shoulder or from shoulder muscles that have been re-innervated with nerve branches that originally innervated muscles controlling hand and wrist movement. Interestingly, touching the skin overlying these re-innervated muscles

gives the subject the sensation of touching the skin overlying the hand or wrist, i.e., the region that it normally innervates.

All of these observations indicate that the potential for reorganization of sensorimotor function after a SCI has not been fully realized as a rehabilitative strategy. Combining this potential for plasticity with new technologies, such as virtual reality and smart robotic devices, seems to be a feasible and logical direction for future efforts in enhancing recovery of sensorimotor function following a wide range of neuromotor disorders. For example, robotic devices can be used to provide more precise and versatile training to SCI subjects (see Chapter 3).

2.8 Conclusion

In the present chapter, we have emphasized the high degree of plasticity of the functional connectivity within the spinal sensorimotor infrastructure in response to an injury and/or step training. We have pointed out that the neural processes involved in the generation of standing and stepping are extremely flexible functionally, and are unlikely to be due to a hardwired, fixed neuronal architecture. Instead, there are many possible pathways and combinations of circuits that can generate movement. This view implies that locomotor-related neural circuits are better defined as the probability of a given assembly of synapses to fire appropriately to produce a successful step that simply by the presence of anatomical connectivity. Such functional flexibility in the activation of the sensorimotor circuits for stepping, in turn, would be responsible for the variability inherent to gait patterns. This variability, in turn, reflects a fundamental feature of the neural control system that should be recognized and accommodated in developing

strategies designed to enhance motor performance by motor training using robotic devices after a SCI.

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CHAPTER 3: Experimental Background

3.1 Robotic Design

Traditionally, rehabilitative training of spinal cord injured patients is done manually, with the trainer manually manipulating the impaired limbs (Klose, Schmidt et al. 1990). This training can be very labor intensive and inconsistent, varying from one trainer to another. Similarly, the task of manual training on SCI animal experiments can be very tedious since most training sessions can last up to thirty minutes per animal; just a few animals can quickly put a toll on the trainer (de Leon, Hodgson et al. 1998). These problems can be solved using robotic devices. In addition, robotics allows us to make the training more task-specific, which when brought into a clinical environment can be adapted to specific patient needs. Thus it is our belief that robotic devices can offer more efficient and effective training. Robotics can also facilitate data collection. Previously, locomotion data were collected using video tracking, which required putting markers on the animals' limbs and manually, frame-by-frame, digitize the marker points. This is a very labor intensive task and can be subjective. With robotics, a lot more data can be recorded and made available in real time. Furthermore, since the data are more accurate and precise, better quantitative measurements can be made with robotics devices. In addition to advantage in SCI rehabilitation research, devices developed for treating spinal cord injury may have application in other areas of research and rehabilitation, such as other models of locomotion deficiency like that caused by stroke, and for use as "smart" exercise machines.

Because the many advantages of robotic devices, recently commercially available robotic orthotics such as the Lokomat™ are already available to facilitate the

rehabilitative training of spinal cord injured and stroke patients with promising results (Hesse, Schmidt et al. 2003; Winchester and Querry 2006). It is our goal to improve our understanding of how learning occurs in the spinal cord following traumatic injury by developing a robotic device for mice to further study the mechanisms that underlie rehabilitative training and thus to further improve upon current training strategies. The other advantage of having a mouse model will be to take advantage of the many strains of transgenic mice available and the ability to genetically manipulate gene expression under controlled conditions. The wide availability of naturally mutated and genetically engineered mice provides us a unique opportunity to identify the biochemical cascades that enable learning-related motor behaviors to occur. Using our device in conjunction with these strains will allow us to observe the phenotypic manifestations of genetic alterations on the learning process, thereby helping us identify factors important to spinal learning. The use of transgenic mice pervades biological research, and thus the utility of a mouse robotic device extends well beyond our research goals. The mouse model will provide researchers with extensive quantitative data directly relating genetic alterations in transgenic mice to motor activity. Understanding gene expression is the goal of proteomics. Our device will help identify proteins related to learning and will enable mapping of their origins in the genetic code. Thus, the mouse stepper will not only help us study spinal learning, it will be a valuable tool for studying other neuromuscular diseases as well.

3.1.1 Hardware Design

A four-axis robotic system (mouse stepper) was developed to both collect hindlimb position data and guide the hindlimbs through complete step trajectories (Figure 3.1). The system enables independent, two-dimensional tracking and control of each ankle in the sagittal plane as the mouse steps on a moving treadmill. The mouse stepper consists of four major components: (1) a pair of robotic arms, (2) a motion controller board, (3) a treadmill, and (4) a body-weight support device. The robotic arms, the primary components of the mouse stepper, serve as the interface between the electronics and the mouse. Each robotic arm is composed of a five-bar leg-guidance linkage (Kazerooni and Her 1994), a pair of motors that drive the linkage (2342-006CR; Micromo Electronics, Clearwater, FL), and a pair of optical encoders that record the rotational position of the motors (HEDM-5500; Agilent, Palo Alto, CA; Micromo Electronics). Forward kinematic equations are used to derive ankle position from the motor angles. The leg-guidance linkage is sized to enable motion tracking and control within a 3.5×3.5 cm workspace, which is sufficient to accommodate all step trajectories associated with mouse treadmill locomotion. The robotic system was used in two modes. In its active training mode, the robotic arms can drive the hindlimbs through any predetermined pattern within the workspace. In its passive recording mode, the linkages move freely in the workspace, allowing the computer to record independent ankle movements generated entirely by the mouse.

To minimize encumbrance on the mouse hindlimbs, precision bearings and motors with low internal friction are used at all revolute joints. The frictional resistance force at the end-effectors is estimated to be 0.032 N. The mass inertia of the robotic arm

linkage, including its actuators, is estimated to be ~0.4 g in its reference configuration, the orientation of the robotic arms in which the stepping workspace is initialized. Mass inertia remains within the same order of magnitude of this value for all orientations within the feasible stepping workspace of the mouse. These are practically negligible values. The robotic arms do not critically hinder stepping. All mice used in the study were tested under the same conditions on the mouse stepper.

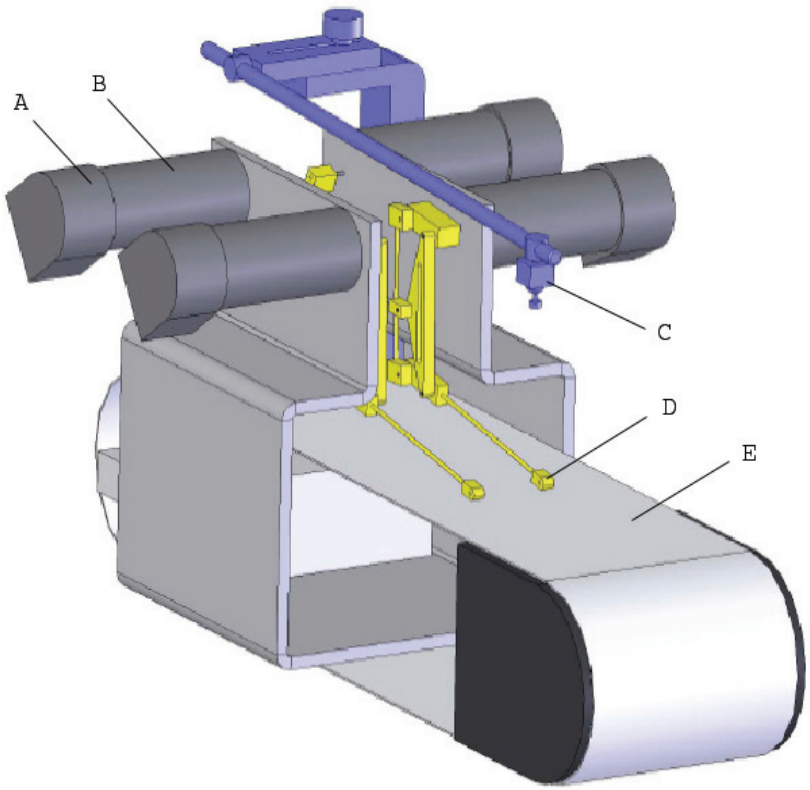

Figure 3.1: Schematic of current step training system. Important components are labeled: A) Optical encoder; B) Motor; C) Weight support; D) Manipulators; and E) Motorized treadmill.

A 4-inch-wide industrial conveyor (GUF-P 2000; MK Automation, Bloomfield, CT) was modified for use as a treadmill. The stock belt was replaced with a slightly tacky treadmill belt that provided a slip-free stepping surface without irritating the skin on the paws, an injury commonly observed with other belt materials. Mice are placed into the mouse stepper using a cone-shaped cloth harness that is magnetically secured to the weight-support system. The hindlimbs are connected to the robotic arms using a drawstring loop attachment. Both ends of a rounded rubber string are fed through an eyelet in the linkage end effector, forming a loop through which the hindpaw is placed. The diameter of the eyelet is sufficient to pass both ends of the string but small enough to resist axial slipping when the string is drawn tight. The thickness of the rubber prevents the ankle from coming into direct contact with the metal linkage, and its elasticity allows an appropriate amount of rotation and lateral motion while the ankle is guided through sagittal trajectories.

The robotic system enhanced both locomotor training and recording, providing several benefits: (1) it enabled precise control of hindlimb movements, (2) it ensured consistent application of a training protocol between mice and across training sessions, (3) it provided a quantitative record of the training history of each mouse, (4) it granted immediate access to the data, and (5) it facilitated application of quantitative analysis techniques.

3.1.1 Software Design

When the robotic device was operating in the active model, the robotic device was controlled via a four-axis dedicated controller board (DMC-2240, Galil Motion Control).

The control output was sent to the motors via an interconnection module with an integrated amplifier (ICM/AMP 1900; Galil Motion Control, Rocklin, CA). The control algorithms were written using LabVIEW™ (National Instruments, Austin, TX) and their output commands were sent to the controller board via an Ethernet connection. The feedback commands were updated at 200 Hz. During passive operation of the robotic device, the positions of each motor was sensed by the corresponding encoder and this information was passed to the controller board by the interconnection module, and decoded by the build in Transistor-Transistor-Logic (TTL) compatible quadrature decoder. The decoded theta positions were then transmitted to a personal computer (PC) via an Ethernet connection, where a custom built LabVIEW™ program used the theta positions from all four encoders to calculate and recorded the position of the end effortor. The sampling rate is again kept at 200 Hz.

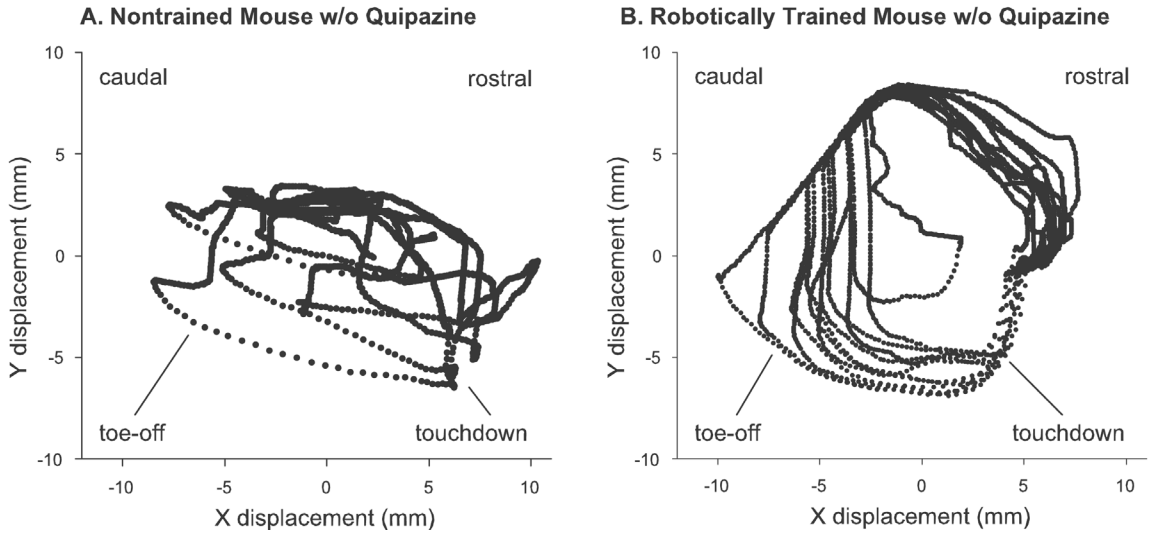

Figure 3.2: Step trajectories. Step trajectories of robotically trained mice showed consistent and rhythmic patterns. Trajectories recorded during the best 12 s periods of treadmill stepping of a representative nontrained mouse (**A**) and a representative robotically trained mouse (**B**) are shown. Ankle velocity is implicitly represented by the spacing of the data points. The rostral and caudal orientations of the mouse are noted, as well as the general regions of touchdown and toe-off.

3.2 Animal Protocol

In each experiment, adult female Swiss-Webster mice obtained from Charles River Laboratories (Wilmington, MA) were used. The mice were housed individually, have access to food and water ad libitum, and were kept on a 12 hour light/dark cycle for the duration of each study. All animal procedures used in these studies were conducted in accordance with the Animal Care Guidelines of the American Physiological Society and were reviewed and approved by the Animal Research Committee at the University of California, Los Angeles.

3.2.1 Anesthetic and Surgical Procedures

Surgeries were performed at approximately postnatal day 60 (P60). Before all surgical procedures, the mice were placed in an induction chamber with isoflurane gas at 5% (or level 5) with 0.4% Oxygen (O_2) for maximum of one minute and then the gas level was lowered to 2.5%. Once the mice were anesthetized for at least ten minutes, they were removed from the induction chamber and placed on the surgical table and anesthesia were administered via a facemask, which provided the same gas mixture to maintain a surgical level of anesthesia. The surgeons monitored the vital signs of the animals (heart rate and breathing) and adjusted the delivered level of anesthetic accordingly. Typically, after 20 min of anesthesia, the isoflurane level was lowered to 2% (or level 2) and was generally maintain at that level throughout rest of the surgery procedure. In the rare occasion when surgery on a mouse took longer then 45 minutes, the isoflurane level was lowered to 1.5%. The level of O_2 was kept constant at 0.4%.

All procedures were performed under aseptic conditions. During surgery, a water circulating heating pad was used to maintain body temperature. A skin incision was made along the dorsal midline from approximately T6 to T9 to expose the musculature overlying the vertebrae, and a laminectomy was performed from approximately T7 to T9. A branch of the thoracodorsal artery in the multilocular adipose tissue above approximately T6 was used as an anatomical landmark. The spinal cord was transected completely at a mid-thoracic level (T7–T9). The location and completeness of the transection were verified visually by two surgeons. Gelfoam was inserted into the gap to ensure complete separation of the proximal and distal stumps (de la Torre and Goldsmith 1990). The musculature was repositioned, and the wound was closed using 5-0 Dexon internal and 5-0 Ethilon external sutures. After surgery, the mice were placed in an incubator maintained at ~29 ± 1°C and observed until they fully recovered from anesthesia. Baytril (40 mg/kg body weight), a broad spectrum antibiotic, was added to the drinking water for 14 d after surgery.

3.2.2 Post-Surgical Care

Post-surgical care and maintenance procedures were similar to those described previously for spinal cord-injured rats and cats (Roy, Hodgson et al. 1992; Ellegala, Tassone et al. 1996). The bladders of all spinal mice were expressed manually twice daily to minimize the risk of bladder infection and related complications. After bladder expression, the hindlimbs of the mice were lightly stretched once through a full range of motion to help sustain joint mobility. Food rewards were given to stimulate positive interaction between the mouse and handler.

3.2.3 Drug Administration

As mentioned in Chapter 3.3, biochemistry plays a key role in locomotor recovery post-SCI. In subsequent experiments, the effects of quipazine, a broad-spectrum serotonin agonist, on facilitation of stepping was examined. We conducted a dose–response study using quipazine doses ranging from 0.2 to 2.0 mg/kg body weight administered intraperitoneally. At all of the doses tested, quipazine did not directly generate stepping in the absence of treadmill-induced sensory stimuli. Based on these results as well as dose-response studies reported for rats (Orsal, Barthe et al. 2002), a dosage of 0.5 mg/kg body weight was selected, which was the smallest dose that evoked robust stepping when the mouse was placed on the moving treadmill belt. This is the same dosage that has been reported to activate the spinal locomotor network in adult spinal rats (Feraboli-Lohnherr, Barthe et al. 1999). During the specified periods of each experiment, quipazine was administered 10 min before each treatment or testing session.

3.2.4 Training Procedure

Manual Training: During manual training, the mice were placed into the weight-support harness and oriented in a manner conducive to hindlimb bipedal locomotion. Manual training protocols vary among laboratories and are inherently difficult to describe. The method used in this study mimics strategies that have been particularly effective in both cats (Lovely, Gregor et al. 1986; de Leon, Hodgson et al. 1998; de Leon, Hodgson et al. 1999) and rats (de Leon, Reinkensmeyer et al. 2002), in which a human trainer grasps the hindlimbs and manipulates them through a trajectory similar to that of normal stepping.

