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SENSORIMOTOR CONTROL OF LOCOMOTION BY INTACT AND INJURED SPINAL CORD

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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This thesis is dedicated to my family –

To my father who always supported free-thinking and belief in my own abilities,
To my mother for her affection and aspiration to share her profound wealth of heart,
To my brother for saving my life.

Я хочу посветить этот тезис моей семье –

Моему отцу за поддержку свободного мышления и веру в мои возможности,
Моей матери за любовь и стремление поделится всем своим душевным богатством,
Моему брату за спасение моей жизни.

Abstract

The control of locomotion is a complex problem of controlling the movement of a mechanical body with multiple degrees of freedom in a variable and often unpredictable environment. This thesis examines questions about the integration of the neuro-musculo-skeletal system for propulsion and stabilization. The five studies are included in this thesis cover the following topics: i.) muscle force generation during static and continuous muscle length changes; ii.) the role and contribution of stretch reflexes during locomotion; iii.) analysis of the spatiotemporal activity of lumbosacral motoneurons during normal locomotion; iv.) variability in the locomotor patterns during fictive locomotion and its implications for organization of the central pattern generator (CPG); v.) a test of the "propriospinal hypothesis" by intraspinal microstimulation (ISMS) of the thoracic spinal cord in rats with complete spinalization.

These studies show that the musculoskeletal system is capable of locomotor stabilization even in the absence of sensory feedback. Intrinsic muscle properties not only resist perturbations, but can also modulate gains in the sensory feedback pathways during locomotion. A finite control system based on multisensory rules can play a crucial role for stabilization of different modes of locomotion and resist to large perturbations. Study of the spatiotemporal activation of the lumbosacral motoneurons supports the hypothesis that activation of the lumbosacral MNs during walking is not wave-like, but it is a switch-like oscillation of activity. This finding led to a study of the patterns of MN activity during fictive locomotion, which is not influenced by motion-related sensory feedback controlling transitions between locomotor phases investigated in Chapter 3. The analysis of the fictive locomotor patterns revealed that the variation in the burst duration during spontaneous changes in cycle duration depends on the percent of the cycle occupied. These results can be explained by a conceptual model of the CPG with two half-centers receiving excitatory bias

from descending inputs and proprioceptive feedback pathways. Finally, the results of the test of the "propriospinal hypothesis" with ISMS of thoracic segments in rats with complete spinal cord injury support the hypothesis.

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List of Abbreviations

adductor femoris magnus	AdFM
one-way analysis of variance	ANOVA
ankle flexors	AF
ankle extensors	AE
contractile element	CE
caudofemoralis	CF
central nervous system	CNS
central pattern generator	CPG
brain-derived neurotrophic factor	BDNF
biceps femoris anterior and posterior	BFa, BFp
extensor carpi radialis brevis	ECRB
extensor carpi radialis longus	ECRL
extensor carpi ulnaris	ECU
extensor digitorum longus	EDL
electromyographic / electromyography / electromyogram	EMG
functional electrical stimulation	FES
flexor carpi radialis	FCR
flexor carpi ulnaris	FCU
flexor digitorum longus	FDL
flexor hallucis longus	FHL
flexor digitorum longus and flexor hallucis longus	FDHL
gluteus maximus and medius	GlutMax, GlutMed
hip extensors	HE
hip flexors	HF
intraspinal microstimulation	ISMS
knee extensors	KE
bifunctional muscle with knee flexor and hip extensor function	KF-HE
L-3,4-dihydroxy-phenylalanine	L-DOPA
gastrocnemius lateralis and medialis	LG, MG
gastrocnemius and soleus	LGS
mesencephalic locomotor region	MLR
motoneuron	MN
maximum voluntary contraction	MVC
motor unit action potential	MUAP
biceps femoris posterior and semitendinosus together	PBST
principal component analysis	PCA
parallel elastic element	PE
peroneus longus	PerL
plantaris	Plan
peripheral nervous system	PNS
rectus femoris	RF
physiological range of motion	ROM
series elastic element	SE

spinal cord injury	SCI
semimembranosus anterior and posterior	SMa, SMp
semimembranosus and biceps femoris anterior	SMAB
short-range stiffness	SRS
semitendinosus	ST
sartorius anterior and sartorius medialis	SRTa, SRTm
tibialis anterior	TA
tibialis posterior	TP
cycle period	Tc
extensor phase	Te
flexor phase	Tf
vastus intermedius, vastus lateralis and vastus medialis / vasti	VI, VL, VM

CHAPTER 1

General introduction

1.1 Introduction

At every moment of our existence the nervous system has to solve multiple control problems. Imagine the level of control involved in successful retrieval of an apple that seems just fingertips beyond your reach, while standing on a tall ladder leaning against an apple tree. It is not surprising then that control of movement involves multiple interconnected systems, organized in hierarchical order. While this system is a convenient adaptation, which reflects multiple steps in evolution, it presents a challenging subject to study.

There are two basic approaches to unraveling the details of the system. The reductionist approach is based on gathering facts about the smallest known components of the system of interest and combining this knowledge to arrive at a full understanding. Alternatively, the systems approach is based on studying the system as whole and deducing information about its components from global facts. Both of these approaches have their advantages and disadvantages; however, a symbiosis of the two is often the most successful. Study of the control of locomotion is a good example of such symbiosis, as will be demonstrated in this thesis.

This chapter contains a general overview of topics focused on the control of movement during locomotion.