Similar strategies are routinely used in physical therapy for human patients with diminished locomotor ability (Wernig, Nanassy et al. 1999; Behrman and Harkema 2000; Dietz 2001; Harkema 2001; Dietz and Colombo 2004). For the manual training used in this study, each mouse was positioned over a moving treadmill and induced to bear some weight. While oriented in this manner, human trainers used cotton swabs to manipulate the hindlimbs through kinematically appropriate stepping patterns. As consistently as possible, the cotton swabs were placed alternately on the ventral surface of the paw, against the back of the heel, and on the dorsal surface of the paw, to lift the paw off the treadmill, to guide the paw forward through swing, and finally to properly place the paw into stance. Because of the small size of the mice, the cotton swabs enabled more precise control of the positioning of the hindlimbs than using the trainer's fingers. Unlike previous works, noxious stimuli (Gwak, Hains et al. 2004; Hutchinson, Gomez-Pinilla et al. 2004), such as pinching of the tail, were not used to elicit locomotor response in experiments describe in this thesis work.

Robotic Training: During robotic training, the mice were placed into the weight-support harness and oriented in a manner conducive to hindlimb bipedal locomotion. The robotic device actively drives the animal's hindlimb using a training algorithm specific to the experiment (describe in detail in subsequent chapters). The training pattern implemented by the mouse stepper was adapted from bipedal stepping patterns recorded from a group of neonatally transected mice of the same age and size as the mice used in this study. Neonatally transected mice can spontaneously recover functional stepping without pharmacological or mechanical assistance (Fong, Cai et al. 2005). Their stepping patterns

are likely more representative of successful spinal stepping than patterns taken from intact, quadrupedally stepping mice.

3.2.5 Testing Procedure

When used, quipazine was administrated to each animal 10 min prior to its testing period. Before each testing session, the mice receiving training, were given a 2 min "warm-up" period, similar to when it was trained during normal training period for 2 min. For control mice, no "warm-up" period was given but quipazine was still administrated 10 min prior to testing when used. The mouse stepper was then used in a passive recording mode to track the ankle position of each leg for 2 min at a treadmill speed of 3 cm/s. Position data were recorded at 200 Hz using a custom acquisition program written in the LabVIEW^{TM}. In addition to kinematics data recorded by robot device, we put reflective markers on the hip, knee and ankle position and captured video footage of both the left and right sides of each mouse during testing and maintained a log of qualitative observations. At a later time, the number of steps executed by each mouse during the test session was counted, and their step rhythm and step shape consistency, stepping parameters that should intuitively improve with locomotor recovery, were quantitatively evaluated using fast Fourier transform (FFT) and principal components analysis (PCA) algorithms, as described below.

3.2.6 Euthanization

At the conclusion of each study, the mice were anesthetized deeply with sodium pentobarbital (50 mg/kg) and transcardially perfused with 1 ml/g body weight of 0.1 M

phosphate buffer, pH=7.4, followed by 2 ml/g body weight of 4% paraformaldehyde for 12 min. The spinal cords then were removed, post-fixed in 4% paraformaldehyde for 5 h, and cryoprotected by incubating in a 30% sucrose solution in 1X phosphate buffered solution overnight to ensure adequate absorption. The cords were then frozen on dry ice, and stored at -80°C.

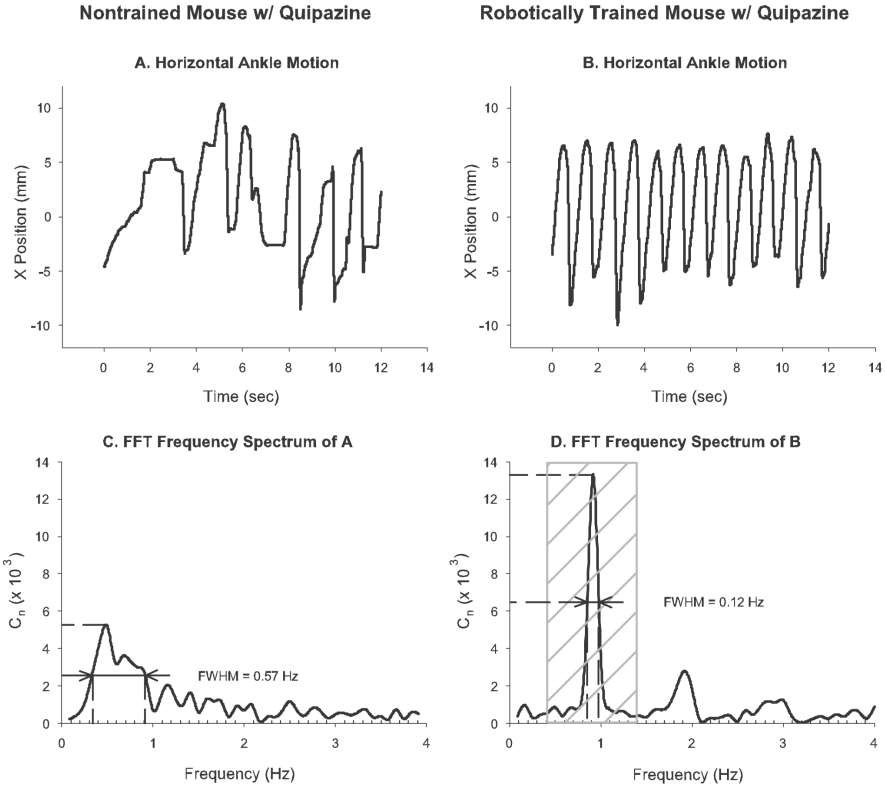

Figure 3.3: Fast Fourier transforms analysis. FFT analysis provides information about step rhythm. Twelve seconds of horizontal ankle motion during stepping are shown for a nontrained mouse given quipazine that stepped arrhythmically (A) and for a trained mouse given quipazine that stepped rhythmically (B). The corresponding frequency spectrum and the FWHM of the peak at the primary stepping frequency for A and B are shown in C and D, respectively. Lower values of FWHM correspond to more rhythmic stepping. For successful spinal stepping on a treadmill at 3 cm/s, the peak corresponding to the primary stepping frequency lies between 0.4 and 1.4 Hz (D, crosshatched region). Cn represents the number of incidences during a test session that the mouse stepped at a particular frequency.

3.3 Data Analysis

Although the Basso, Beattie and Bresnahan (BBB) locomotor rating scale is widely used to test behavioral consequences of spinal cord injury, we did not use it in our animal

studies for several reasons. First, the BBB scale is originally developed for contusion rats that are mildly or moderately injured. In our experiments describe below, mice with a complete mid-thoracic transection are used. Hence many of the locomotor performance criteria such as hindlimb and forelimb coordination, an important criterion in the BBB scale, can not be used directly. Antri et al (Antri, Orsal et al. 2002) has altered the BBB scale to studied complete transected rats, it still does not alleviate the second fact – the BBB scale is developed to evaluate open-field movements resulting in poorly control of factors known to be important in locomotion. Lastly and most importantly, the BBB scale is a qualitative measurement and thus inherently subjective. It is possible for two evaluators to give two different scores for the same animal. With the use of the robotic device, we can measure locomotor performance more objectively and quantitatively. Below are the three methods that we used to quantify locmotor recovery.

3.3.1 Number of Steps

The number of steps performed by the each mouse during its testing period was counted using the following criteria for successful stepping. Video footage and plots of ankle position data were used to identify and count the number of steps performed by each mouse. Steps were identified based on predetermined criteria for step length, height, duration, and degree of interlimb coordination. The 12 s stepping interval containing the most steps was recorded for subsequent analyses. Both plantar and dorsal steps were accepted. Better performing subjects performed primarily plantar steps, whereas poorer performing subjects exhibited dorsal steps and paw drag. Examples of different quality step trajectories are depicted in Figure 3.2.

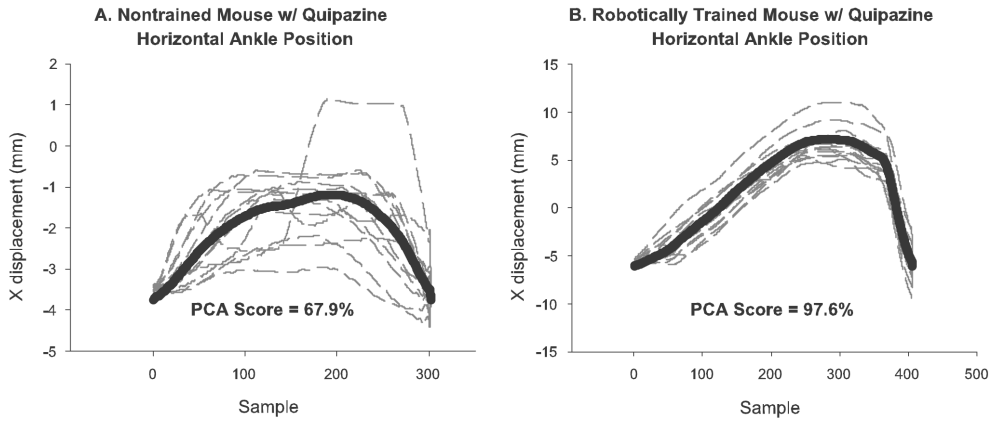

Figure 3.4: Principal component analysis. The first principal component from a PCA identifies the most representative step pattern executed by a mouse.Amouse with a high PCA percentage for its first principal component stepped with a more consistent step shape than a mouse with a lower PCA percentage score. Plots of several x trajectories (dashed lines) and their corresponding first principal component (solid lines) are shown for a quipazine-treated nontrained mouse (A) and for a quipazine-treated trained mouse (B). The corresponding PCA percentages, 67.9% (A) and 97.6% (B), respectively, indicate that quipazine facilitated greater improvement in spatial stepping consistency in trained than nontrained mice. Note also from the different scales of the x-displacement (ordinate) axis that trained mice generally took much longer steps than nontrained mice.

3.3.2 Step Periodicity

Given their periodic nature, locomotor stepping cycles are well suited for FFT analysis (Turkey and Cooley 1965). The FFT spectrum of ankle position during the best 12 s interval of stepping for each mouse was determined for each test session. Peaks in the FFT spectrum correspond to the most common stepping frequencies exhibited during the test interval (Figure 3.3C,D). Sharp, distinct spikes correspond to very consistent, rhythmic stepping (Figure 3.3B,D). Broad peaks, or the lack of a dominant peak, correspond to inconsistent, arrhythmic stepping and are characteristic of frequent stumbling and paw drag (Figure 3.3A,C). Because constant treadmill speed requires mice to step rhythmically, we expected that the width of the dominant peak would progressively decrease as more aggressive treatment strategies were applied. To examine step rhythm, we measured the full-width at half-maximum (FWHM) of the tallest peak in the FFT spectrum between 0.4 and 1.4 Hz. FWHM is the width of the selected peak at

half its maximum amplitude and is measured in Hertz, the same unit used for stepping frequency. Note that lower values of FWHM indicate better relative step rhythm. At the treadmill speed used in this study (3 cm/s), the expected stepping frequency, i.e., the location of the tallest peak, is ~1 Hz. The range of stepping frequencies from which the measured peak was selected (0.4 –1.4 Hz) accounts for variations in mouse limb lengths and small fluctuations in treadmill speed. Peaks at lower frequencies are inconsistent with successful stepping and were ignored. Peaks at higher frequencies approach the physical limit of mouse hindlimb motion and generally were not observed. A minimum of three steps was required to perform FFT analysis. When stepping was so poor that no dominant peak was found or when the mouse did not perform at least three steps, a default FWHM value of 1.2 Hz was assigned. This value is slightly larger than the highest FWHM values that we measured empirically (~0.95 Hz). We found that the FWHM of the dominant peak within the normal range of stepping frequencies provided a key measure of stepping performance.

3.3.3 Shape Consistency

Improved spatial stepping consistency was characterized by increasingly consistent repetition of a nominal trajectory. PCA is a multivariate analysis technique that picks out patterns in a dataset and reduces the dimensionality of the data without significant loss of information (Dunteman 1989). Given a series of step trajectories, PCA extracts the fundamental trajectory as the first principal component (Figure 3.4). The PCA score reported here is the percentage of the total variance that is captured by the first principal component. Hence, the higher the PCA score, the more consistent the stepping. The raw

stepping data were preprocessed for PCA. First, the successful step cycles from the selected best 12 s intervals of stepping were isolated and separated into their *x* and *y* components. Each step component was resampled to a consistent number of data points per step, thus removing the temporal information. The data were then arranged into two $m \times n$ matrices, with each column containing the data for a single step and each row containing the interpolated position values at each time step:

x component of steps

$$\begin{bmatrix} x_{1,1} & x_{1,2} & \cdots & \cdots & x_{1,n} \\ x_{2,1} & x_{2,2} & \cdots & \cdots & x_{2,n} \\ \vdots & \vdots & \ddots & & \vdots \\ \vdots & \vdots & & \ddots & \vdots \\ x_{m,1} & x_{m,2} & \cdots & \cdots & x_{m,n} \end{bmatrix}$$

y component of steps

$$\begin{bmatrix} y_{1,1} & y_{1,2} & \cdots & \cdots & y_{1,n} \\ y_{2,1} & y_{2,2} & \cdots & \cdots & y_{2,n} \\ \vdots & \vdots & \ddots & & \vdots \\ \vdots & \vdots & & \ddots & \vdots \\ y_{m,1} & y_{m,2} & \cdots & \cdots & y_{m,n} \end{bmatrix}$$

A custom program written in Matlab (MathWorks, Natick, MA) development environment was used to identify the principal components of each dataset and to calculate the PCA score. A minimum of three successful steps was required to conduct a statistically significant PCA. Mice that could not perform at least three steps in any 12 s interval were assigned a PCA value of 35%, which is approximately the lowest score that we obtained for mice that could perform at least three steps.

3.4 Summary

In this chapter, experimental procedures for studying SCI using a mouse model were described in detail. New technology and techniques were developed to overcome the challenges created by the smell size of mice. A robotic system based on a design by David Reinkensmeyer (Nessler, Timoszyk et al. 2005) was built and used as both a

training and evaluation device. With this device, we also implemented new analysis techniques to evaluate the recovery of locomotion more quantitatively then methods currently used.

3.5 Chapter Reference

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CHAPTER 4: Spinal Cord-Transected Mice Learn to Step in Response to Quipazine Treatment and Robotic Training

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4.1 Abstract

In the present study, concurrent treatment with robotic step training and a serotonin agonist, quipazine, generated significant recovery of locomotor function in complete spinal cord-transected mice (T7–T9) that otherwise could not step. The extent of recovery achieved when these treatments were combined exceeded that obtained when either treatment was applied independently. We quantitatively analyzed the stepping characteristics of spinal mice after alternatively administering no training, manual training, robotic training, quipazine treatment, or a combination of robotic training with quipazine treatment, to examine the mechanisms by which training and quipazine treatment promote functional recovery. Using fast Fourier transform and principal components analysis, significant improvements in the step rhythm, step shape consistency, and number of weight-bearing steps were observed in robotically trained compared with manually trained or nontrained mice. In contrast, manual training had no effect on stepping performance, yielding no improvement compared with nontrained mice. Daily bolus quipazine treatment acutely improved the step shape consistency and number of steps executed by both robotically trained and nontrained mice, but these improvements did not persist after quipazine was withdrawn. At the dosage used (0.5 mg/kg body weight), quipazine appeared to facilitate, rather than directly generate, stepping, by enabling the spinal cord neural circuitry to process specific patterns of sensory information associated with weight-bearing stepping. Via this mechanism,

quipazine treatment enhanced kinematically appropriate robotic training. When administered intermittently during an extended period of robotic training, quipazine revealed training-induced stepping improvements that were masked in the absence of the pharmacological treatment.

4.2 Introduction

In recent years, it has become clear that manual training can be used effectively to recover hindlimb motor function in complete spinal animals (Edgerton, 1997a, 2001, 2004a, 2004b). For example, adult spinal cats (Lovely, 1986, 1990; Edgerton, 1991a, 1991b; Hodgson, 1994; de Leon, 1998; Roy, 1998; Rossignol, 2002) and, to a lesser extent, adult spinal rats (de Leon, 2002; Moshonkina, 2002; Timoszyk, 2003) that are regularly trained to step can regain the ability to generate full weight-bearing treadmill stepping over a range of speeds. The underlying mechanism responsible for their recovery, however, is poorly understood.

The wide availability of genetically modified strains makes the mouse an attractive model for dissecting the adaptive mechanisms of training-induced locomotor recovery. Data to date suggest, however, that in the absence of any pharmacological intervention, the level of recovery is lower in smaller species such as rats (de Leon, 2002) and mice (Guertin, 2004) compared to cats. Although there is one report of significant spontaneous recovery in complete spinal mice without any pharmacological intervention (Leblond, 2003), we have been unable to duplicate these observations. The lower recovery in small species may be due to the increased difficulty required to manipulate

small hindlimbs through kinematically appropriate step cycles, or there may be less adaptive potential in rodents following a spinal cord injury (SCI).

Species differences in training-induced locomotor improvement may be related to the varying difficulty required to apply appropriate spatial and temporal patterns of proprioceptive cues during training in different-sized animals. Training with robotic devices enables locomotor patterns to be imposed with levels of precision and consistency that cannot be attained by human hands. In addition to improving training, robotic devices can provide an accurate, quantitative, and immediate assessment of locomotor performance.

Previous studies have shown that quipazine improves treadmill locomotion in adult chronic spinal cats (Barbeau 1990; Brustein 1999), and rats (Feraboli-Lohnherr, 1999; Antri, 2002), and recently, similar findings were reported for spinal mice (Guertin, 2004). We hypothesized that quipazine acutely elevates the sensitivity of the spinal locomotor circuits to proprioceptive inputs, thereby facilitating locomotor drive patterning when the spinal cord is presented with appropriate sensory cues.

The objectives of this study were three-fold: 1) to determine the relative effectiveness of manual and robotic training in improving stepping in complete spinal mice, 2) to determine whether daily, acute administration of quipazine would improve stepping, and 3) to determine whether combining quipazine and robotic training would produce an interaction effect on improving stepping. The results demonstrate that 1) complete spinal mice can be robotically trained to step, whereas manual training, as performed in the present study, is ineffective, 2) quipazine (0.5mg/kg) effectively facilitates, but does not directly generate stepping, and 3) when applied concurrently, quipazine and robotic step

training produce an interaction effect, resulting in stepping performance that exceeds that achieved when either intervention is used alone.

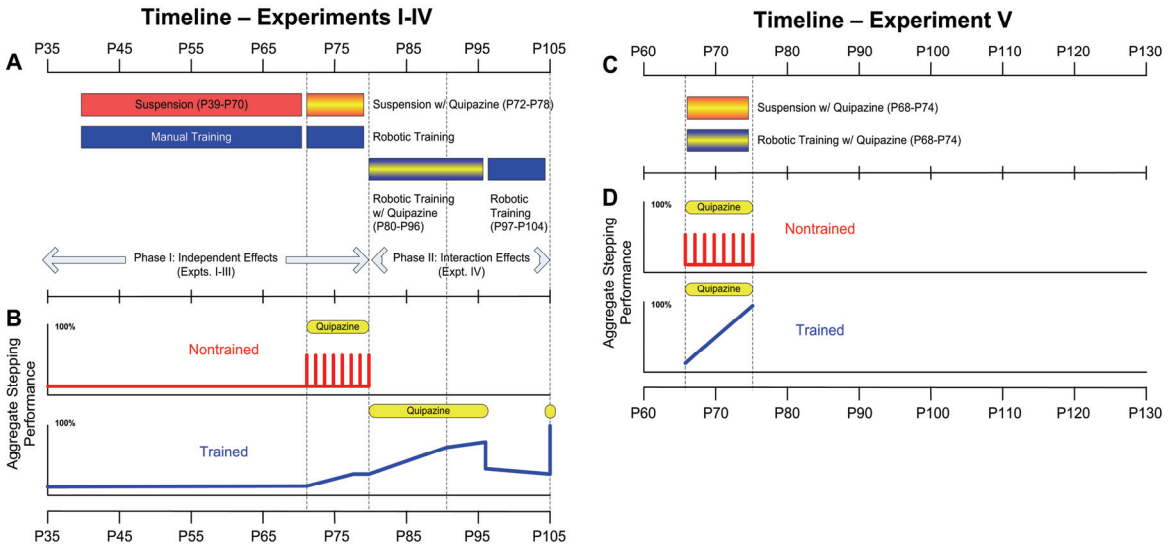

Figure 4.1: Experimental timeline. The sequence of experiments conducted on the step trained (blue bars) and nontrained (red bars) groups are shown for experiments I–IV (***A***) and experiment V (***C***). Test dates are indicated by the vertical dashed lines. Yellow stripes denote the periods during which quipazine was administered as a single bolus daily. Time course plots of the aggregate stepping performance of the trained (blue line) and nontrained (red line) mice are shown for experiments I–IV (***B***) and experiment V (***D***). Aggregate stepping scores were determined as a qualitative weighting of the three stepping measurements used in the study (number of steps, step rhythm, and step shape consistency) and were normalized against the best stepping observed during each group of experiments (denoted as 100%). Number of steps performed was the predominant factor in determining the aggregate score. During the periods labeled as "Suspension," the nontrained mice were placed in the harness with their hindlimbs unloaded for 15 min/d. This amount of unloading is extremely unlikely to have adversely affected stepping ability. Open circles, open squares, and filled triangles denote the time points used for examining the effects of manual training, quipazine treatment, and robotic training, respectively, in experiments I–III. It is important to note that the baseline scores against which these treatments were compared are time independent and represent the maximal performance that can be expected for nontrained, untreated mice. Open diamonds denote the time points compared in the longitudinal experiment IV. Double daggers denote the time point studied in the parallel experiment V.

4.3 Experimental Methods

The study consisted of five experiments, each consisting of a trained group and a nontrained group. The same trained and nontrained groups were maintained throughout Experiments I-IV. Experiment V utilized a different set of trained and nontrained mice.

For Experiments I-IV, sixteen Swiss-Webster mice (mean weight 21.5±0.3 g at spinal cord transection) obtained from Charles River Laboratories (Wilmington, MA) were used. For Experiment V, an additional twenty Swiss-Webster mice (mean weight 25.3±0.3 g at spinal cord transection) were used. The mice were housed individually, had access to food and water ad libitum, and were kept on a 12 hour light:dark cycle for the duration of the study.

Following each of the two sets of spinal cord transection surgeries (see Chapter 3.2.1), the mice were equally and randomly divided into trained and nontrained groups. The trained groups were provided with various training paradigms, with and without quipazine, throughout the study and were tested periodically to assess their stepping performance. During the corresponding sessions, mice in the nontrained groups were placed into a weight support harness. The reasons for suspending the nontrained mice were two-fold: 1) to approximate the same amount of handling that was given to the trained mice by placing them into the harness, and 2) to unload their hindlimbs, thus eliminating proprioceptive and cutaneous stimuli through the paws, thereby removing the possibility that the nontrained mice received even minimal training during "treatment" sessions. It is extremely unlikely that such short periods of unloading would generate muscle atrophy or similar effects that would be detrimental to their locomotor performance. The nontrained mice received one interval of quipazine treatment during the study and were robotically tested periodically, but were never trained. The sequence of experiments and the intervention history of the mice used in Experiments I-IV are depicted in Figure 4.1. All animal procedures used in this study were conducted in accordance with the Animal Care Guidelines of the American Physiological Society, and

were reviewed and approved by the Animal Research Committee at the University of California, Los Angeles.

Baseline Performance Scores. In this study, the performance of nontrained, untreated (no quipazine) mice served as the standard against which the effectiveness of robotic training and quipazine treatment were compared. Typically, however, the nontrained, untreated mice were unable to perform the minimum three steps in a 12-sec interval required to perform FFT or PCA analyses. For the purpose of defining a baseline standard, we assigned the following scores for nontrained, untreated spinal mice: steps = 16, FFT = 1.2 Hz, PCA = 35.0%. The value selected for the baseline number of steps is the average number of steps performed by nontrained, untreated mice (see Results section). The baseline FFT and PCA values are the values assigned for mice that could not perform at least three successful steps in a 12-sec interval (discussed above). Consistent with findings in adult spinal rats (Commissiong, 1993; Molinari, 1993), in the absence of treatment, complete spinal Swiss-Webster mice (P39) do not spontaneously recover locomotor ability. Hence, these baseline scores are independent of time post-lesion.

4.4 Results

Experiment I: Effects of Manual Training

The purpose of Experiment I was to determine whether manual training could be used to improve locomotor performance following a complete mid-thoracic spinal cord transection in mice. Manual training was performed for 15 min/day, 5 days/week for five weeks beginning at P43, four days after the spinal cord transection surgery. On the sixth day of each of the first four weeks, the hindlimbs of both the trained and nontrained mice

were attached to the robotic arms for acclimatization. On the last day of the experiment, P71, the hindlimb movement patterns of all of the mice were recorded for two minutes with the robot operating in passive recording mode. Quipazine was not administered at any time during Experiment I.

After five weeks of manual training, the locomotor performance of the trained mice was not statistically different from that of the nontrained mice. Mice in both groups were largely unable to initiate a swing phase and thus dragged their paws on the treadmill belt. On the isolated occasions that they were able to bring a paw forward, they typically landed on the dorsal surface of the paw, a proprioceptive trigger for stumbling and collapse. The mean total number of steps performed in a 2-min test period in the trained mice was 16.0±5.1 compared to 15.8±5.5 for the nontrained mice. Neither PCA nor FFT analysis could be performed at this juncture because the animals were unable to perform the critical number of successful steps required to implement these measures. Manual training did not produce a statistically significant improvement compared to no training.

Experiment II: Effects of Robotic Training

The purpose of Experiment II was to determine whether robotic training could be used to facilitate locomotor improvement in spinal mice. The mice that were manually trained in Experiment I from P43-P70 were used for this experiment and underwent robotic training for 15 min/day between P71-P78. On P79, the hindlimb movement patterns of the trained mice were recorded for two minutes with the robot operating in passive recording mode. Quipazine was not administered at any time during Experiment II.

Rrobotic training of the previously manually trained rats in Experiment I generated statistically significant improvement in locomotor performance compared to the assigned baseline scores for nontrained spinal mice that basically did not step in Experiment I (Table I). At P79, the mean number of steps executed by the robotically trained mice had increased from 16 to 31.6±7.1 ($p=0.023$). Based on FFT analysis, their mean step rhythm score had improved from 1.2 to 0.81±0.15 Hz ($p=0.011$). Likewise, PCA analysis showed that their mean step shape consistency score had improved from 35.0% to 76.4±3.8% ($p<0.001$). Therefore, robotic training improved stepping performance.

Experiment III: Effects of Quipazine Treatment

The purpose of Experiment III was to determine whether daily quipazine treatment could be used to facilitate locomotor improvement in spinal mice. During the same period that the trained mice were robotically trained, P71-P78, the nontrained mice were administered a bolus dose (0.5 mg/kg) of quipazine i.p. daily. After quipazine was administered, the mice were suspended for ~15 min in the weight support harness in order to provide them with similar sensory stimuli as that given to the robotically trained mice, which were similarly supported during training. On P79, the hindlimb movement patterns of the nontrained mice were recorded for two minutes with the robot operating in passive recording mode.

Within five minutes of the initial quipazine treatment, mice that were previously unable to step were able to execute long periods of uninterrupted, successful stepping at treadmill speeds ranging from 3–10 cm/sec (please refer to videos located online at:

http://robotics.caltech.edu/jneurosci). No air stepping was observed. The mice only stepped when their paws were placed on the moving treadmill belt. Qualitatively, the most significant improvements of the quipazine-mediated stepping were 1) robust toe-off into swing phase, 2) pronounced, perhaps exaggerated, toe extension, and 3) frequent plantar paw placement. Although quipazine acutely improved stepping immediately after each treatment, no persistent or progressive enhancements of stepping ability were obtained with repeated quipazine treatment alone.

Table I. Quipazine and robotic training independently improve stepping performance

	Base Line	**Experiment II**	**Experiment III**
	NT, -Q	**RT, -Q**	**RT, +Q**
Number of Steps	16.0	31.6 ± 7.1[c]	55.1 ± 13.0[d]
FFT FWHM (Hz)[a]	1.2	0.81 ± 0.15[c]	0.96 ± 0.12[d]
PCA (%)[b]	35.0	76.4 ± 3.8[c]	77.4 ± 3.9[d]

NT, Nontrained; **RT**, robotically trained; **+Q**, treated with quipazine; **-Q**, not treated with quipazine. FWHM is of the dominant peak in the FFT spectrum between 0.4 and 1.4 Hz. SEM values are reported.

a Lower values of FWHM correspond to improved step rhythm.

b Higher values of PCA percentage correspond to improved step shape consistency, up to a practical maximum of ~90%.

c Based on all three measures of stepping ability, i.e., number of steps performed ($p<0.05$), step rhythm ($p<0.05$), and step shape consistency ($p<0.001$), mice that received robotic training performed statistically better than nontrained, untreated mice.

d Mice administered quipazine statistically improved the number of steps performed ($p<0.05$) and step shape consistency ($p<0.001$) compared with nontrained, untreated mice. Quipazine did not affect step rhythm.

Compared to the baseline scores assigned for these same mice earlier in the study when they were the nontrained group (Experiment 1: P43-P70) that basically did not step, quipazine treatment acutely generated statically significant improvements in number of steps performed and step shape consistency on P79 (Table I). At P79, the mean number

of steps executed by the quipazine treated mice had increased from 16.0 to 55.1±13.0 ($p=0.046$). Similarly, PCA analysis showed that their mean step shape consistency score had improved from 35.0% to 77.4±3.9% ($p<0.001$). Unlike robotic training, however, quipazine treatment did not statistically improve step rhythm. Therefore, quipazine treatment improved two of the three aspects of stepping performance measured.

Experiment IV: Interaction Effects of Combining Robotic Training and Quipazine Treatment

Having established that robotic training and quipazine treatment were each able to generate locomotor improvement in spinal mice, the goal of Experiment IV was to determine whether combining the treatments would produce a net interaction effect that was greater than either of the independent effects. To maximize the use of the animals, we implemented an experimental plan in which the trained mice were continuously robotically trained while quipazine was repeatedly administered and withdrawn. The goal of this procedure was to observe whether the stepping performance of the mice would fluctuate when they were sequentially administered or not administered quipazine. The mice used for Experiment IV were the same trained mice used sequentially in both Experiment I & II that had been manually trained from P43-P70 (Experiment I) and then robotically trained from P71-79 (Experiment II). Between P80-P96, these mice were provided a daily regimen combining quipazine and robotic training using the same procedures described above (Figure 4.1 A). Stepping performance was tested after quipazine administration on P91. Between P97-P104, quipazine was withheld, while

robotic training continued. The mice then were tested both before and after a bolus dose of quipazine on P105.

The time-course variation in the number of steps executed by the mice is consistent with an interaction effect (Figure 4.2 A). At P79, following the first period of robotic training without quipazine, the mice executed 31.6±7.1 steps. At P91, during the combination treatment period, the mice performed 78.2±20.0 steps in the 2-min test interval, a statistically significant improvement ($p=0.015$). At P105, following the second period of robotic training without quipazine, the number of steps decreased to 24.3±21.6 steps, a statistically significant decline compared to that at P91 ($p=0.021$), and similar to the number of steps performed at P79. After being given the additional bolus dose of quipazine, their number of steps again markedly increased to 146.0±16.9 ($p<0.001$, compared to before quipazine), marking their best performance of the study ($p=0.013$, compared to P91).

The step shape consistency results also support an interaction effect (Figure 4.2 B). At P79, the mean PCA score of the mice was 76.4±3.8%. As a result of the combined treatment, their score statistically increased to 87.1±3.2% ($p=0.031$). The score decreased to 55.0±9.7% when quipazine was withheld ($p=0.007$ compared to P91), but rose again to 78.5±5.5% ($p=0.035$, compared to before quipazine) following the bolus dose of quipazine at P105.

In contrast to number of steps performed and step shape consistency, the step rhythm scores for the mice improved steadily throughout the course of treatment (Figure 4.2 C). The mean FFT score of the mice decreased continuously from 0.81±0.15 Hz at P79 to 0.15±0.02 Hz at P105 ($p=0.001$). These results are consistent with the results of

Experiment III, which indicate that quipazine has a smaller effect on step rhythm compared to robotic training.

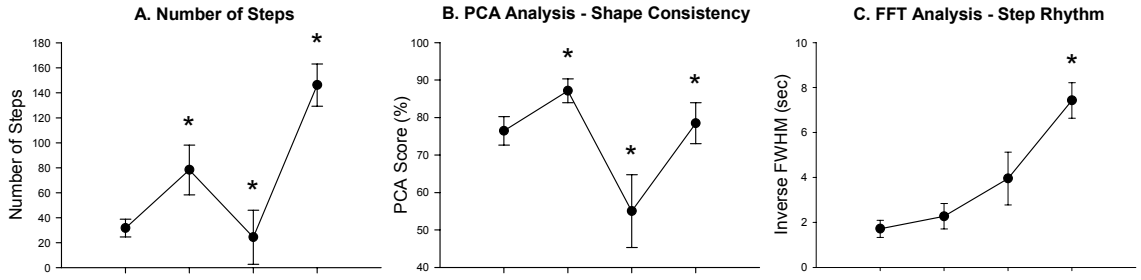

Figure 4.2: Progression of locomotor performance attributable to administration and withdrawal of quipazine during continued robotic training. After the initial period of robotic training, which ended at P79, the number of steps (***A***) and the step shape consistency (***B***) of the mice continued to improve when robotic training and quipazine treatment were used together (P91) and then decreased when quipazine was withdrawn (P105a). This suggests that the improved performance observed after the combination treatment was primarily mediated by quipazine and, hence, that the net performance was attributable to an interaction effect between quipazine and robotic training. Moreover, an additional bolus treatment with quipazine (P105b) immediately generated stepping that was significantly better than that exhibited at P91, despite the fact that quipazine had not been administered during the preceding 9 d. This finding suggests that the acute quipazine treatment improved stepping by facilitating effects of chronic robotic training that were masked in the absence of drug treatment. Unlike the number of steps and step shape consistency, the step rhythm (***C***) improved steadily throughout the course of robotic training, as depicted by the plot of inverse FFT score. This is consistent with the results of experiments II and III, which indicated that robotic training has a greater effect on step rhythm than quipazine. RT, Robotically trained;_Q, treated with quipazine;_Q, not treated with quipazine.