1.2 Muscle

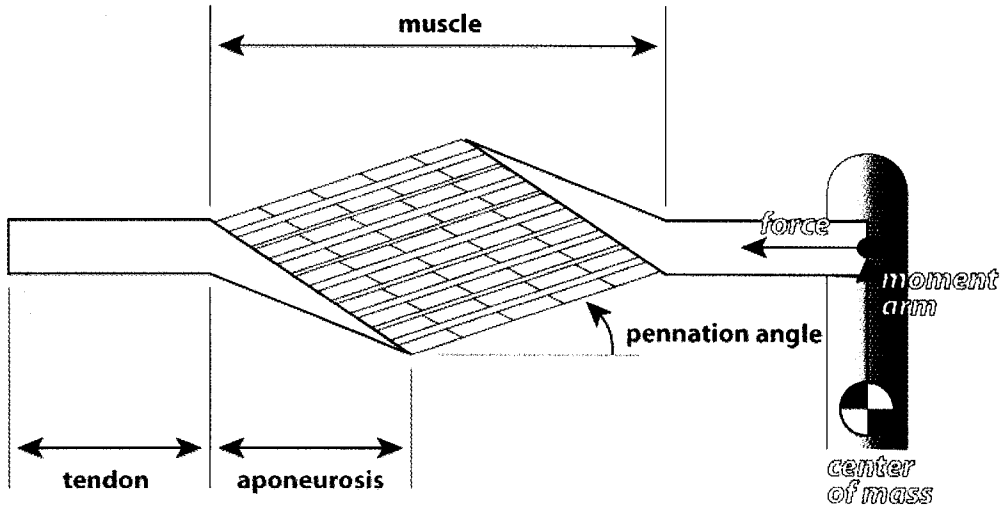
> "Nature sets in motion by signs and watchwords, which are made with little momentum.... Just as in the army the soldiers are set in motion by one word as if by a given signal and continue to move until they receive another signal to stop, so the muscles move in order and harmony from established custom."
> – William Harvey (1578-1657)

1.2.1 Basic morphology and properties of the contractile machinery

The neural system delivers its action through biological actuators, muscles. There is a considerable diversity in muscle structure. Here we will overlook properties specific to cardiac and smooth muscles and concentrate on striated skeletal muscle. The word "striated" comes from light microscope observations of striped patterns of dark and light bands in muscle fibers formed by sarcomeres arranged in series. Muscle fibers are arranged in parallel and attached to a connective tissue sheet, called aponeurosis (Figure 1.1A). The force generated by muscle is transmitted through the aponeurosis to a tendon, which in turn is attached to the body skeleton. The points of muscle attachment, called origin and insertion, determine the line of muscle action. In some muscles, muscle fibers are arranged at an angle, called a pennation angle, to the line of muscle action. This organization increases the moment arm of muscle fibers and is common for muscles bearing high loads, such as gastrocnemius and vasti. However, this organization comes at the expense of the decreased range of motion. Muscles bearing lower loads, like the hip flexors ilio-psoas and sartorius, have their fibers arranged in parallel with the line of action (He *et al.*, 1991).

Over 100 years ago, Blix (Blix, 1893) observed that the force generated by active muscles depends not only on the activation level but also on muscle length. He plotted length-force relationships of frog muscles from a series of isometric tetanic contractions performed by the muscle at different lengths.

A.



B.

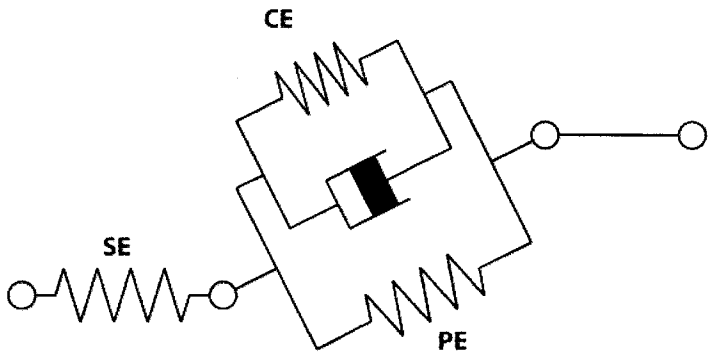


Figure 1.1. Schematic of muscle model. ***A.*** Muscle attaches to skeleton through aponeurosis and tendon. Some muscle fibers are arranged at an angle to the line of muscle action. ***B.*** Hill-type muscle model, where CE is a contractile element, PE is an elastic element in parallel and SE is an elastic element in series.

Repeatedly reproduced (e.g. Abbot & Wilkie, 1953), the profile of the isometric length-force curve consisted of an ascending limb, followed by a plateau, a descending limb and a final ascending limb. With the exception of the final ascending limb, which is attributed to passive muscle stiffness (Huijing, 1985; Zajac, 1989), the initial ascending and descending limbs are attributed respectively to increases and decreases in the overlap of actin and myosin filaments within sarcomeres during active muscle contractions (Gordon *et al.*, 1966). The muscle length corresponding to the maximum value of active force is called the muscle's optimal length, at which the overlap between the muscle filaments is the greatest (Gordon *et al.*, 1966; Huxley, 1974).

In addition, Hill determined experimentally the dependence of active force generation on the velocity of shortening, by measuring the force exerted by a muscle at a given length, as the muscle actively shortened under a series of loads (Hill, 1938). Similarly, the dependence of active muscle force on the velocity of lengthening was characterized (Katz, 1939). During shortening (concentric) and lengthening (eccentric) contractions the muscle force-velocity relationship is similar to that of a viscous energy-dissipating element.

The force-length-velocity relationship of active muscle may be explained to a large degree by the sliding-filament theory (Huxley & Niedergerke, 1954; Huxley & Hanson, 1954; Huxley, 1957). It suggests that as sarcomeres change their length and the contractile filaments slide past each other, myosin cross-bridges can form connections with attachment sites on actin. The theory states that muscle force depends on the number of attached actin-myosin cross-bridges and the rate of cross-bridge attachment and detachment. If these parameters are independent, then the force-length and force-velocity properties of a single sarcomere are independent of each other as well. Further studies demonstrated that the profile of the force-velocity curve varied only marginally with

length (Bahler *et al.*, 1968; Edman & Andersson, 1968) and the profile of the force-length curve was independent of shortening velocity (Abbot & Wilkie, 1953). Thus, muscle can be conceptually represented by a "dashpot" in parallel with a force generator, which behaves like a "spring", at least on the initial ascending limb of the isometric force-length curve (Figure 1.1B). This description became the foundation for Hill-type muscle models.

1.2.2 Hill-type muscle model

Muscle modeling is a challenging field of systems physiology. The most broadly used muscle models are based on the classic paper by Nobel Prize laureate Archibald V. Hill in 1938 (Hill, 1938). The central principal of these models is that skeletal muscle behaves as a lumped sarcomere with the following independent properties: muscle activation, force-length and force-velocity relationships. Generally, this relationship can be described by the equation:

$$F_{ce} = F_{\max} \cdot f_v \cdot f_l \cdot a_t \qquad \text{Eq. 1.1}$$

where F_{ce} is force exerted by the contractile element, F_{max} is the maximum isometric force, f_v and f_l are the normalized force-velocity and the force-length relations respectively and a_t is the normalized muscle activation.

In this phenomenological model, which deals with macroscopic properties, the contractile element (CE) is often connected in series and in parallel with two elastic elements. The series elastic element (SE) is usually stiff and represents properties of tendon, while the parallel elastic element (PE) represents passive properties of connective tissue (Figure 1.1B).

The main challenges of muscle modeling include optimization of model structure and realistic representation of built-in parameters. For example, until recently, most of the studies in biomechanics have used Hill-type models with force-length curves based on *isometric* (static) measurements to describe *continuous* active movements (e.g. Hoy *et al.*, 1990; Pandy *et al.*, 1990; Veltink *et al.*, 1992). The isometric force-length curve described above predicts that a muscle stretched beyond its optimal length, therefore on the descending limb of the curve, generates less force with further stretch, i.e. muscle stiffness is negative (Stein *et al.*, 1999). How realistic is this prediction? In control systems, negative stiffness in a force actuator would lead to unstable behavior. For example, if an actuator with negative stiffness were loaded, the stretch would decrease its force production and lead to further stretch, causing instability. From experiments in healthy human subjects, wrist extensors, but not flexors, exhibit isometric force-length curves with descending limbs and decreasing moment arms at muscle lengths beyond the optimal length (Lieber *et al.*, 1994; Loren *et al.*, 1996; Lieber & Friden, 1998). This would suggest that as the wrist is extended, the torque of muscles like extensor carpi radialis brevis (ECRB) would decrease. This prediction seemed at odds with observations of these muscles under functional electrical stimulation (FES) control (Prochazka *et al.*, 1992). Chapter 2 of this thesis shows that in situations involving continuous movement, muscle stiffness remains positive throughout the physiological range of motion (ROM), even when the isometric force-length curve contains a pronounced descending limb. A similar result was found for rat medial gastrocnemius muscle (Meijer *et al.*, 1997). Thus, isometric length-tension curves do *not* adequately represent muscle properties during continuous movements.

Once the level of model complexity is chosen, the next challenge is to find a realistic representation of model parameters, e.g. muscle or tendon length and stiffness, maximum velocity of concentric contraction, moment arms, etc. Some of these parameters can be estimated indirectly. For example, tendon stiffness can be determined from its cross-sectional area, the tendon slack length and Young's modulus (Hoy *et al.*, 1990). Physiological range of motion can be used to estimate muscle rest length and vice versa (Brown *et al.*, 1996). Another intriguing indirect method of parameter estimation is based on known or predicted optimization of natural movements, e.g. based on minimal jerk, which can be used to predict muscle response to a known perturbation (Bertram *et al.*, 1999; Raasch & Zajac, 1999; Neptune *et al.*, 2000). However, direct experimental measurement of muscle properties used in a model is likely to be the most reliable approach.

1.2.3 History-dependent muscle properties

History-dependent muscle properties, such as short-range stiffness (SRS), yielding (Joyce *et al.*, 1969), force enhancement (Edman *et al.*, 1978; Herzog & Leonard, 2002) and depression (Herzog *et al.*, 2000) are usually omitted in Hill-type muscle models. However, these properties can greatly influence muscle performance. Short-range stiffness is observed as an instantaneous increase in muscle stiffness when muscle is lengthened through its initial ~1% of rest length (Joyce *et al.*, 1969). The mechanism of SRS can be explained based on the cross-bridge theory. It is thought that at the onset of stretching a proportion of cross-bridges effectively remain attached and continue to deform elastically. When muscle is stretched beyond the initial ~1% of rest length, the number of attached cross-bridges reduces and muscle stiffness declines (Rack & Westbury, 1974). It has been proposed that SRS shares the same mechanism as yielding, a transient decrease in exerted force during ramp and hold experiments, and that together they play an important role in postural

stabilization (Malamud *et al.*, 1996). Finally, force enhancement is an increase in exerted isometric force immediately after stretching (Abbot & Aubert, 1951; Edman *et al.*, 1978, 1982). Since this property is most likely to manifest itself when the muscle is stretched beyond its optimal length (Edman *et al.*, 1978), this phenomenon together with SRS and yielding may explain the positive stiffness of muscle during continuous movement, even when the isometric force-length curve has a negative slope (Gillard *et al.*, 2000).

In light of new evidence, some of the assumptions of Hill-type muscle models have been criticized. The assumption of an independent relationship between muscle activation and the profile of the force-length curve has been criticized based on studies using the single frog muscle fiber preparation. It was shown that during submaximal contractions, lengthening increased force production beyond its isometric level (Edman & Reggiani, 1984; Stennett *et al.*, 1996). The mechanism of this enhanced activation at longer muscle lengths was linked to coupling between two processes, Ca^{2+}-troponin binding and actin-myosin binding (Fuchs, 1977; Rassier *et al.*, 1999). One way this effect can be taken into account is by defining a_f in Eq. 1.1, a parameter relating activation to force, as a function of length (Cheng *et al.*, 2000).

Another debatable assumption of Hill-type muscle models is that all sarcomeres in a muscle fiber change their length uniformly, which justifies describing muscle as a single lumped sarcomere. Edman and Reggiani were the first to demonstrate experimentally that the lengths of sarcomeres along a single fiber vary both when the muscle is active and at rest (Edman & Reggiani, 1984). Several observed muscle phenomena may be explained primarily by inhomogeneities in the length of sarcomeres in series, a state called sarcomere nonuniformity. For example, force enhancement, described above, may result from the redistribution of sarcomere lengths. After lengthening, some

sarcomeres may be stretched beyond their optimal length and act as stiff transducers of load, while others would exert higher forces because they continue to operate on the ascending limb of the force-length curve (Edman & Reggiani, 1984). The phenomenon of force depression after active shortening was also attributed to sarcomere nonuniformities (Meijer *et al.*, 1997). Using variable amounts of shortening prior to isometric contractions, the authors demonstrated a population of force-length curves, which showed force depression dependent on the amount of preceding shortening at high lengths. It is remarkable that even in the presence of the descending limb in isometric force-length curves, the lines connecting points with the same previous shortening history had positive slopes. Recently, finite-element modeling, an analysis of stress distribution within materials, was successfully used to describe sarcomere length inhomogeneities in single fibers (reviewed in Huijing, 2000).