Experiment V: Quipazine Treatment vs. Robotic Training with Quipazine Treatment

To further examine the existence of an interaction effect, we compared the effects of quipazine treatment alone to those of the combination treatment (quipazine with robotic training) in an additional set of mice. For Experiment V, twenty mice were spinally transected at ~P60 (mean weight 25.3±0.3 g) and were equally and randomly divided into a trained and a nontrained group. Between P68-P74, the trained mice were provided a daily regimen combining quipazine and 15 min/day robotic training. During the same interval, the nontrained mice were administered daily bolus doses of quipazine (0.5

mg/kg) and were suspended in the weight support harness for 15 min/day. At P75, the stepping performance of each group was evaluated for two minutes using the robot in passive recording mode.

At P75, the group that received the combination treatment outperformed the group that was administered quipazine only. The combination treatment mice executed more steps (32.9±7.0) than the quipazine only mice (17.1±6.0) ($p=0.050$). The combination treatment mice had a lower FFT score (0.12±0.01 Hz) than the quipazine only mice (0.66±0.18 Hz), indicating that they stepped more rhythmically ($p=0.006$). PCA analysis also showed that the combination treatment mice (83.9±3.8%), displayed a more consistent step shape than the quipazine only mice (60.7±8.9%) ($p=0.015$). These results indicate that the locomotor enhancement mediated by combining robotic training with quipazine was larger than that of quipazine treatment alone.

Summary of Results:

Figure 4.1 B summarizes the principal findings of this study, which are: 1) manual training did not affect stepping ability, 2) robotic training significantly improved stepping performance, 3) daily acute quipazine treatment transiently facilitated locomotor improvement, but did not generate a long-lasting effect on stepping ability, 4) stepping performance improved further when robotic training was combined with concurrent quipazine treatment, and 5) acute quipazine treatment of robotically trained mice revealed training-induced stepping improvements that were masked in the absence of drug treatment.

4.5 Conclusions and Discussions

4.5.1 Quipazine facilitated spinal processing of sensory information associated with weight-bearing stepping

Complete mid-thoracic spinal cord transection induces a substantial loss of spinal cord serotonin content (Anden, 1964; Laporte, 1995). One pharmacological strategy for treating SCI is to recreate an intraspinal chemical environment that enables the spinal circuitry to generate functional locomotor patterns. Systemic and intrathecal applications of noradrenergic (Barbeau, 1991; Chau, 1998; Giroux, 2001), dopaminergic (Goldberger, 1977; Barbeau, 1991), serotonergic (Feraboli-Lohnherr, 1999; Machacek, 2001; Antri, 2002), and glycinergic drugs (Edgerton, 1997b; de Leon, 1999b) have been effective in eliciting locomotor patterns in spinal cats, rats, and mice. We observed that quipazine enabled hindlimb bipedal stepping in complete spinal mice, an observation consistent with findings reported by Guertin (2004). Quipazine and another serotonin agonist, m-chloropiperazine, have been shown to facilitate locomotion in cats (Barbeau, 1990) and rats (Kim, 2001), respectively, and similar improvement was reported when serotonergic embryonic raphe cells were implanted in spinal rats (Feraboli-Lohnherr, 1997; Kim, 1999; Dumoulin, 2000; Ribotta, 2000).

The mechanism by which serotonin mediates locomotor improvement following SCI is not well understood. Although it has been suggested that serotonin induces fictive locomotion (Cazalets, 1992; Grillner, 2001), the present results indicate that serotonin facilitates, but does not generate, stepping in the in vivo spinal mouse. Spinal mice administered quipazine did not “air step.” On the contrary, quipazine-treated mice only

initiated stepping when triggered by sensory stimuli derived from placing the hindlimbs on the moving treadmill belt.

Combinations of therapeutic treatments may be more beneficial than individual treatments. When quipazine was administered concurrently with weight-bearing step training, the performance of the spinal mice exceeded that obtained with either quipazine treatment or robotic training alone. Although spinal mice markedly improved their stepping ability after one week of robotic training, their rate of improvement and overall stepping performance further increased when robotic training and quipazine were combined (combination treatment). The data indicate that this additional improvement was mediated primarily by quipazine treatment, not by extended robotic training, since stepping ability immediately regressed when quipazine was withdrawn. This transient nature of quipazine-mediated stepping facilitation is consistent with findings describing a decline in locomotor performance after termination of chronic intrathecal quipazine treatment in spinal rats (Antri, 2002).

Analysis of the stepping data confirmed that each treatment made distinct contributions to the net locomotor scores. In Experiment IV, consistent with the findings in Experiments II and III, step rhythm improved steadily with continued robotic training, but step shape consistency fluctuated in accordance with quipazine administration and withdrawal. The combination treatment produced a positive interaction effect that was superior to robotic training alone.

The results of the parallel comparison in Experiment V also support an interaction effect. After one week of treatment, the number of steps, step rhythm, and step shape consistency of mice given the combination treatment were significantly better than those

of mice administered quipazine only. Thus, the combination treatment was more successful than quipazine treatment alone.

Despite the difference in the ages of the mice at spinal transection, both Experiment IV (P35) and Experiment V (P60) demonstrated an interaction effect between robotic training and quipazine treatment on stepping performance. Thus, the specific age of the mice at the time of each experimental intervention was most likely not the critical factor responsible for producing the observed outcomes.

When administered in conjunction with weight-bearing stepping, quipazine may modulate the relative levels of sensory information "perceived" by the spinal cord, favoring interneuronal pathways that are linked to proprioceptive and cutaneous stimuli. This putative sensory modulation role is consistent with the presence of 5-HT receptors in the dorsal horn of the lumbosacral spinal cord (Liu, 2002). Furthermore, numerous findings associate 5-HT with sensory input modulation (Machacek, 2001; Meuser, 2002; Miquel, 2002; Shay, 2002; Bosco, 2003; Hains, 2003). In intact cats, monoamine release selectively increases the excitability of some reflex circuits while decreasing the excitability of others, e.g., potentiating transmission from Group I spindle fibers and tendon organs (Edgley, 1988; Bras, 1990), while depressing transmission from nociceptors and Group II fibers (Headley, 1978; Fleetwood-Walker, 1985). The near absence of serotonin caudal to a complete spinal cord lesion may limit the ability of the spinal cord to discriminate sensory inputs, and may contribute to the spasticity associated with spinal injury. During locomotion, quipazine may restore appropriate sensory processing, "tuning" the spinal cord to relevant sensory cues, while suppressing extraneous inputs such as pain.

Serotonergic agonists hyperpolarize the action potential threshold (Wikstrom, 1995; Grillner, 2001; Hill, 2003) and shorten the duration of afterhyperpolarization (Fedirchuk, 2004). These changes frequently bring the membrane potentials of spinal neurons near the threshold required to generate plateau potentials, which have been hypothesized to be the basis for locomotor drive potentials. In this hyperexcitable state, even minimal amounts of sensory input can initiate plateau potentials (Kiehn, 1996; Gorassini, 1999). Thus, short-term modulation of the membrane potential may be a mechanism by which quipazine acutely facilitates locomotor recovery.

By repeatedly activating sensory circuits and the interneurons to which they project, chronic, consistent training of spinal cord injured subjects may continue to modify spinal locomotor circuits even after functional improvements are no longer apparent. In the present study, quipazine treatment may have revealed masked positive effects of weight-bearing step training. During the longitudinal robotic training experiment (Experiment IV), the stepping ability of mice administered the combination treatment rapidly declined to their pre-quipazine performance when quipazine was withdrawn, and remained at that level even when robotic training was continued. Initially, this suggested that the positive effects of robotic training had plateaued. At the end of the extended robotic training period, however, an additional bolus dose of quipazine immediately increased overall stepping ability, eclipsing the performance after the combination treatment period. Since the mice were not administered quipazine during the nine days preceding this final test, chronic quipazine treatment effects were ruled out. Consequently, the observation that the same quipazine treatment elicited a substantially greater level of performance after than before the additional robotic training

suggests that the acute quipazine treatment may have facilitated masked effects of chronic step training.

For studies in which pharmacological treatments are investigated for improving motor function after an injury, the present results indicate that repeated handling of subjects in a manner that consistently activates relevant sensory pathways may be critical to generating recovery. These findings support the hypothesis that spinal learning is highly sensitive to specific spatial and temporal patterns of sensory cues, and suggest that providing appropriate types and levels of sensory information during step training is essential for optimal recovery after a SCI. In contrast, poorly designed or poorly implemented training may yield no effect, and may even impair locomotor performance.

4.5.2 Manual training did not improve stepping performance

Adult cats (Lovely, 1990; Edgerton, 1991a, 1991b; de Leon, 1998; Rossignol, 2002) and rats (de Leon 2002; Moshonkina, 2002) that are completely spinalized at a mid- to low-thoracic level can relearn to step via manual training. Trainer-assisted guidance of the hindlimbs through a kinematically appropriate trajectory, the manual training method used here, did not improve locomotor performance in complete spinal mice. This was probably due, in part, to our inability to control small mouse hindlimbs with sufficient consistency. It is possible, although unlikely, that other methods of manual training would yield different results.

No significant recovery of stepping ability was observed in either the nontrained or manually trained mice. These observations contrast with those of Leblond et al. (2003), who described spontaneous locomotor recovery in adult spinal CD1, BALB/c,

and C57BL/6 mice without training or pharmacological facilitation. While we have observed spontaneous recovery in neonatally (P5) transected mice, we have not observed spontaneous recovery in Swiss-Webster mice transected at P35 or later, a result that parallels observations in spinal rats (Commissiong, 1993; Molinari, 1993).

4.5.3 The robotic system significantly enhanced training effectiveness and enabled quantitative locomotion analysis

The results of the present study demonstrate the significant value that robotic systems can bring to locomotor training and evaluation. In active training mode, the mouse stepper significantly improved stepping ability in spinal mice within one week of training. In passive recording mode, the mouse stepper elucidated subtle differences in stepping ability, distinguishing the effects of robotic training and quipazine treatment. By providing precise and consistent training, and enabling rapid quantitative recording and analysis of stepping data, robotic systems can greatly improve rehabilitation after a SCI.

4.6 Summary

The combination of robotic training and pharmacological treatment utilized in the present study illustrates how a multimodal strategy can be used to reinstate functional locomotion in mice after a complete spinal cord transection. Clearly, the neurotransmitter environment of the post-injury spinal cord is critically important in defining the functionality of the spinal circuitry and the extent to which it can be modified by specific sensory cues. The present results illustrate the feasibility of using robotic systems to train and to examine locomotor performance quantitatively in spinal mice, and of extending

these studies into relevant transgenic models. Future studies will examine the long-term effects of robotic training and/or quipazine treatment on functional recovery, and will further quantify their interaction effect.

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CHAPTER 5: Implications of Assist-As-Needed Robotic Step Training after a Complete Spinal Cord Injury on Intrinsic Strategies of Motor Learning

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5.1 Abstract

Robotic training paradigms that enforce a fixed kinematic control might be suboptimal for rehabilitative training as they abolish variability, an intrinsic property of neuromuscular control (Jezernik et al., 2003). In the present study, we introduce "assist-as-needed" (AAN) training paradigms using a robotic training device for rehabilitation after a spinal cord injury. To test the efficacy of this strategy of robotic control to teach spinal mice to step, 27 adult female Swiss-Webster mice were divided randomly into three groups. Each group was robotically-trained using one of three control strategies: a fixed training trajectory (Fixed Group), an AAN training paradigm without interlimb coordination (Band group), and an AAN training paradigm with bilateral hindlimb coordination (Window group). Beginning 14 days after a complete mid-thoracic spinal cord transection, the mice were trained daily (10 min/day, 5 days/week) to step on a treadmill 10 min after the administration of quipazine (0.5 mg/kg), a serotonin agonist for a period of six weeks. During weekly performance evaluation, the mice trained with the Window paradigm generally showed the highest level of recovery, as measured by the number, consistency, and periodicity of steps during the testing sessions. In all three measurements, there were no significant differences between the Band and the Fixed training groups. These results indicate that the window training approach which includes

loose alternating interlimb coordination is more effective than a fixed trajectory paradigm with rigid alternating interlimb coordination or an AAN paradigm without any interlimb constraints in promoting robust post-injury stepping behavior.

5.2 Introduction

It has been shown that adult spinal mice can be trained to step on a moving treadmill belt using a robotic device (Fong et al., 2005). In addition, there was a positive interaction effect between robotic training and quipazine, a broad serotonin agonist, administration. To date, the algorithms that have been used for locomotor training with robotic devices have primarily focused on repeated movements of the limbs through fixed kinematic trajectories. However, these types of training abolish the variation in the kinematics and activation patterns of motor units from cycle to cycle, a fundamental feature of the neural control of repetitive movements such as stepping (Hausdorff, 2005). This feature of neural control has direct implications for the development of the controls used in designing robotic devices to assist the neuromuscular system in learning a motor task. Robotic orthosis driven in a fixed pattern effectively limit the degrees of freedom of the leg's motion relative to that of the naturally occurring muscle activation patterns (Hidler and Wall, 2005). Thus, fixed trajectory training will likely produce an extensive level of habituation to sensory input, resulting in markedly reduced sensory responses associated with weight-bearing locomotion. As a consequence, the training could become counterproductive, resulting in a decrease in the activity of sensorimotor systems that should be highly active. In turn, this is likely to reduce the activity of the spinal neural control circuits that control locomotion (Wirz et al., 2005). In addition, we believe that a

fixed, repetitive training paradigm may lead to "learned helplessness" i.e., the lower spinal cord habituates to repetitive activation of the same sensory pathways during a training session (Skinner, 1979; Wool et al., 1980).

In the present study, we tested the hypothesis that the post-SCI spinal cord can relearn to step more effectively if it is constantly challenged during locomotor training by introducing flexibility in the training pattern. We implemented this flexibility in the form of "assist-as-needed" (AAN) training paradigms. Similar forms of robotic control algorithms have been used for rehabilitative training of upper extremities in stroke patients (Hogan and Krebs, 2004; Patton and Mussa-Ivaldi, 2004; Patton et al., 2006). In the present study, we have implemented such training algorithms for gait training of the hindlimbs of complete spinalized animals. Our AAN algorithms allow the animal to largely control its own motions when it is performing well. In this way, some variability in the stepping trajectory is experienced during training after an SCI, as occurs during normal locomotion.

Although there are many variations of the AAN theme, we compared the efficacy of two AAN robotic training algorithms and a fixed trajectory robotic training paradigm on the recovery of locomotor ability in complete spinalized adult mice that also were administered quipazine daily, just prior to each training session. The results indicate that mice undergoing AAN robotic training with loose controls of interlimb coordination exhibit faster and more pronounced recovery of stepping ability than mice trained using a fixed robotic paradigm or an AAN robotic training paradigm without interlimb coordination constraints.

5.3 Materials and Methods

Animals and animal care. Adult female Swiss-Webster mice (mean body weight of 25.3±1.3 g on the day of spinal cord transection) obtained from Charles River Laboratories (Wilmington, MA) were used. The mice were housed individually, had access to food and water *ad libitum*, and were kept on a 12 hour light/dark cycle for the duration of the study.

Surgical procedures and post-surgical care. Surgeries were performed at approximately postnatal day 60 (P60). The mice were maintained in a deep anesthetic state throughout the surgery using isoflurane gas (2-5% isoflurane mixed with 0.4% O_2 via face mask). All procedures were performed under aseptic conditions (See chapter 4.2.1).

Quipazine administration. Quipazine, a broad-spectrum serotonin agonist, was used to facilitate stepping in all mice. Based on previous dose-response studies (Orsal et al., 2002), a dose of 0.5 mg/kg body weight was administered intraperitoneally 10 min before each training or testing session.

Robotic training algorithms. The mice were divided randomly and equally into three groups (n = 9/group). Each group received a different robotic training algorithm, i.e., a repetitive training algorithm with a fixed and tightly controlled trajectory or one of two AAN training algorithms. The two AAN algorithms differed in the amount of interlimb coordination that was imposed during training. The AAN training algorithms were implemented using a velocity field approach where the velocity of the distal tip of the linkage was commanded to a specific speed defined by a velocity field. Using the linkage Jacobian matrix, the distal velocities were converted to the desired motor velocities. All mice received quipazine injections prior to each training and testing

session (see above). Each mouse was trained for 10 min/day, 5 days/week, for 6 weeks. Stepping performance was evaluated on the 6th day of each training week.

Mice in the Fixed group received a rigid robotic training algorithm, where a Proportional-Integral-Derivative (PID) controller continuously tracked a desired training pattern. When attached to the mice, the robotic arms actively moved the ankles along this fixed trajectory which included a fixed alternating interlimb coordination. Since neonatally transected mice can spontaneous recover functional stepping without pharmacological or mechanical assistance (Fong et al., 2005), the imposed X and Y trajectory of each hindlimb was obtained from a neonatally transected mouse that stepped well. This pattern was recorded when the neonatally transected mouse was approximately the same age as the adult mice used in the present study.

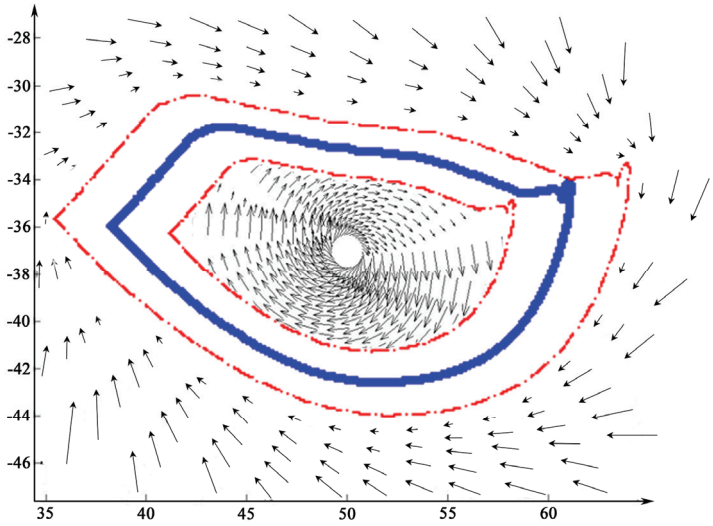

Figure 5.1: AAN training paradigm I (Band). The solid thick line shows the desired training trajectory of the animal's ankle position in the sagittal plane. The dotted thin lines represent the boundaries within which "soft control" (see Materials and Methods) is applied to the limbs. The arrows outside the boundaries correspond to the convergent velocity fields that drive the legs to the band region. Modified from (Cai et al., 2005).

The Band group of mice received an AAN strategy which implements two fixed boundaries, an inner bound and an outer bound, forming a band surrounding the desired trajectory. When the ankle lies in saggital plane regions inside (or outside) the band, an outward-spiraling (or inward-spiraling) converging velocity field drives the ankle to the band region. When the ankle leaves the band, the convergent velocity fields will rapidly move the ankle back into the band region (Figure 5.1). Within the band, the ankle is guided by a small constant velocity field tangent to the desired trajectory, i.e., the robot provides a gentle guidance at a constant rate, but it does not enforce specific timing of leg movement nor enforce the ankle to be at a specific location (soft control). In this way, the mouse largely dictates its own motions inside the band, with only a small bias provided by the robot. Note that this particular instantiation of the AAN paradigm does not impose an interlimb coordination constraint.

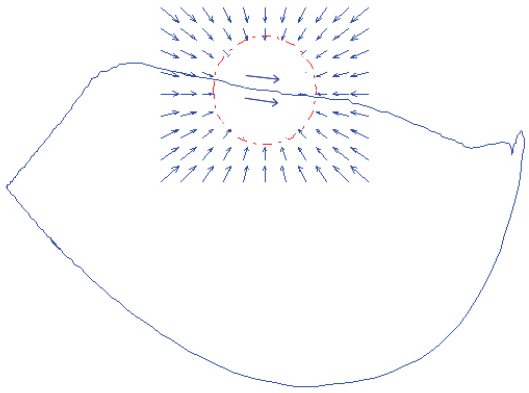

Figure 5.2: AAN training paradigm II (Window). The solid line represents the desired training trajectory of the animal's ankle position in the sagittal plane and the moving window is outlined by the dotted red circle within which "soft control" is applied to the limbs. The arrows outside the circle correspond to the radial force fields. Modified from (Cai et al., 2005).

The last group of mice (the Window group) received an AAN training paradigm analogous to the second group, but based on a moving window geometry. In this

approach, a circular window moves along the desired trajectory (Figure 5.2). The 4 mm diameter window size, which was fixed throughout the experiment, was chosen because it was close to the maximum variation observed during stepping of a neonatally transected mouse (Figure 5.3). Within the window, a small constant velocity field tangent to the desired trajectory biases the robot's motion, but without spatial or temporal enforcement inside the window. Outside the boundary, the robotic movement is guided by a radial velocity field that points inward with a magnitude proportional to the distance from the center of the circle: $v = k\,(d\text{-}r)$, where v is the velocity field magnitude, d is the distance between the ankle and the center of the moving window, r is the window radius, and k is a constant. Hence, when the ankle of the mouse deviates from the window, it is quickly returned to the window. Within the window, the ankle is gently guided in the direction of the trajectory, thus providing loose timing control. The same strategy was used on both hindlimbs, and the control systems for each leg were timed to provide coordination that was consistent with weight-bearing stepping.

Data analysis and evaluation methods. Testing was performed on the 6th day of each training week. The mice were given a 2 min "warm-up" period before each testing session using the same training algorithm associated with that particular group. The mouse stepper was used in a passive recording mode to track the ankle position of each leg for 2 min at a treadmill speed of 3 cm/s. Position data were recorded at 200 Hz using a custom acquisition program written in LabVIEW^{TM} (National Instruments, Austin, TX). In addition to the robot data, video footage of both the left and right sides of each mouse was captured during testing and a log of qualitative observations was maintained. Using these data, the quality of stepping was assessed in terms of: 1) the number of steps

performed; 2) the periodicity of the steps, i.e., the ability to maintain a regular stepping frequency; and 3) the regularity of the stepping patterns. The following analyses were used for these assessments of stepping ability.

Data Analysis. The number of steps performed in the best twelve second interval during the two min. testing session is counted. Then step periodicity and step shape consistency are analyzed using FFT and PCA respectively (See Chapter 4.3). Locomotor performance scores are reported as the mean ± standard error of the mean. One-way ANOVA analysis was used to compare the three training groups within each training day. A *p*-value less then 0.05 was used to define statistical significance, which corresponded to a critical *F*-value of 3.44. To measure statistical difference between groups, the least significant difference (LSD) was calculated.

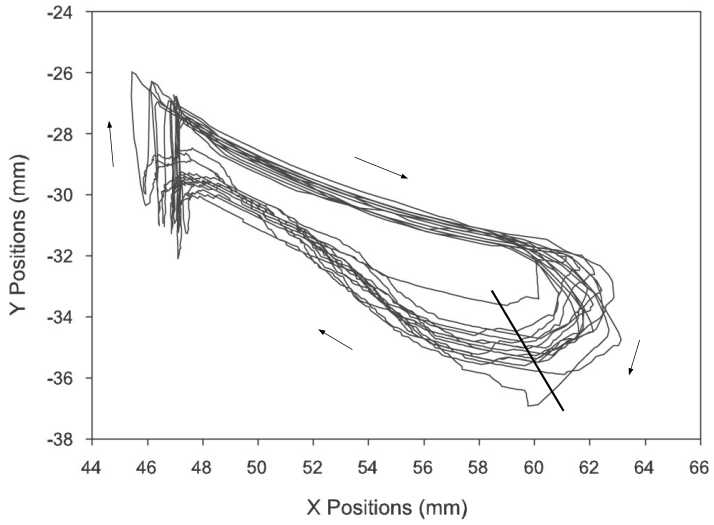

Figure 5.3: The stepping trajectories of the ankle of a neonatally spinal cord transected mouse at approximately three months of age. The diagonal line through the trajectories shows where the most deviation (~ 4 mm) occurs. The arrows represent direction of travel.

5.4 Results

All groups showed improvement over the 6-week training period based on the average

number of steps taken in the best 12-sec interval during each testing session (Figure 5.4). The mice in the Window group, however, had a faster rate of recovery than in the other two groups. The average number of steps taken by the Window group was higher than in the Fixed group from weeks 1 to 3 and higher than in the Band group at weeks 1 and 3. There were no significant differences between the Band and Fixed groups at any time point, and the average number of steps was similar in the three groups after 6 weeks of training.

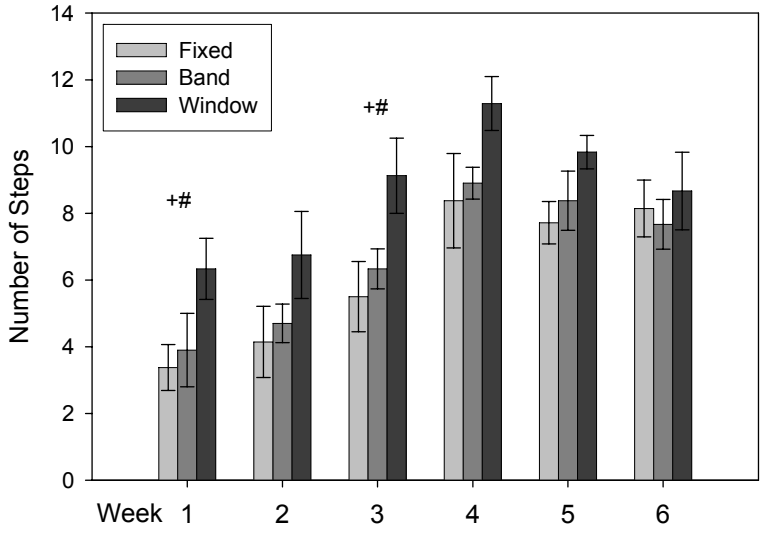

Figure 5.4: Average number of steps performed during the best 12-sec interval by each of the three groups during the weekly tests. After four weeks of training, all three groups showed a significant increase in the average number of steps taken compared to week 1. On average, mice in the Window group performed better compared to the other two groups. +, denotes significant difference between the Window and the Fixed group; #, denotes significant difference between the Window and Band group.

Inverse FWHM scores for the mice in the Window group were significantly higher than in the Band and Fixed groups after 4, 5, and 6 weeks of training (Figure 5.5). There were no significant differences between the Band and Fixed groups at any time point. The maximum level of step rhythmicity was achieved after 6 weeks of training: Window group (8.3) was higher than the Band (6.4) and Fixed (6.3) groups.

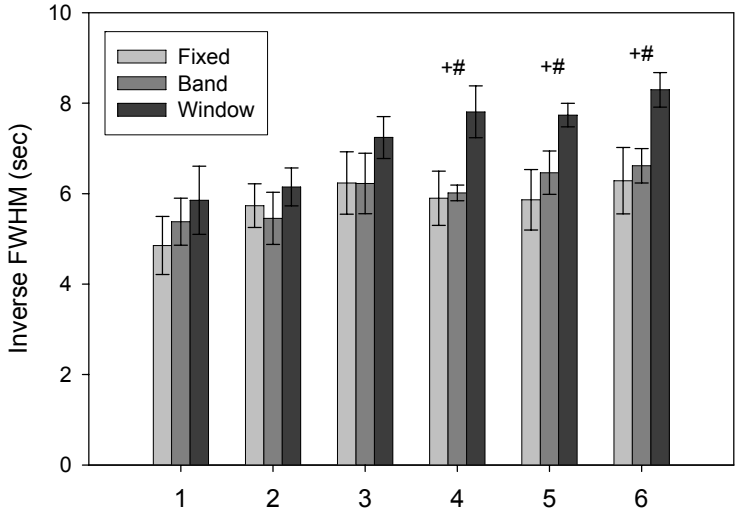

Figure 5.5: Step rhythmicity as depicted by the plot of the inverse FWHM. Although the mice trained with the Window algorithm performed more consistently than the Fixed and Band groups, the differences are not significant until the fourth week. +, denotes significant difference between the Window and the Fixed group; #, denotes significant difference between the Window and Band group.

All three groups showed progressive improvement in step shape consistency, based on PCA analyses, throughout the first 5 weeks of training and then a slight decrease at 6 weeks (Figure 5.6). There were no significant differences among the three groups at any time point.

5.5 Discussion

5.5.1 All step training algorithms improved stepping beyond the level that is achieved without any step training.

We have demonstrated previously that a combination of quipazine administration and robotic training can significantly improve the locomotor performance of adult spinal mice. Without pharmacological and/or mechanical interventions, the average number of steps performed in a full 2-min interval was 16.0±5.1 and no mouse was able to perform the minimum of three consecutive steps in a 12-sec interval that were required for FFT

and PCA analysis (Fong et al., 2005). All of the mice in the current experiment received quipazine treatment and robotic training. Consequently all training paradigms improved stepping and the level of improvement was greater than the initial test at week 0 and the level of performance reported previously. Even after only one week of training, the lowest average number of steps for the best 12-sec interval among the three groups was 3.7±0.7 steps. These results demonstrate the effectiveness of robotic systems in enhancing locomotor training after a SCI, even when using sub-optimal training algorithms.

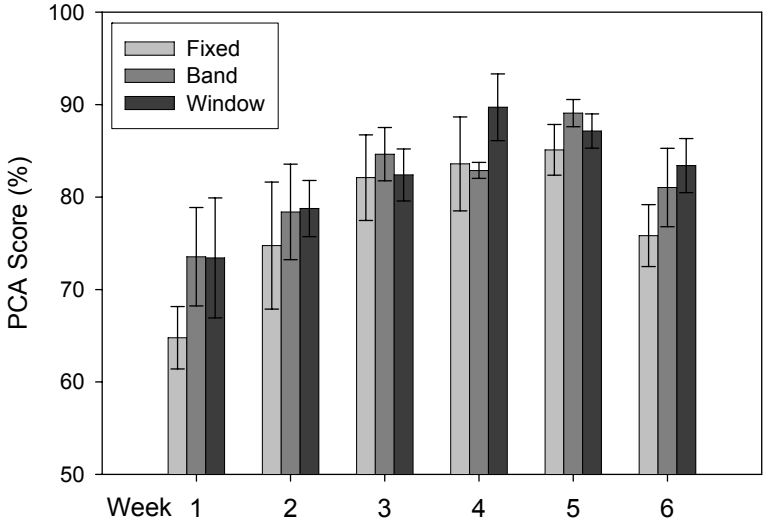

Figure 5.6: Step shape consistency as measured by PCA. There were no significant differences among the three groups at any of the weekly tests.

5.5.2 Permitting an intrinsic network solution facilitates stepping more effectively after a SCI than imposing an extrinsic motor solution.

A key objective of this study was to ascertain whether permitting variability during step training enhances stepping recovery after a complete spinal cord transection in adult mice. We hypothesized that a fixed trajectory training strategy would drive the spinal

circuitry into a state of "learned helplessness" (Wool et al., 1980; Grau et al., 1998). This occurs when the spinal cord is not permitted to explore potential solutions to stepping patterns and, thus, defers to the fixed training pattern with the neural circuits of relevance habituating. Figure 5.3b shows that even though the fixed training paradigm is one in which the periodicity of stepping is most tightly controlled, the animal fails to produce a consistent stepping period during testing. In contrast, when the training paradigm allows the stepping period to vary, the mice were able to adapt to a consistent stepping period. These results, combined with number of steps performed, suggest that the window training paradigm significantly improved the stepping ability of the mice compared to the band and the fixed training paradigms.

Sensory information is critical to motor learning. The pattern and timing of assistance provided during step training seems to play a critical role in specific sensorimotor pathways that become reinforced after a SCI (de Leon et al., 1998). Regardless of the level of practice of a task, some variability in the patterns and levels of activation of motor units within a motor pool persists even during the simplest repetitive actions. Thus, there also must be variation in the efficacy of the ensemble patterns of sensory input to the spinal circuitry from step to step. Given this intrinsic variability, when a mechanically-fixed pattern is imposed the sensory input is highly unlikely to match the subsequent motor output. From this perspective, it appears that a continuous incongruity between the input and output signals will occur when a fixed trajectory is imposed. Thus, a training algorithm that is incompatible with the basic feature of variability during stepping seems likely to hinder the ability of the spinal cord to learn to step after a SCI.

Conversely, a training algorithm that "permits" the intrinsic variability in the activation of motor pools may allow the spinal circuitries to explore the multiple patterns of activation and thereby optimize training effectiveness. In this experiment, we have tested only two such algorithms. Spinal mice recovered stepping ability more effectively with the window AAN algorithm than the band AAN or fixed training paradigms. Even the window algorithm, however, is unlikely to be the optimum solution as demonstrated by the peak in locomotor performance reached after four weeks of training in the current study. One can imagine many variations of the AAN training algorithm, but it will be difficult to experimentally test all of these variations in attempts to find an optimum rehabilitative training strategy. One approach will be to develop a learning model for the plasticity within the spinal cord derived from machine learning theories. Having such a model will allow us to explore many more parameter spaces, such as window size and shape, than would otherwise be possible experimentally. Once candidate training algorithms are identified analytically, these algorithms can be validated experimentally using an experimental paradigm as in the present study.

5.5.3 An imposed interlimb coordination pattern facilitates learning to step.

Another observation from this study was that control of interlimb coordination can improve locomotor recovery. Although it had been shown that spinal cats can adapt to different walking speeds on a split treadmill (Barbeau and Rossignol, 1987), de Leon et al. (de Leon et al., 1999) found that in examining the cause of failure to continue stepping in chronic spinal cats that had been trained to step, the most consistent contributing factor was a gradual loss of the appropriate interlimb coordination. Rarely was failure due to

poor intralimb kinematics. Similarly, the current experiment suggests that maintaining interlimb coordination plays an important role in training adult spinal mice to step. The steps were typically arrhythmic and frequently interrupted by dragging in mice trained with the band algorithm, which is evident from the low inverse FWHM value (Figure 5.3b). In contrast, stepping executed by the mice in the Window group was rhythmic and prolonged. In many cases, the mice were able to step throughout the entire 2-min testing period. This was reflected by the average number of steps taken in the best 12-sec interval by the mice, which showed that stepping in the Window group converged to a frequency near 1 Hz as the study progressed, and is consistent with constant speed treadmill locomotion at 3 cm/sec (Figure 5.3a, week 4).

5.5.4 Distinction between shape consistency and quality of stepping.

There were no significant differences in step shape consistency among the three groups throughout the study based on PCA scores (Figure 5.3c). Even after one week of training, the average PCA scores of the Band and Window groups were over 70%. By week 3 the average PCA score of all three groups was greater than 80%, indicating that all of the mice could perform consecutive rhythmic movements that were similar in shape within that animal. Therefore, the PCA results reflected consistency in cyclic movements, but did not differentiate the quality of stepping between the different training groups**.** Combined with previous result, which reports an average PCA score of 77±4% with just quipazine administration alone (Fong et al., 2005), one reasonable hypothesis from these results is that quipazine may have a greater effect on the shape consistency of the stepping than robotic training, especially when the training itself is not rigid.