Muscle shows a great capacity for adaptation to changes in functional demands due to development, exercise or trauma (Belanger & McComas, 1989; Yang *et al.*, 1990; Gordon *et al.*, 1993; Navarrette & Vrbova, 1993). It is well known that lack of use leads to gradual deterioration of muscle and bone, e.g. after paralysis or prolonged exposure to zero gravity (Keller *et al.*, 1992; Gordon & Mao, 1994). Motor unit type can change due to electrical stimulation and exercise (Scott *et al.*, 1985; Gordon & Mao, 1994). It was shown that both exercise and change in muscle excursion lead to changes in the number of sarcomeres in series and a consequent adaptation of the force-length curve (Lynn & Morgan, 1994; Koh & Herzog, 1998; Lynn *et al.*, 1998). For example, downhill running exercise for several days increases the number of sarcomeres in myofilaments of vastus intermedius, a knee extensor, in rats (Lynn & Morgan, 1994; Lynn *et al.*, 1998). The same adaptation was reported after an increase in muscle range of motion following

surgical retinacular release of tibialis anterior, an ankle flexor (Koh & Herzog, 1998). Thus, it is not surprising that force-length curves of flexor and extensor muscles might be different (Gareis *et al.*, 1992; Gillard *et al.*, 2000). This suggests that force-length properties of a muscle are optimized to its functional demands.

To conclude, muscle modeling is challenging because of the complex morphology and nonlinearity of the musculoskeletal system. When assessing the results of muscle modeling, a balance should be achieved between oversimplification of muscle behavior on one hand, which may under-represent some important properties, and too much complexity on the other, which can lead to difficulties in implementation and hidden errors.

1.3 Musculo-skeletal system – the plant

> "The power of locomotion is that which contracts and relaxes the muscles whereby the members and joints are moved, extended or flexed. This power reaches the limbs by way of the nerves and there are as many forms of power as there are of movement. Each muscle has its own peculiar purpose and it obeys the decree of the composite sense."
> – Avicenna (Ibn Sina) (980-1037)

1.3.1 Passive dynamic properties of skeletal system

The skeletal system is sometimes called in engineering terms the *plant*. It is controlled by the nervous system through non-linear force actuators, the muscles. The challenge of movement coordination is to manage redundant degrees of freedom of this system (Bernstein, 1967). It is conceivable that each individual degree of freedom, or independent direction of movement, *may* be individually controlled by the nervous system. However, there are many indications that structure and control of complex biological motor systems are organized hierarchically (Philippson, 1905; Ghez & Krakauer, 2000). The first system to consider is the skeletal system, which consists of mechanical segments connected to each other by joints. In the block diagram of Figure 1.2,

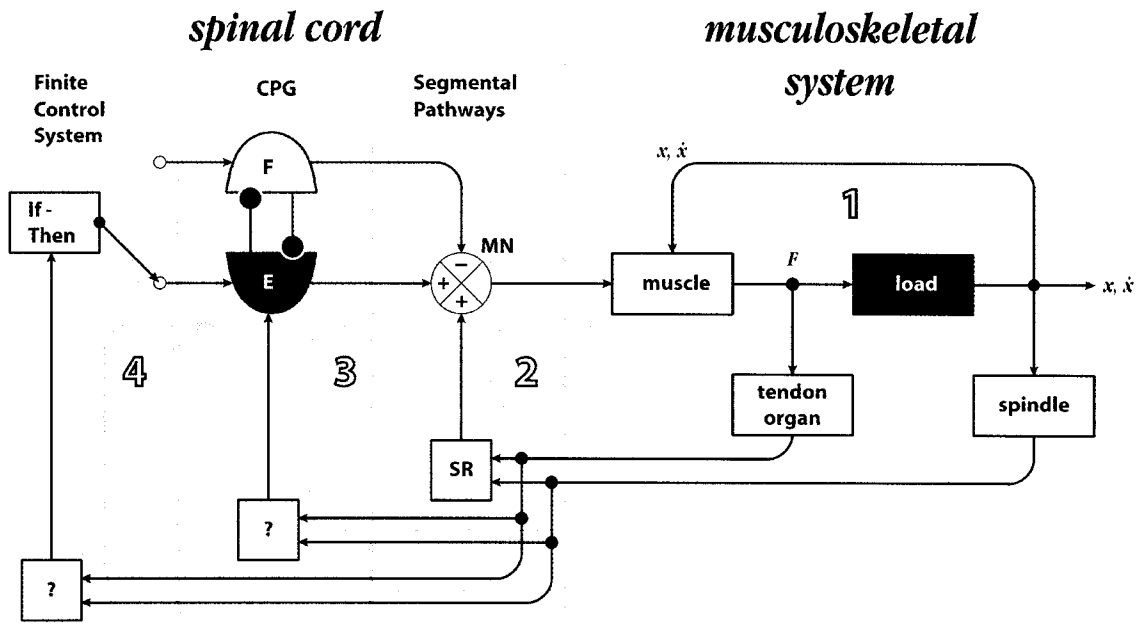


Figure 1.2. Simplified diagram of the locomotor control system. Loop **1** represents intrinsic muscle negative displacement feedback. Loop **2** contains delayed sensory feedback from tendon organ and spindle afferents. Loop **3** describes sensory pathways capable of modifying amplitude and / or timing of the locomotor CPG. Finally, loop **4** describes multisensory pathways, which may contribute to the control of phase transitions of the locomotor CPG.

the skeletal system is represented as *load*. It was recognized for a long time in biomechanics that purely mechanical systems are capable of "self-organizing" behaviour through interactions with the environment (Schoner & Kelso, 1988). For example, it was shown that simple, completely passive anthropomorphic models without actuators or sensory feedback can preserve stability while walking down shallow slopes (McGeer, 1990; Garcia *et al.*, 1998). Robots have been developed with passive dynamic properties and simple control algorithms that allowed them to hop, run or trot (Raibert, 1990). Thus, the skeletal system alone may contain intrinsic properties, which stabilize the system even in the absence of active control.