5.6 Summary

The present results provide strong evidence that a fundamental strategy of the neural control of a given motor task (stepping) is to incorporate a degree of variability in the sensorimotor pathways. These data suggest that when the intrinsic variability is overridden, e.g., when a "fixed" pattern is imposed, learning of a task is suboptimal relative to the condition when the training is "assist-as-needed". Beyond the insight provided by these results on the strategy for neural control of movement, the practical implications may be highly significant for future efforts to develop robotic devices that can be used to facilitate recovery from neuromotor impairments.

5.7 Chapter References

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5.8 Chapter Appendix: Computation of the Band Vector Fields

To generate the velocity fields for the "Band" algorithm seen in Figure 5.1, we transformed a simple convergent velocity field for a circular trajectory to the desired stepping trajectory via a numerical conformal mapping procedure.

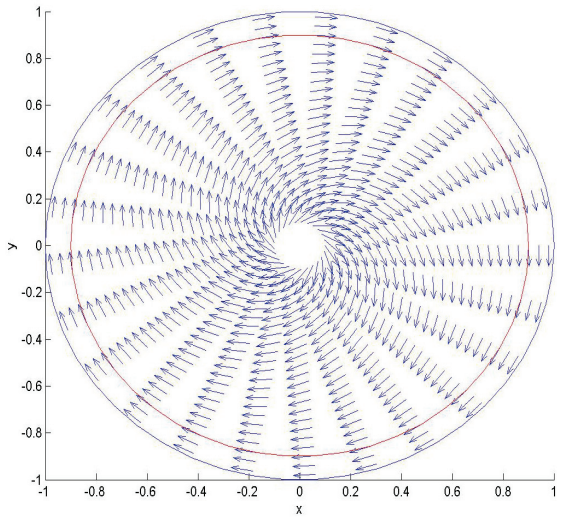

Figure 5.7: Convergent velocity field of a unit circle.

Figure 5.7 shows a clockwise outward spiraling velocity field inside a unit circle which will cause a point in the interior that follows the integral of the velocity fields to converge to the circular trajectory. The velocity field is determined by the following equations in polar coordinates r, α:

$$\vec{v}_r(r,\alpha) = (1-\delta-r)(\vec{i}\cos(\alpha) + \vec{j}\sin(\alpha)), r \le 1-\delta$$
$$\vec{v}_r(r,\alpha) = 0, \qquad r > 1-\delta$$
$$\vec{v}_a(r,\alpha) = k(-\vec{i}\sin(\alpha) + \vec{j}\cos(\alpha))$$

Distance ($0 < \delta < 1$) defines a band along the unit circle. If the point is within the distance δ to the unit circle, the velocity field just follows the trajectory. Outside the band, the velocity field tends to move towards the band.

By reversing the direction of the angular component of the velocity field and mapping it onto the area outside the training trajectory, the inward spiraling velocity field component of Figure 5.1 is realized.

The numerical conformal mapping is implemented via the Zipper program developed by Prof. D. E. Marshall of University of Washington: (http://www.math.washington.edu/~marshall/zipper.html).

CHAPTER 6: Computational Model of Motor Learning Based on Intrinsic Variability in Stepping

6.1 Abstract

In this chapter, we propose a model for motor learning using a form of reinforcement learning derived from machine learning theory to examine the effects of variability on motor learning. Our model consists of traditional reinforcement learning plus a modifiable intrinsic variability parameter (IVP) that is drawn from a Gaussian distribution. The objective is for the system to learn a target function despite the uncertainty created by the IVP. Simulations using this model show that learning rate is highly dependent on the training paradigm. If training is rigid, the system is continuously being "punished" due to the imposed errors generated by the IVP and fails to learn the target function. Such a fixed training algorithm induces an effect equivalent to "learned helplessness." Alternatively, if the training allows for variability, much like our AAN training algorithm in animal experiments, the system learns more effectively than a rigid training paradigm. The simulation results from our model are consistent with experimental evidence suggesting that learning rate is dependent on the level of variability allowed by the training and that there is a critical level of variability for obtaining an optimal training effect (see Chapter 5).

6.2 Introduction

Increased evidence suggest that robotic training paradigms that enforce a fixed kinematic control are suboptimal for rehabilitative training as they abolish variability, an intrinsic

property of neuromuscular control (Jezernik, Scharer et al. 2003; Hogan and Krebs 2004; Patton and Mussa-Ivaldi 2004; Emken and Reinkensmeyer 2005; Hausdorff 2005; Hidler and Wall 2005). Chapter 5 demonstrated that the locomotor recovery of adult spinalized mice is improved by incorporating AAN robotic training compared to fixed-trajectory training, suggesting that the motor learning can occur in the isolated spinal cord level. Therefore, it is theoretically possible to find a more optimal rehabilitative training paradigm by permitting the spinal circuitries that generate stepping to utilize all of the intrinsic properties inherent in its neural control.

The automaticity in the control of locomotion as well as the importance of sensory feedback in neuromuscular systems (see Chapter 2.4) resembles that of a simple mechanical control system, where the goal is to use feedback to guide the performance of a movement such that the error between the command input and the resulting output is minimized (Zhou, Doyle et al. 1996) (Figure 6.1A). Although, such controllers have proven to be versatile and reliable in the engineering world, it is unlikely that biological neuromuscular systems are controlled in such a manner. First of all, such a control system cannot adapt to changes in the environment and can become unstable quickly when there are large disturbances. Secondly, such a control system lacks the ability to learn to improve its performance from past experience. In contrast, the vertebrate spinal cord neural circuitry is capable of adjusting to disturbances (Barbeau, Fung et al. 2002) and learning from repetitive training (Edgerton, Roy et al. 1992; de Leon, Hodgson et al. 1998; de Leon, Hodgson et al. 1998), even in the absence of supraspinal control.

Another robust property of the normal neural control system of locomotion is variability, where the activation pattern of neural circuitries controlling locomotion from

cycle to cycle is not deterministic but governed by a probabilistic component that can be altered with training (see Chapter 2). Nevertheless, even with this variability, the success of hundreds or even thousands of steps can be predicted in the uninjured individual under normal circumstances. Following a SCI this variability in both the activation patterns and the resulting kinematics of the hindlimbs increases, and the probability of generating consecutive successful steps will be quite low and, in many cases, near zero. Step training reduces the variability in the kinematics of the limb motions (de Leon, Hodgson et al. 1998). Presumably, increasing the occurrence of a given pattern of sensory information associated with load-bearing stepping increases the probability of pattern-recognition by those neural networks that are linked to the sensory patterns. In addition, more frequent occurrence increases the probability of generating a predictable kinematic pattern whenever that sensory pattern is recognized. In essence, the likelihood of a given set of neurons being activated in a given condition may change from near randomness to one that is highly predictable, reflecting properties that are typical features of learning systems.

Most of the theories of learning systems are developed based on "supervised learning," the kind of learning most widely studied in machine learning. Supervised learning is the process of learning from examples provided by a knowledgeable instructor, where the input and output examples are provided as a classified pair (Anderson, Michalski et al. 1983). Although this type of learning is significant in knowledge acquisition, alone it is not adequate to explain locomotor learning. In a neuromuscular system, most of the learning processes are "unsupervised" and involve skill refinement. Although the idea has been around for decades, a relatively new set of

learning theories called “reinforcement learning” have been developed in recent years to address this problem.

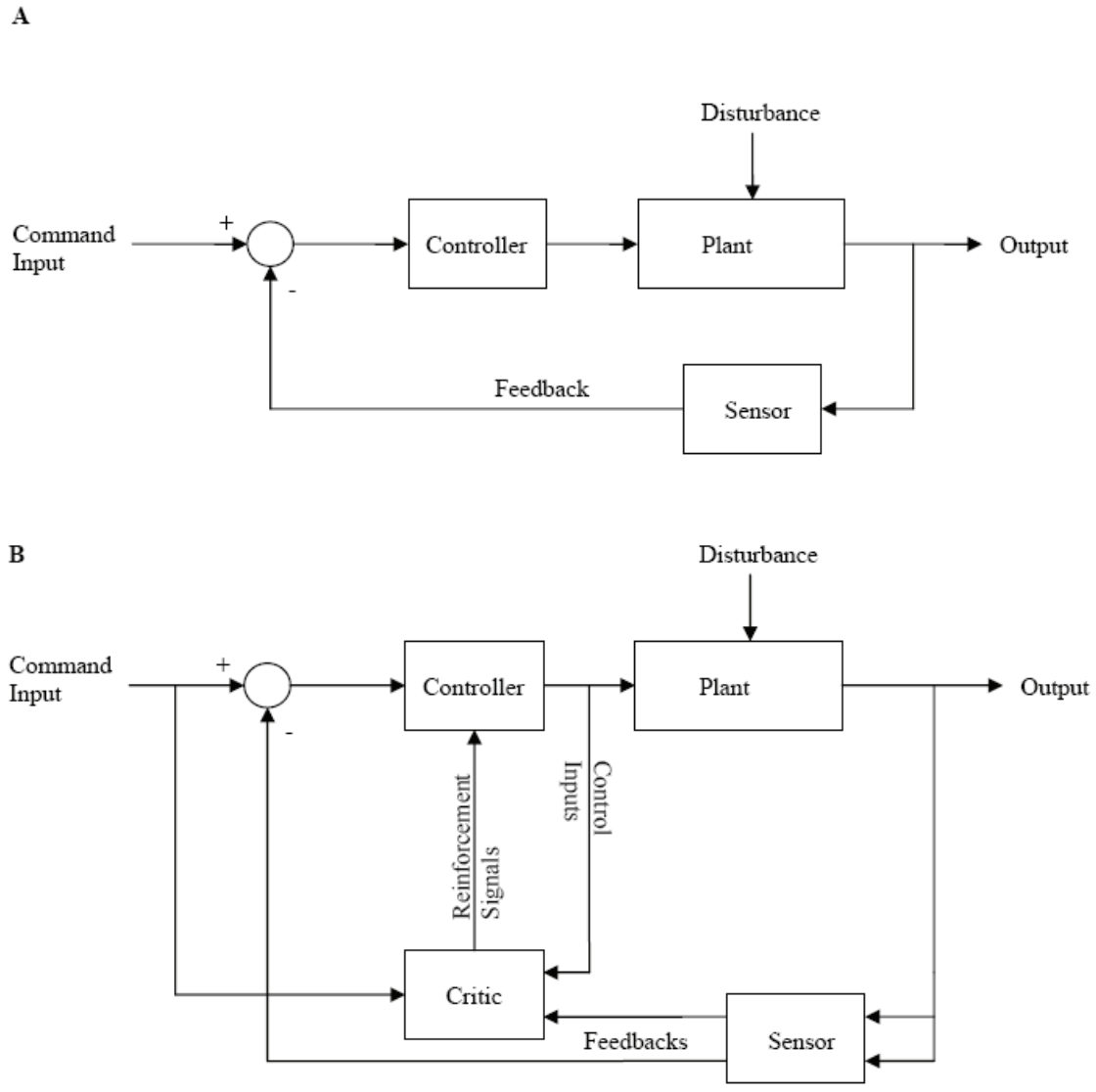

Figure 6.1: (**A**) Block diagram for a simple mechanical controller. In neuromuscular systems, the controller would be the motor neurons, the plant would be the muscles, and the sensor would be all of the proprioceptive feedback to the motor neurons. The information provided by the sensor is a negative feedback (denoted by the – sign) and is used to minimize the error between the output of the plant and the command input, a positive input (denoted by the + sign). In controls, the disturbance generally refers to unmodeled dynamics of the plant. However in neuromuscular systems, this would represent perturbations that the system might encounter. (**B**) Block diagram for an adaptive controller incorporating reinforcement learning. In neuromuscular systems, the controller, plant and sensor will be the same as in (**A**). The critic will be the input from all of the interneurons, e.g., Ia, Ib, Renshaw cells, etc., affecting the efficacy and excitability of the motor neuron (the controller), which is represented by the reinforcement signals.

In contrast to supervised learning, reinforcement learning emphasizes learning feedback that evaluates performance without providing a standard of correctness in the form of behavioral targets, i.e., reinforcement learning gives an index of how well the system performed relative to it previous trials without giving any indication of the correct response (Barto 1994). Therefore, to maximize reward, the reinforcement learning paradigm requires the system to actively try alternatives, evaluate the results, and then use a selection mechanism to guide behavior toward the best alternative. The fundamental process is analogous to "trial and error". However, the search is not random or undirected. Instead, the system takes into account results acquired from previous trials to decide how and where the next increment in stepping will be taken, choosing a path that will give the highest probability for future success. In this concept of reinforcement learning, randomness is often utilized to create behavioral variety, which is called exploration. The consequential actions, however, are strongly guided by evaluation of earlier experiences and often the system will prefer an option that has produced favorable results in the past, such a move is called *exploitation*. As a result, reinforcement learning algorithms are selection processes, but there must be variability in the action-generation process so that the consequences of alternative action can be compared to select the best alternative.

By incorporating the concept of reinforcement learning into a control system, such a system is able to use feedback to evaluate the performance for improving subsequent movement by changing the controller itself (Figure 6.1B). This is analogous to sensory inputs changing the efficacy of spinal circuitries that control locomotion as a result of training. In most artificial reinforcement learning systems, the critic's output at

any time is a number that scores the controller's behavior: the higher the number, the better the behavior. If the behavior being scored is immediately preceding a subsequent unit of behavior produce by the controller based on the critic's score, there must be enough variability in the controller's behavior so that the critic can evaluate many alternatives for this process to work. A learning mechanism then must adjust the controller's behavior so that it tends toward behaviors that are favored by the critics (Sutton and Barto 1998). Applying these learning theories to the neuromuscular system, it is apparent that variability is a necessity for motor learning to occur most effectively.

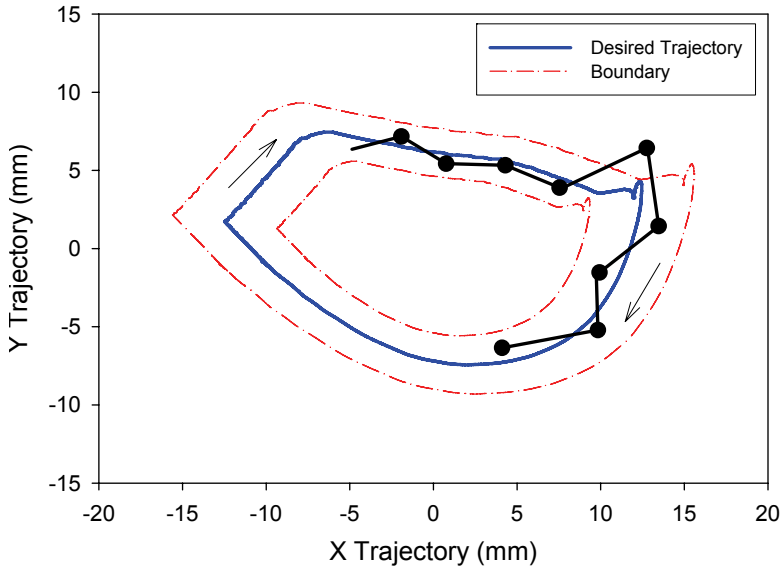

Figure 6.2: Schematic of a semi-active fixed-trajectory paradigm for step training, where the desired ankle trajectory (blue) is bounded by both inner and outer boundaries (red). The actual trajectory (black) that the neural circuits might induce is allowed to vary within the boundary. However, once the trajectory falls outside of the boundary, the robot will actively bring the ankle back within the boundaries. The black line with periodic dots illustrates a hypothetical trajectory of generated by an animal that is sampled at discrete times. The probability that the neural control would move the limb to the exact position defined by the blue line, representing a fixed trajectory, is highly unlikely. As a result, theoretically, the neural control system is continuously disrupted by the fixed trajectory paradigm. This fixed trajectory, therefore, does not allow the neural control circuitry to respond to any of its intrinsic activation patterns, but rather forces the intrinsic circuitry to continuously respond to external perturbations. This strategy for control would seem to unnecessarily disrupt the spinal circuitry and in the process minimize or even preclude the intrinsic circuitry from interpreting relevant proprioceptive information required to generate a solution (i.e., make choices) and, thus, presumably prevent the circuitry from meaningful learning phenomena.

To better understand the role that variability plays in motor learning, we develop a learning system that tries to abstractly model the process of whereby the intact portion of the lower spinal cord in a spinalized mouse recovers locomotion during robotic training with an assist-as-needed (AAN) training paradigm. The training allows the subject to learn the target within a given tolerance in order to accommodate the inherent variability in animal locomotion.

6.3 Summary of the learning/training procedure

During AAN training, at any giving point of the training cycle, the isolated spinal cord will integrate all of the available sensory information coming into the spinal cord and produce a motor output for the next point in the cycle (Figure 6.2). However, this input and output set is not deterministic but rather has a probabilistic range of values. Immediately after injury this range can be quite large, but through repeated activation with training, the range will gradually decrease. Macroscopically, this process can be modeled using an idealized one dimension learning system as summarized in Figure 6.3. The goal is for the system to learn how to reach a target position, labeled "c," to within a tolerance, or "window" around c of radius δ, a parameter of our own choosing. This window is termed the "goal window." Assume that the learning system starts in an initial state μ_0, which is located a distance x_0 away from the desired goal, c.