Do animals and humans use the passive dynamic properties of their skeletal system for motor control? Supporting evidence was adduced by Hoy and Zernicke (Hoy & Zernicke, 1985) using inverse dynamics methods to study active muscle moments and inertial moments during the swing phase of gait in cats. They demonstrated that the inertial moments due to angular accelerations of the limb segments contribute to knee flexion in early swing (F phase) and knee extension in late swing (E1 phase). Humans similarly show passive knee flexion during the swing phase (Mochon & McMahon, 1980). Another example of passive generation of locomotion is provided by the remarkable ability of relatively simple passive spring-mass models to describe behaviours like walking, running and jumping (Blickhan, 1989; Lee & Farley, 1998; Herr *et al.*, 2002; Seyfarth *et al.*, 2003). Taken together, these results suggest that the nervous system combines passive reactions of the skeletal system with active muscle forces in the control of movement. The challenge in neurophysiology is to disentangle the relative contributions of intrinsic musculoskeletal properties, active muscle forces and the neural activity that orchestrates the whole.

1.3.2 Stabilizing role of muscles

The inherent mechanical properties of muscles, and their arrangement across joints, act to resist postural perturbations and simplify control in the face of the multiple degrees of freedom of the skeletal system. Often muscles are attached to control several independent directions of joint excursion and to maximize automatic levering of the mechanical system when it is perturbed from its control position. For example, the arrangement of flexor and extensor muscles around the ankle joint is such that large yaw displacement (abduction-adduction), can be counteracted with large extrasaggital levering of these muscles to increase torques needed to stabilize quadrupeds and bipeds during locomotion (Lawrence *et al.*, 1993; Lawrence & Nichols, 1999).

Ankle flexors are prone to generate adduction torques (Lawrence & Nichols, 1999), which would tend to place the leg more medially during the swing phase of locomotion, a strategy linked to energy efficient walking (Donelan *et al.*, 2001). Finally, it was shown that during postural perturbations in cats, the muscles of the leg exert stabilizing *passive* lateral forces before active muscle contractions mediated by the nervous system occur (Macpherson, 1988).

The spring-like property of muscle actuators has long been recognized as equivalent to negative displacement feedback. This has led to the development of motor control theories that incorporate muscle stiffness as an integral component, e.g. the equilibrium point (λ) theory (Feldman, 1966). Spring-like force-length and viscous force-velocity behaviour serves to resist muscle lengthening or shortening and tends to return the system to its control position. In this sense mechanical properties play an important stabilizing role during coordinated behaviours such as locomotion (Gerritsen *et al.*, 1998; Prochazka & Yakovenko, 2002). Note that the negative feedback loop 1 in Figure 1.2, which corresponds to the intrinsic muscle properties, belongs to the feedforward and feedback pathways. Because of that, it can be predicted that muscle shortening,

which decreases the gain of loop 1, automatically attenuates the loop gains of all feedback pathways (Prochazka *et al.*, 1997a; Yakovenko *et al.*, 2004). Alternatively, muscle lengthening produces the opposite effect; it increases the gains of loops 1-3 in Figure 1.2. The intrinsic muscle properties not only contribute to automatic load compensation, but they may also play an important role of modulating loop gains of sensory feedback.

1.4 Sensory feedback

> Spinal motoneurons are "the final common pathway."
> – Sir Charles Sherrington (1857-1952)

1.4.1 Proprioceptors involved in the regulation of locomotion

In the early days of neuroscience, it was recognized that sensation of movement, muscle sense, originates in muscles and is caused by mechanoreceptors located in the muscle belly, ligaments and joints. These receptors were unified in a single class called proprioceptors (Sherrington, 1906). Since then, experimental studies have shown that other receptors respond to movement and also contribute to kinaesthesia. Thus, they may be included in the proprioceptor group. For example, skin receptors, initially classified as exteroreceptors together with auditory, visual and olfactory systems, can generate powerful illusions of movement in response to stretch (Moberg, 1983; Collins & Prochazka, 1996). In order to direct the discussion, we will concentrate on an overview of the muscle spindle and tendon organ receptors only.

Muscle spindles are spindle-shaped encapsulated sensory organs located in the muscle belly. The human body contains 25,000 - 30,000 muscle spindles, most of which are located in the extremities (Hulliger, 1984). Each spindle contains two types of sensory endings embedded in specialized intrafusal muscle fibers, which in their turn attach to extrafusal muscle fibers. Though intrafusal fibers may sometimes share innervation by ß-skeletofusimotor axons with extrafusal

fibers, spindle fibers receive input from specific small-diameter γ-motoneurons (Emonet-Denand *et al.*, 1975). The two types of sensory endings belong to primary group Ia fibers (conduction velocity 72–120 m/s in cats) and secondary group II fibers (20–72 m/s) (Matthews, 1972).

Spindle afferents respond to changes in muscle length even in the absence of fusimotor drive. Group Ia afferents are more sensitive to dynamic changes, i.e. they are more sensitive to changes in velocity and acceleration, while group II afferents respond to static length changes and have a low dynamic sensitivity. Similar to the surrounding muscle, intrafusal fibers possess non-linear properties resulting in non-linear aspects of spindle afferent response, e.g. Ia sensitivity depends on the amplitude, offset and velocity of stretch (Hulliger, 1984; Poppele & Quick, 1985; Prochazka, 1996). When fusimotor drive is present, the sensitivity of spindle afferents to variations in length changes. Generally, dynamic fusimotor drive increases the sensitivity (gain) of response of Ia sensory endings. Static fusimotor drive increases the firing bias of both group Ia and II endings of sensory afferents and reduces Ia stretch sensitivity (reviewed in Prochazka, 1996).