The learning process proceeds in a general way as follows, and many variations of the basic scheme are explored. The "system" generates a random "step" or "probing move." The probing move is drawn from a random distribution, which is assumed to be a Gaussian with variance σ. Here σ is the intrinsic variability parameter (IVP) and is

modifiable (see below). The IVP models the probabilistic component of motor output, which is modifiable through training. The learning algorithm then accepts or rejects this probing move (different rules for accepting/rejecting a step are discussed below). If the move is accepted, then the system adjusts its state to the position of the random move. Additionally, the variance of the probing distribution is potentially adjusted during each iteration. We call the combination of the probe acceptance/rejection rule and the variance update rule the "learning rule." This probing/updating process repeats until a stopping criterion is satisfied. Note that generally, a differnt learning rule may be invoked as the system state enters the "window" around the goal.

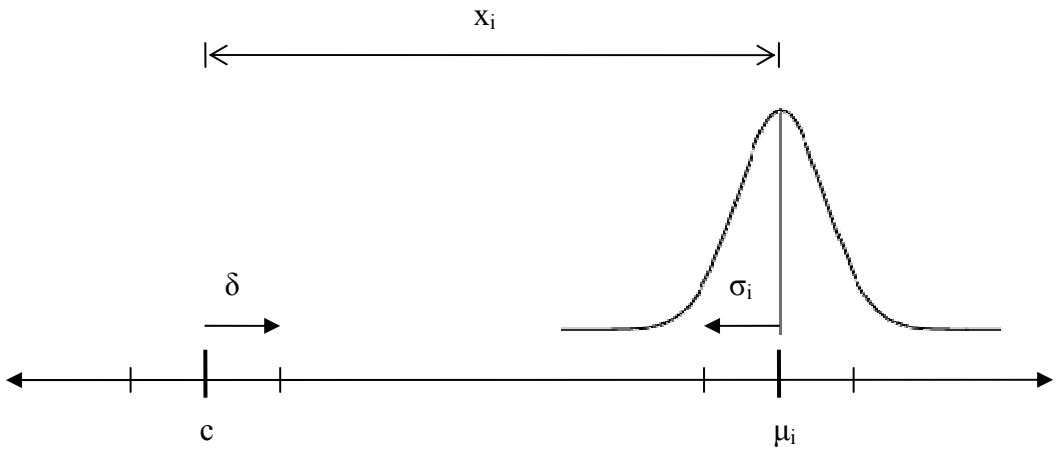

Figure 6.3: Idealized one-dimensional learning model where c is the target and δ is a fixed window around the target. The goal is for the system state to reach c and stay within the window δ. σ_i is the intrinsic variability parameter (IVP), which determines the variability of the next probing move μ_{i+1}. The IVP is updated depending on whether the probing falls inside or outside of the target window. x_0 is the measurement of the initial position from the target c.

6.3.1 First Learning Rule

The following version of the learning rule described above is the simplest prototypical version of this learning approach. Assume that at the end of the i^{th} step of the learning

process, the system is located at position μ_i. The system generates a random probing point $\hat{x}_{i+1}$ from a normal distribution with a mean of μ_i and standard deviation σ_i

$$\hat{x}_{i+1} = \mu_i + \varepsilon_i \quad \text{where } \varepsilon_i \in N(0\ , \sigma_i)$$

The system does not necessarily move to this new point. Instead, the probe is accepted or rejected according to the following rule

$$\text{If } |\hat{x}_{i+1} - c| < |\mu_i - c| \text{ then } \mu_{i+1} = \hat{x}_{i+1}$$

$$\text{else, } \mu_{i+1} = \mu_i$$

That is, if the probing step is closer to the goal, c, then the step is accepted. Else, the probing step is rejected, and no move is undertaken in the i^{th} iteration. For simplicity, assume that c is at the origin. In this case, the acceptance/rejection rule can be stated in terms of the Heaviside function:

$$\mu_{i+1} = \mu_i + H(|\mu_i| - |\hat{x}_{i+1}|) \cdot (\hat{x}_{i+1} - \mu_i) \qquad (1)$$

At the end of each step, the variance of the probing distribution is also updated according to the following rule:

$$\sigma_{i+1} = \begin{cases} \sigma_i(1+\alpha) & if\ |\hat{x}| > \delta \\ \sigma_i(1-\alpha) & if\ |\hat{x}| \le \delta \end{cases}$$

where α is a constant parameter that is chosen in advance. That is, the step variance is continually increased until the system samples fall within the desired window of the goal, whereupon the variance is continually decreased. This procedure can be express as a sign (sgn) function:

$$\sigma_{i+1} = \sigma_i(1 + \alpha(\text{sgn}(\hat{x} - \delta))) \qquad (2)$$

The goal of the system is to learn the target c or $\mu_{i+1} \approx c$, thus for simplicity, the stop criterion is set to:

$$\left|\mu_{i+1} - c\right| < 0.1\ \delta$$

That is, if the system state is nearly in the center of the window, then learning is considered to be successful.

6.3.2 The Second Learning Rule

In the previous learning rule, the system state μ_i is modified by comparing the terms $\left|\hat{x}_{i+1} - c\right|$ and $\left|\mu_i - c\right|$. This rule implies that the system knows the exact location of target c. This assumption does not model the experimental setup well, and is perhaps not biologically plausible. Instead, we want to modify the state μ_i in fashion similar to the modification of the parameter σ in the first learning rule and simulation.

Here, like the previous learning rule, the system generates a random probing point $\hat{x}_{i+1}$ from a normal distribution with a mean of μ_i and standard deviation σ_i

$$\hat{x}_{i+1} = \mu_i + \varepsilon_i \quad \text{where } \varepsilon_i \in N(0\ , \sigma_i)$$

Next, determine if the probing point lies within the goal window. If $\left|\hat{x}_{i+1}\right| \le \delta$ then μ_{i+1} moves a fraction closer to the goal, else it will stay put. More specifically:

$$\mu_{i+1} = \begin{cases} (1-\alpha)\mu_i & if \quad \left|\hat{x}\right| \le \delta \\ \mu_i & if \quad \left|\hat{x}\right| > \delta \end{cases}$$

where α is a constant. This procedure again can similarily be expressed as a Heaviside function:

$$\mu_{i+1} = \mu_i\left(1 - \alpha H(\delta - \hat{x})\right)$$

The variance σ is updated similar to the first learning rule with two exceptions. First we want to be able to adjust the growing and shrinking rate of σ independently. So the updating rule is modified to be:

$$\sigma_{i+1} = \begin{cases} \sigma_i(1+\alpha\beta) & if\ |\hat{x}| > \delta \\ \sigma_i(1-\alpha\gamma) & if\ |\hat{x}| \le \delta \end{cases}$$

where β and γ are constants of our own choosing. Second, we want to set a upper and lower bound for σ. The lower bound will correspond to any inherent variability that exist in any animal locomotion, and it is set to be 10% of σ_0 in the ensuing simulations. Physically, this constant corresponds to the fact that even in a perfectly adapted neural system, the participating neurons always produce a minimal amount of intrinsic variability. The upper bound for σ will physically correspond to the movable space that is physiologically possible for the animal to reach, and it is set to 10 times σ_0 in the simulations below.

The stopping criterion is the same as the first learning rule, where we consider the system to have converged after N iterations when:

$$|\mu_N - c| < 0.1\delta$$

Conversely, to shorten simulation time, the system is considered to have diverged if the stopping criterion is not met after 10000 iterations of the algorithm. In practice, we implemented a stopping criterion that checks to see if μ_i stay with in the window on average:

$$\frac{\sum_{i=0.9N}^{N} |u_i - c|}{0.1N} < 0.1\delta$$

6.3.3 Applying the Learning Model to a Biomechanical Model

Next, we want to see if the learning model describe previously can be apply to a simple biomechanical model of the animals hindlimb, such that it could learn a desire trajectory. Assuming that the point of interest is the ankle position, we can express the ankle trajectory in Cartesian coordinates as a function of the hip (θ_h) and knee (θ_k) angles using a two bar mechanical system model: (Figure 6.4):

$$X = l_f \cos(\theta_h) + l_t \cos(\theta_h + \theta_k)$$
$$Y = l_f \sin(\theta_h) + l_t \sin(\theta_h + \theta_k)$$

where l_f and l_t corresponds to length of the femur and tibia bone respectively.

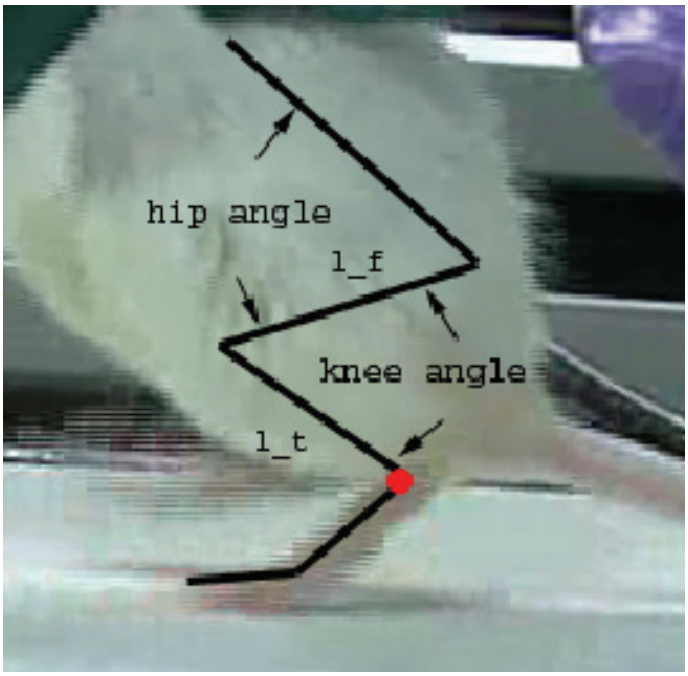

Figure 6.4: A simple biomechanical model of the mouse hindlimb where the red dot represents the ankle position. l_f and l_t corresponding length of the femur and tibia respective and the hip and knee angle is as labeled.

Using inverse kinematics, for any desired ankle trajectory we can get the corresponding angle trajectory, which can then be parameterized using the following equations.

$$\begin{aligned}\theta_h &= a_{h1}\sin\left(b_{h1}t + c_{h1}\right) + a_{h2}\cos\left(b_{h2}t + c_{h2}\right) + d_h \\ \theta_k &= a_{k1}\sin\left(b_{k1}t + c_{k1}\right) + a_{k2}\cos\left(b_{k2}t + c_{k2}\right) + d_k\end{aligned} \quad (3)$$

Doing so, we can apply the one-dimension learning rule to the coefficients of the above parameterizations.

6.4 Results

As equations (1) and (2) showed, even the one idealized one dimensional case reduces to a non-linear stochastic differences equation (SDE), where there no analytical solution is possible. Hence, we have to rely on numerical simulation, first for the one dimensional model, follow by the two dimensional case.

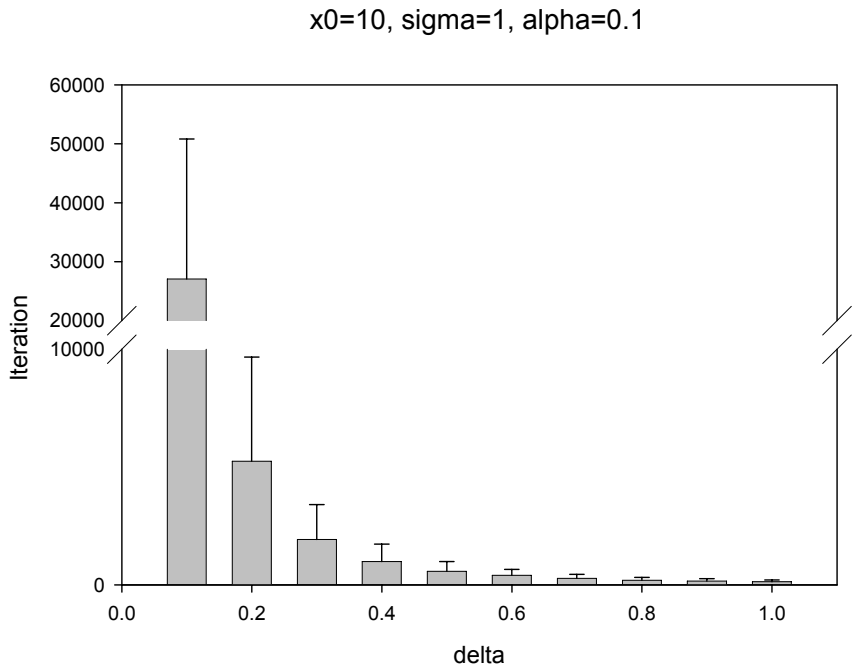

Figure 6.5: Numerical simulation of the first learning rule. Number of iteration before the stopping criterion is met with various delta sizes. Mean and standard deviation of 500 tries is plotted. x_0 is normalized to 10 and sigma is set to 1. Note the number of iterations increase drastically as delta approaches 0.

6.4.1 First Learning Rule Simulation Results

To study the first learning rule, a brute force simulation carried out, with the simulation repeated 500 times at each of several different values of δ sizes. The averaged number of iterations before the system converged is used to assess the performance. The initial simulations results suggest that this system always converges to the target given sufficient time. Note that on average the convergence rate is highly sensitive at small values of the parameter δ. As δ approaches 0, the convergence time increases exponentially (Figure 6.5). However, since the probing point is accepted immediate, there are times where the system will have a "lucky try" and find the target in relatively few iterations, which accounts of the large standard deviation.

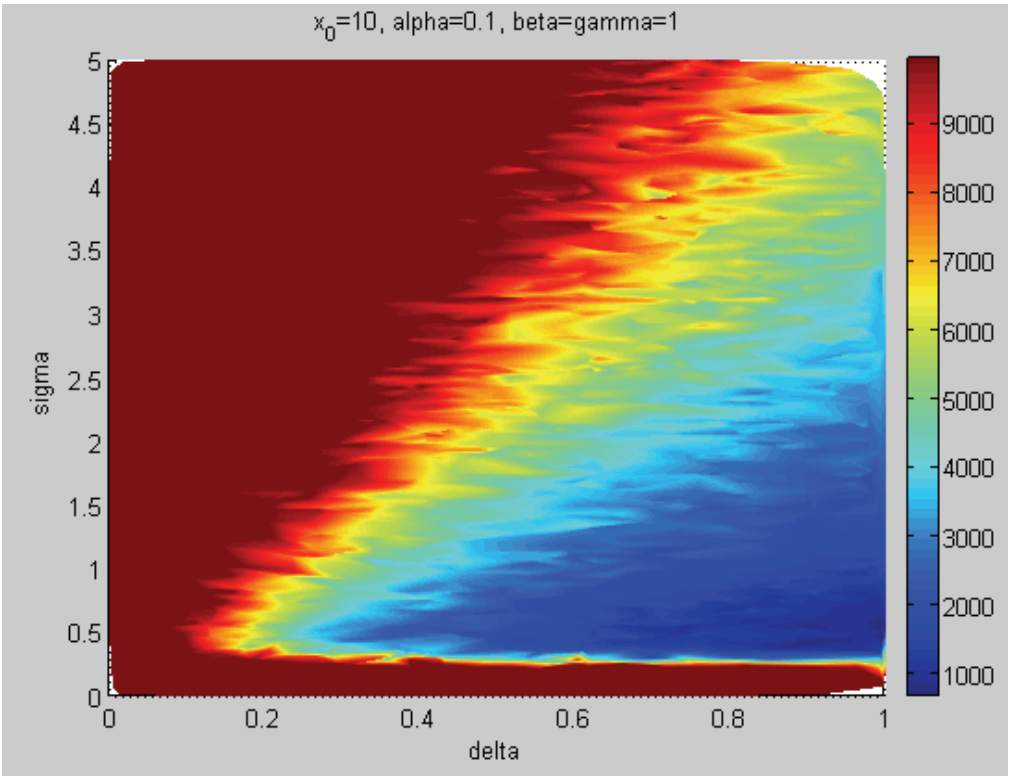

Figure 6.6: Numerical simulation of the second learning rule. Number of iterations before the stopping criterion is met as a function of δ and σ, x_0 is normalized to 10. The color bar corresponds to the number of the iterations. There were 2500 combinations of random δ and σ values in this simulation.

6.4.2 Second Learning Rule Simulation Results

Simulation results for the second learning rule are similar to the first learning rule. The major differences are: 1) the upper limit on number of iteration allowed is capped at 10000, after which the system is consider divergent, hence the system does not always converge like the first learning rule, although given enough time it might; 2) the movement toward the target is done at an incremental pace, so that there are no "lucky tries," resulting in a smaller standard deviation from trial to trial with the same parameters. Hence, a Monte Carlo's simulation is possible and appropriate; and 3) since there are an upper and lower bound on σ, the learning rate is sensitive to the σ size as well. The simulation results are summarized in Figure 6.6. In this simulation, the initial distance x_0 is kept fixed, while σ and δ are randomly varied using Monte Carlo method. The results (Figure 6.6) show given an initial σ and x_0, the system convergence depends on the choice of δ. In addition, there seems to be a minimum intrinsic variability value, below which the system fails to converge regardless of window size and it is very sensitive.

6.4.3 Biomechanical Model Simulation Results

Assuming that the desired target trajectory is a circle (which approximates ankle trajectories of the animal experiments), the parameterized hip angle is shown in blue in Figure 6.7. Using the same parameters from 7.4.2, we show that the system can learn the desired ankle trajectory over time, shown in red in Figure 6.7. Similar to the one dimensional case, the system's ability to track the desired trajectory is dependent on the target window size (Figure 6.8). It is interesting to note that if one takes a cross-sectional

view of any giving point of the trajectory, all the features of the learning system demonstrated in the one-dimension scenario is preserved.

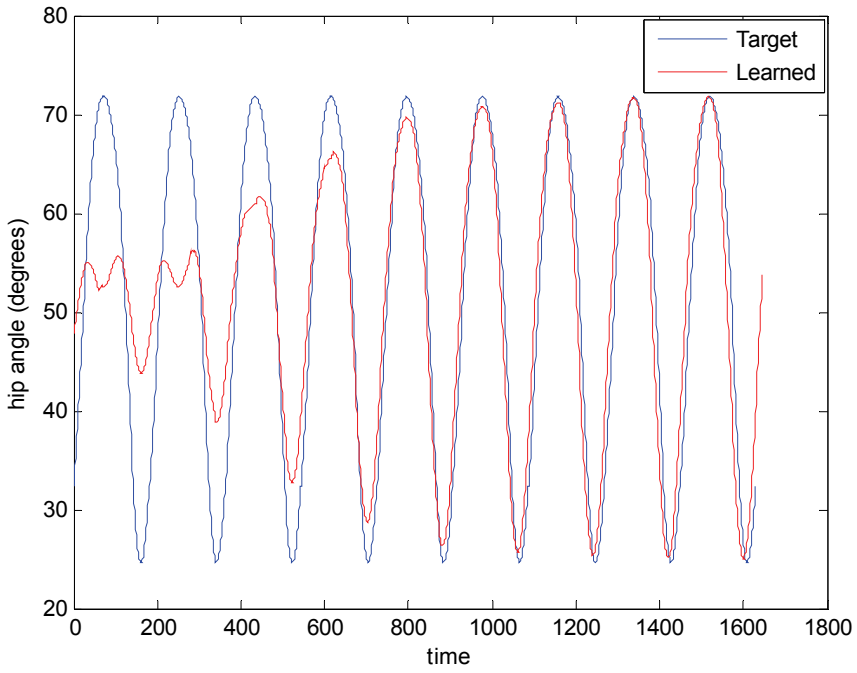

Figure 6.7: Applying the learning rule to the coefficients of the hip joint angle parameterization (eq. 3). The target hip angle trajectory for an ankle trajectory of a circle is shown in blue. The red line represents the learned trajectory.

A **B**

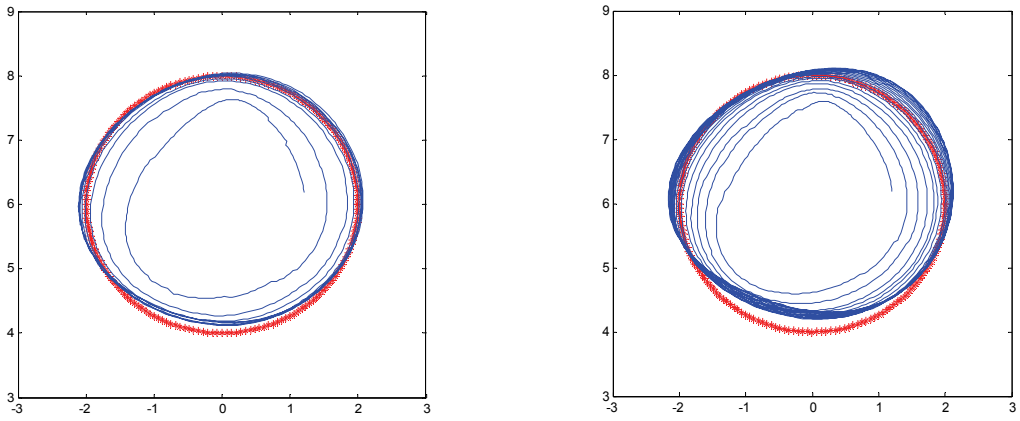

Figure 6.8: Application of the learning rule to the biomechanical model. The desired trajectory is a circle, shown in red. With all other parameters fixed, in **A**, the window size is normalized to 1 while the window size in **B** is normalized to 0.5. Note the significant increase in iterations before the system tracks the target. Also note the system tracks less accurately with a smaller window size, due to the drift cause by the variability parameter.

In addition, to learning an ideal trajectory such as a circle, we also tried to demonstrate that the system can learn a more physiological possible trajectory. Figure 6.9, shows using the same parameterization for joint angle positions as in the circular case, the system can track a trajectory similar to an actual trajectory recorded from an animal (Figure 6.10). Note, however, the trajectory is not a perfect match. This is probably due to the simple parameterization function that we have chosen.

One of the goals of developing this model is to see if we can predict what the optimal training paradigm might be. From looking at Figure 6.6, we see a ratio of δ/σ where the learning is most efficient. Hence, we tried to improve on the learning rate by constantly altering δ to keep a constant δ/σ ratio, hence making the training adaptive. In simulation, it seems that by doing so, the learning rate significantly increased (compare Figure 6.11 to Figure 6.8).

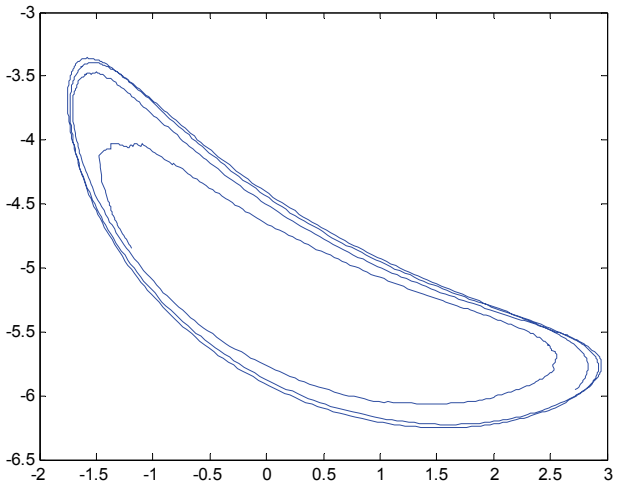

Figure 6.9: The learning system tracking a more physiological ankle trajectory. The window size is normalized to 1 in this simulation.

6.5 Discussions and Conclusions

6.5.1 Similar to the Animal Experiments, Learning Models Show that Motor Learning Is Dependent on the Amount of Variability the Training Allows.

Variability in neuromuscular control and locomotion is well documented (Lovely, Gregor et al. 1990; Hooper 2004; Horn, Zhurov et al. 2004; Hooper, C. et al. 2006). Chapter 5 showed that rehabilitative training is more effective when using a training algorithm that allows the animal to vary its trajectory from cycle to cycle. This chapter developed a learning model to examine the importance of this variability. In the first simulation of the one-dimensional learning system, the learning rate increased exponentially as the target window size decreased. This suggests that as the training approaches a fixed pattern, it becomes much harder for the system to find the desired target. This is due to the fact that there are always some tracking errors caused by the intrinsic variability built into the learning system. When the target window decreases, the system will be punished more and more for deviating outside of the range of variability allow by the trainer. However, since the simulation is by "brute force" numerical simulation and only a limited number of window sizes are tried, we could not draw any relationship between the size of the target window and convergence.

Simulation results from the second learning rule are more revealing compared to simulation results from the first learning rule, partly because many more combinations are tried using a Monte Carlos simulation method. These simulations showed that given a level of intrinsic variability, there is a critical window size below which the system will fail to converge. The ratio between the levels of variability and the critical window size

δ_c seems to be linear, where an increase in variability parameter σ will lead to a higher δ_c. This conceptually makes sense because, similar to the first learning rule, if there is a large variability and the window size is too small, the system will constantly be "punished" for venturing outside of the target window. Perhaps less clear is why learning fails when the variability drops below a critical level. One possible explanation is that once the variability drops to a critical level, there are fewer explorations by the system and hence stuck in a local minimum, much like the traditional reinforcement learning case (Sutton and Barto 1998).

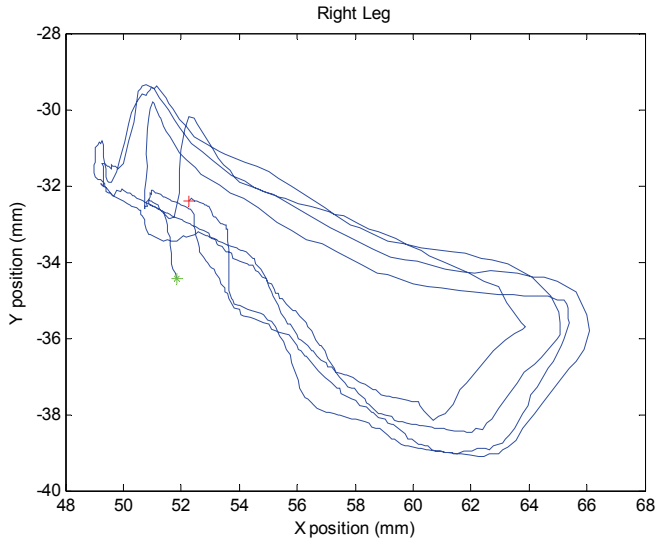

Figure 6.10: Actual ankle trajectory recording from a neonatal mouse. Note cycle to cycle variation.

When the above learning rule is applied to the simple biomechanical model of the mouse lower limb, results are similar to the one dimensional case. As show in Figure 6.8, when the target window is small, the animal fails to learn the desired target. These results are consistent with those from the animal study described in Chapter 5. As the target window width approaches 0, the training simulates the "Fixed Training" algorithm

described in Chapter 5. Hence, the learning models actually capture some of the fundamental characteristics of neuromuscular control such as of "learned helplessness" (Wool, Siegel et al. 1980; Grau, Barstow et al. 1998).

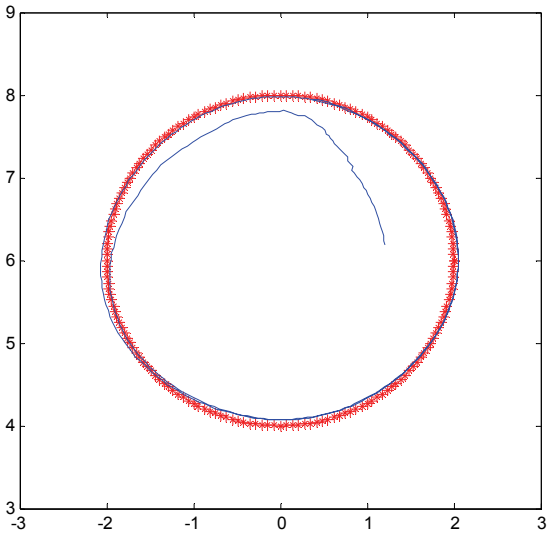

Figure 6.11: Tracking of a circular target using a variable window size. Here the ratio of δ/σ is fixed to be 1/2. This is a ratio where the convergence appears to be the fastest according to Figure 6.6. Note that the convergence is much faster than when a fixed window size training was used, shown in Figure 6.8. Again, delta is normalized to 1.

6.5.2 Learning Models Allows Us to Find More Optimal Rehabilitative Training Paradigm.

One advantage of having a learning model described in this chapter is to predict how an animal might respond to a specific training algorithm. In the current model, the target window size can be selected to be near zero width to simulate a fixed trajectory training, or one can choose a predetermined window size to simulate the AAN training described in the pervious chapter. In addition to testing our experimental results, it is desirable to make some theoretical predictions on how parameters such as window size and window shape might affect the effectiveness of the training. As Figure 6.6 demonstrates, there is

a critical window size for the amount of intrinsic variability the system exhibits. This is an interesting result on its own because it suggest that when designing an AAN training algorithm as described in Chapter 5, the size of the target window can be critical, especially during the initial training period immediately post-SCI when the variability of the animal is large (de Leon, Hodgson et al. 1998). The direct corollary to these observations is that there seems to be an optimal window size where the learning is most efficient given an σ value. Data from Figure 6.6 suggest that learning is much more efficient if the ratio of σ to δ is kept below 2. Using the biomechanical model described in 7.4.3 and varying the size of δ with respect to σ, we show that the learning rate is improved (Figure 6.11). This suggest that in AAN training, it might be more optimal if one can vary the window size to closely match the amount of variability that the animal exhibits at a giving level of recovery.

6.5.3 Principle of Neuromuscular Control and Sensorimotor Integration Resemble that of Adaptive Control Theory.

One of the fundamental principles of neuromuscular control is the ability to integrate vast amounts of sensory information to produce a functional motor output. However, sensory measurements are inherently noisy, both in biological and engineering systems. As such, it is unlikely that the sensorimotor integration process happens in a deterministic manner, but rather has a stochastic component that allows for cycle to cycle variability. In both experimental and simulation results, this thesis has shown that in the presence of such variability, motor learning is best achieved when the training does not enforce a desired trajectory, but rather challenges the system to use feedback more intelligently to reinforce

the coupling between sensory information and functional motor output. The underlying principle resembles reinforcement learning where if an action is favorable, then the tendency of producing that action is strengthened. Using a variation of reinforcement learning method called Q-Learning, Sutton et al has presented a convincing argument that reinforcement learning is direct adaptive optimal control (Sutton, Barto et al. 1992). This and other frameworks such as Hebbian feedback covariance control and Bayesian Learning framework should facilitate experimental elucidation of the mechanisms of internal models and the reverse engineering of such neural mechanisms into novel brain-inspired adaptive control paradigms in future (Tin and Poon 2005).

6.6 Summary

The learning model introduced in this chapter provides strong validation for our animal experiments that suggest post-SCI motor learning can occur in the spinal circuitry. These results also suggest that optimization schemes can be evaluated computationally to identify parameters that can be used to develop more effective training strategies for rehabilitation. Furthermore, models such as the one described in this chapter can provide a novel tool for identifying those mechanisms which underlie neuromuscular control and sensorimotor integration in the spinal cord. By improving our understanding of neuromuscular control, the learning model will have implications in not just biological researches, but in machine learning, artificial intelligence, and adaptive optimal control researches as well.

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CHAPTER 7 – Concluding Remarks

7.1 Summary

In this thesis work, a mouse model was developed to study neuromuscular disorders such as spinal cord injury. Using this model, we showed for the fist time that adult mice with a complete spinal cord transection can be robotically trained to step, even though manual training failed to elicit any recovery response. This confirmed the importance of the role that robotic devices play in rehabilitation post-SCI and other neuromuscular disorders such as stroke. Instead of relying on imprecise qualitative methods such as the BBB scale, using the robotic device developed in this thesis work, we were able to quantitatively measure locomotor recovery post-SCI. This allowed us to using numerical techniques such as Fast Fourier Transform (FFT) and Principle Components Analysis (PCA) to examine different aspects of stepping such step periodicity and shape consistency respectively.

Using these measurements, we showed that in addition to robotic training, quipazine administration can have a positive interaction effect on locomotor recovery. In addition, quipazine induce a long term retention effect similar to learning within the isolated spinal cord. This led us to believe that locomotor recovery can be training specific in the presence of quipazine administration. With the versatility that robotic devices provide, we developed more complex and precise training algorithms and showed that locomotor recovery can better achieved using an "assist-as-needed" (AAN) training paradigm. Based on our quantitative measurements, results showed that an AAN training paradigm that loosely controlled interlimb coordination elicited better recovery then a fixed trajectory training paradigm or an AAN training paradigm that did not

impose interlimb coordination. This suggested that a poorly designed training algorithm can be suboptimal and can actually have a negative effect. For the most effective training, there needed to be a close coupling between the trainers, robotic devices in our case, and the intrinsic neuromuscular control mechanism of the trainee. If intrinsic properties of the neuromuscular control such as variability were abolished by the training, motor learning failed to occur, leading to a condition similar to "learned helplessness." Thus, one of the most important contributions of this thesis was to show that robotic devices can be used not only as a tool for physical therapy, they can be used as a tool for research scientists to gain insight into the fundamental mechanism behind the recovery of neuromuscular control, which will lead to development of more optimal rehabilitative training strategies.

The other major contribution of this thesis work was to show that theories from learning models can be used to explain the plasticity observed in the lower spinal cord. Using a learning model derived from reinforcement learning, we demonstrated that theoretically variability is needed for motor learning to occur. Using an idealized one-dimensional model, we were able to capture many macroscopic properties of motor learning observed in our animal experiments. Using this model, we were even able to predict what the optimal rehabilitative training strategy might be.

7.2 Future Work

One of the most logical extensions of this thesis work is to get experimental validation of our learning model. Based on the modeling results, it is suggested that robotic rehabilitative training is more effective if the AAN training window constantly varies to

closely match the intrinsic variability of the trainee. Although this is a very important next step, the technical difficulties involved are beyond the scope of this thesis work. To implement this experiment, one will need to be able to measure the intrinsic variability of the animal, which could be a separate thesis work in itself, and adjust the training algorithm accordingly in real time.

Another long term goal of the author is to better understand neuromuscular control and motor learning in general. What makes the neuromuscular system so adaptive yet robust? What is the role of variability in motor learning? Understanding these important questions will allow us to develop more biologically inspired control algorithms that can be applied in the engineering world and perhaps lead to advancements in the field of adaptive optimal controls.