Overall, there are about 80% more tendon organs than spindles. Tendon organs are encapsulated structures, which can be found at the junction of muscle and tendinous structures and are about 1 mm long and 0.1 mm in diameter (Barker, 1974). Within the capsule, free sensory endings are intertwined with the collagen fibers of tendon in braided fashion, so that tendon stretches deform the nerve endings. Each tendon organ , which gives rise to a group Ib afferent axon outside of the capsule, interacts with 10-20 motor units and each motor unit affects 1-6 tendon organs (Proske, 1981; Jami, 1992). Unlike spindles, tendon organs are relatively sensitive to changes of length or velocity, but respond to force generated actively by the muscle (Houk & Henneman, 1967; Stephens *et al.*, 1975). Tendon organs may possess discontinuities in their force

response characteristics and signal whole muscle force non-linearly (Jami et al. 1985). For example, because of the parallel arrangement of muscle fibers, contraction of fibers not "sampled" by a tendon organ can in some cases unload it, reducing the firing of the Ib afferent (Houk and Henneman 1967; Stuart et al. 1972).

1.4.2 Models of spindles and tendon organs

Mathematical models of spindles and tendon organs have been developed based on input-output characteristics collected in acute experiments. In the case of muscle spindles, the receptor-bearing muscles were detached from their surrounding structures, their tendons were cut and attached to length servos and the muscles were subjected to sinusoidal or "ramp and hold" stretches, while the activity of the sensory afferents was recorded from teased dorsal root filaments (Matthews & Stein, 1969; Poppele & Quick, 1985). The response characteristics of Ia afferents were usually fitted with functions of muscle length and velocity. Tendon organs have been studied by stimulating single motor units, groups of motor units, or the whole muscle and recording the Ib responses in dorsal root filaments. Frequency analyses of their response to feedback-controlled forces applied to the muscle have also been performed (Houk & Simon, 1967; Anderson, 1974; Stephens *et al.*, 1975). These models are in the form of transfer functions between the force signal and the firing frequency of Ib afferents. The tendon organ transfer function is that of a high-pass filter with several zeros and poles. The Bode plots of Ib afferents resemble those of muscle spindle group II afferents more than Ia afferents, in that their high-pass characteristics are less marked. At low levels of active force, the firing of single motor units and the recruitment of new motor units cause irregularity and steps in Ib firing rates (Jami *et al.*, 1985).

Spindle and tendon organ models have been successfully tested in freely moving normal cats (Prochazka *et al.*, 1976; Loeb & Duysens, 1979; Prochazka & Gorassini, 1998b, a). Accompanied by measurements of muscle length, velocity and EMG activity, ensemble activity of spindle group Ia and II afferents and tendon organ group Ib afferents were chronically recorded in dorsal root ganglia. Spindle models relating muscle length to afferent firing produced passable predictions of Ia and II responses to fast muscle stretches that occur during locomotion. Predictions of the afferent firing rates were further improved by the addition of EMG-dependent offsets, which represented biasing of Ia firing rate due to alpha-gamma linkage (Emonet-Denand *et al.*, 1975). Though muscle force was not directly measured in these studies, tendon models produced good predictions of Ib firing rates from EMG profiles as well.

The development of representative mathematical models of sensory feedback and their use in combination with musculo-skeletal models may prove to be crucial for our understanding of the role of sensory feedback during complex behaviours such as locomotion, control of balance and voluntary movement of the upper extremities.

1.4.3 Stretch reflexes

Muscle stretch gives rise to reflex responses through sensory feedback to the spinal cord and brain from muscle receptors. Since Sherrington's time, it has been realised that so-called reflex responses to muscle stretch are not invariant, and may be modified by descending and segmental CNS mechanisms (Loeb, 2000).

The contribution of segmental Ia-mediated stretch reflexes and their electrically-elicited counterparts, H-reflexes, to ongoing activity has been extensively studied (Dietz, 1996; Prochazka, 1996; Brooke *et al.*, 1997; Dietz, 1998; Duysens *et al.*, 2000). It was found that human H-reflexes

are bigger during standing than during locomotion and their amplitude is phase-dependent within the locomotor step cycle (Garrett *et al.*, 1981; Garrett & Luckwill, 1983; Capaday & Stein, 1986). H-reflexes were also found to be further reduced during running and in difficult beam-walking (Capaday & Stein, 1987; Llewellyn *et al.*, 1990). It remains unclear whether the origin of this modulation is controlled centrally (Schneider *et al.*, 2000) or through sensory feedback pathways (Misiaszek *et al.*, 1998). Similarly, stretch reflexes elicited during locomotion by rapid stretching of muscles also show phase-dependent modulation (Orlovsky & Shik, 1965; Akazawa *et al.*, 1982; Hiebert *et al.*, 1996; Sinkjaer, 1997). However, stretch-evoked responses show a higher variability than H-reflexes, which is probably due to the fact that a variety of pathways contribute to stretch responses (Sinkjaer, 1997; Sinkjaer *et al.*, 1999; Christensen *et al.*, 2001).

Stretch reflexes have been subdivided into groups based on their latencies (Pearson *et al.*, 1999; Gritsenko *et al.*, 2001). Short-latency responses are produced by the firing of spindle and tendon organ afferents in response to muscle stretch. Spindle group Ia afferents monosynaptically excite homonymous alpha motoneurons and cause the receptor-bearing muscle to resist the imposed stretch. While Ib afferents reflexly inhibit homonymous motoneurons in static postures, this switches to homonymous excitation during locomotion, at least in cats (Conway *et al.*, 1987; Pearson & Collins, 1993; Gossard *et al.*, 1994; Guertin *et al.*, 1995; Pratt, 1995). The evidence for switching of Ib reflex sign in humans remains ambiguous (Dietz, 1998; Stephens & Yang, 1999; Capaday, 2000; Misiaszek *et al.*, 2000; Pang & Yang, 2000). Spindle group II afferents may also contribute to stretch reflexes through di- and oligo-synaptic pathways (Matthews, 1991; Sinkjaer *et al.*, 2000; Grey *et al.*, 2001).

Key concepts of the stretch reflex system can be presented in the form of the control system diagram shown in Figure 1.2 (*loops 1 & 2*). The contribution of spindle group Ia and II afferents to stretch reflexes is represented as negative feedback (loop 2), which augments the intrinsic stiffness of active muscles (*loop 1*). The diagram shows that intrinsic stiffness can be viewed as negative displacement feedback (Partridge, 1966). As mentioned above, Ib feedback switches from inhibition to excitation of homonymous motoneurons during locomotion (Gossard *et al.*, 1994; McCrea *et al.*, 1995). Ib homonymous excitation represents positive force feedback. Positive feedback is generally associated with instability in engineering systems. However, a theoretical study (Prochazka *et al.*, 1997a, b) showed that the force-length properties of muscles tend to stabilize what would otherwise be an unstable system with positive force feedback. In control systems, positive force feedback with embedded negative displacement loop (positive muscle stiffness) behaves similarly to a negative displacement loop. For example, load can increase muscle force through the positive feedback loop to homonymous motoneurons. The muscle can then exert higher forces at the same muscle length, which may lead to muscle shortening or negative displacement. To summarize, during locomotion, stretch reflexes (*loop 2*) augment the automatic load compensation provided by intrinsic muscle stiffness (*loop 1*).

How much do the stretch reflexes contribute to the ongoing activity of muscles during locomotion? Several research groups have attempted to answer this challenging question. Selective nerve block of fusimotor drive was shown to reduce EMG activity of ankle extensors by about 50% in a locomotor cat preparation, indicating that spindle input is a major contributor to motoneuronal excitation (Severin, 1970). However, some alpha motoneurons may have been blocked along with gamma motoneurons in these experiments. In another set of acute experiments

in cats, the contribution of stretch reflexes was estimated by comparing force responses to sinusoidal stretches before and after deafferentation (Bennett *et al.*, 1996). The difference in the force generated by ankle extensors was about 25%. In humans, it was estimated that stretch reflexes induced by abrupt lengthening of soleus, an ankle extensor, contributed 30-60% of the total EMG activity during stance (Yang *et al.*, 1991) and reached about 50% in the late swing phase (Sinkjaer *et al.*, 1996). However, it is difficult to separate the force attributable to intrinsic muscle stiffness alone and the additional force attributable to stretch reflex augmentation of that stiffness in the closed-loop system, where muscle is allowed to change length during load compensation. On one hand, the above estimates indicate that stretch reflexes contribute significantly to locomotor control. On the other hand, the intrinsic musculoskeletal capacity for stabilization should not be underestimated. Interestingly, it was demonstrated recently that large-fiber sensory loss and absent tendon jerks in mammals, including humans, did not abolish weight-support and even locomotion, provided lateral support was present (Allum *et al.*, 1998; Pearson *et al.*, 2003).

1.5 Control of locomotion

> "If then the soul in pigeons, frogs, vipers, and tortoises, is not confined to the brain, but can continue for a long time to actuate their bodies independent of that organ ... why should we deny that, in man and such animals that resemble him most, the parts may continue to be actuated by the soul or sentient principle for some few minutes after their communication with the brain has been cut off?"
> — Robert Whytt (1714-1766)

1.5.1 Simple locomotor reflexes

The study of locomotion dates back to La Mettrie's observations of rhythm generation in animals with cervical spinalization (Mettrie, 1745). A century later, Spencer (Spencer, 1855) and Sechenov (Sechenov, 1863) proposed that movements are composed of chains of reflexes. Further experimental evidence for this hypothesis was provided by studies of flexion and extension reflexes

in spinalized or decerebrated dogs and cats (Freusberg, 1874; Sherrington, 1910). At about the same time, Muybridge was the first to publish cinematic recordings of animal movement and introduced an objective kinematic method of analysing locomotion (Muybridge, 1887).

Later, Philippson (Philippson, 1905) proposed not only a comprehensive subdivision of the step cycle based on kinematics, but also he suggested the locomotor mechanism based on interactions with environment and reflex responses. He stated that extensor thrust was evoked by ground contact. This reaction was reinforced by the flexor crossed-extensor reflex of the contralateral limb, until exteroceptive stimuli caused by the increased pressure on the foot caused flexion of the ipsilateral limb and extension of the contralateral limb. The cycle then repeated for the contralateral limb. In spite of this appealing description, Sherrington and Brown (Sherrington, 1910; Brown, 1911) recognized that spinalized dogs were capable of "movements of progression" or "reflexive stepping" without ground contact and even after cutaneous deafferentation.

The remarkable study by Sherrington (Sherrington, 1910) remains a reference work of biomechanical actions of cat hindlimb muscles and their participation in flexion and extension reflexes and air-stepping even to this day. In this study, Sherrington suggested that the locomotor pattern is generated by the interplay of "primary" and "secondary" reflexes. While the "primary" reflex is continuous and can be evoked by electrical stimulation of the rostral stump of the transected cervical spinal cord or by mechanical stimulation of various peripheral points outside the limb, the "secondary" reflex is antagonistic to the "primary" reflex and is evoked by the "primary" reflex through a proprioceptive response. Thus, the "secondary" reflex interrupts the "primary" reflex until the proprioceptive signal is abolished and then the cycle repeats. Essentially, Sherrington reconciled the purely peripheral scheme of pattern generation with the contradictory

results of the deafferentation studies by introducing a central factor that could be affected by descending or peripheral inputs.

A study by Brown, Sherrington's student, provided fundamental evidence of a central origin of locomotor activity (Brown, 1911). He demonstrated that decerebrate and low-spinal animals can generate alternating flexion and extension activity in completely deafferented hindlimb muscles. Though recognizing an important modulatory role of sensory feedback, Brown proposed that the spinal cord contains a pattern generating apparatus with at least two antagonistically organized half-centers or a "pair of antagonistic opposites". He called this mechanism "the intrinsic factor." It was later renamed "the central pattern generator" (CPG) (Grillner & Zangger, 1975).

1.5.2 Locomotor central pattern generator (CPG)

A number of rhythmic behaviours are produced by stereotypic periodic patterns of muscle activity. After the pioneering work of Sherrington and Brown, many studies established that isolated neuronal networks in the CNS can generate commands not only for walking, but also for swimming, flying, scratching, paw-shaking, breathing, chewing and even micturition and ejaculation (Grillner, 1975; Prochazka, 1996; Kiehn *et al.*, 1998; Orlovsky *et al.*, 1999; Carro-Juarez *et al.*, 2003).

Because of the complexity of the nervous system, it is challenging to study structural details and function of the locomotor CPG in vertebrates. Simpler invertebrate neural systems have proved to be a valuable source of information on endogenous characteristics of single CPG neurons and their network interactions. Using intracellular recording techniques, single-cell oscillators have been identified in *Aplesia*, *Helix*, leech and crustacean to name a few. These oscillators produce stable periodic bursting and can be perturbed by synaptic inputs or neuromodulators, which can

modify membrane properties and synaptic strength (Marder & Bucher, 2001). Mathematical models based on realistic membrane properties contributed considerably to our understanding of these "simple" systems (Kopell, 1988). For example, the role of reciprocal inhibitory connections in a dynamically coupled network of two endogenous oscillators regulating the heartbeat in the leech has been recently examined using bifurcation analysis (Cymbalyuk *et al.*, 2002). This study demonstrated how the half-center configuration of the CPG controls bursting characteristics such as period, phase and duty-cycles and incorporates characteristics of single-neuron oscillators to ensure its stable operation.

One of the intriguing properties of many endogenously bursting neurons and their follower neurons is the mechanism of the plateau potential. This enables neurons to switch between two stable membrane potentials. Short depolarizing currents can trigger a sustained membrane depolarization or plateau, whose duration outlasts the stimulus and depends on persistent, slow inward currents. In the depolarized state, the "plateau" can be rapidly terminated by a short hyperpolarizing current (Marder & Calabrese, 1996). This membrane property was found in invertebrates (Pin *et al.*, 1990; Ramirez & Pearson, 1991) as well as vertebrates (Conway *et al.*, 1988; Hounsgaard *et al.*, 1988; Hounsgaard & Kiehn, 1989; Bennett *et al.*, 1998; Gorassini *et al.*, 1999). If these bistable potentials correspond to inactive hyperpolarized and active depolarized states, then the neuron may exhibit bursting activity. In fact, plateau potentials were found to be critical for heartbeat generation in leech (Angstadt & Calabrese, 1991; Opdyke & Calabrese, 1994). Because of susceptibility to neuromodulation, e.g. by serotonin (5-hydroxytryptamine, 5HT), noradrenergic agonists (Katz & Harris-Warrick, 1990; Lee & Heckman, 1999), plateau potentials were implicated as a mechanism of reorganization of the neural networks to produce rhythmic

behaviors, e.g. escape behavior in mollusks and pyloric activity in crustaceans (Katz & Harris-Warrick, 1990). Thus, plateau potentials may be responsible for endogenous bursting and shaping of the CNS output.

The location, structure and properties of neural networks implicated in locomotor pattern generation have been investigated in many different species (Kiehn *et al.*, 1998). Generally, there are two alternative opinions about localization of the locomotor pattern generator in vertebrates. It may be confined to the rostral segments of the lumbosacral enlargement (L4-L5 spinal segments in cats) (Cazalets *et al.*, 1995) or it may be distributed throughout the lumbosacral enlargement (Deliagina *et al.*, 1983; Ho & O'Donovan, 1993; Kjaerulff & Kiehn, 1996; Arshavsky *et al.*, 1997; Cowley & Schmidt, 1997; Kremer & Lev-Tov, 1997). Because it is technically challenging to monitor the activity of large neural networks, all of the studies on localization of CPGs have been carried out in reduced preparations including isolated embryonic spinal cords of rat (Lev-Tov & O'Donovan, 1995), spinalized cat (Andersson *et al.*, 1978; Grillner & Zangger, 1979; Deliagina *et al.*, 1983), lamprey (Zhang *et al.*, 1996; Grillner *et al.*, 1998) and mudpuppy (Cheng *et al.*, 1998). Rhythmic activity of motoneuron pools which often had the specific temporal features observed in locomotion, was evoked by administration of pharmacological agents such as dihydroxy-phenylalanine (DOPA), N-methyl D-aspartate (NMDA), serotonin, or by electrical stimulation of descending or peripheral sensory pathways. However, these preparations lacked either descending inputs or sensory inputs from actively moving limbs or both. It was shown that descending pathways originating in cerebral, cerebellar and midbrain areas exert a significant influence on the amplitude and timing of muscle contractions during normal locomotion (Armstrong & Marple-Horvat, 1996; Rho *et al.*, 1997). Structures within the brainstem can neuromodulate spinal cord

circuitry using descending noradrenergic and serotonergic pathways (Barbeau & Rossignol, 1990, 1991). Moreover, sensory feedback from moving limbs delivers phase-dependent modulation of motoneuron activity and initiates phase transitions (Grillner, 1975; Grillner & Rossignol, 1978; Prochazka, 1996).

One popular version of the "distributed" hypothesis describes the hindlimb CPG as a series of autonomous "unit burst generators" distributed along the neuraxis and dynamically coupled by propriospinal connections (Grillner & Wallen, 1985). Taking this representation literally, one might have assumed that the potential for rhythmogenesis is equally distributed throughout the lumbosacral enlargement. However, there is evidence that the more rostral segments of the enlargement may produce rhythmic activity more robustly than the sacral segments in response to administration of excitatory amino acids and adrenergic agonists (Kiehn & Kjaerulff, 1998; Marcoux & Rossignol, 2000). In contrast, a recent study of rhythmogenesis in response to intraspinal microstimulation showed the opposite result, namely that the caudal lumbosacral spinal cord produces rhythmic activity more readily than its more rostral segments (Guevremont *et al.*, 2003). Unfortunately, there is no clear answer to this controversy at this time. It is possible that the rostral neural networks are more susceptible to neuromodulators, while the caudal neural networks are more sensitive to electrical stimulation.

1.5.3 Locomotor patterns generated in the presence of sensory feedback

For a hundred years, two opposing hypotheses of the role of sensory reflexes in stereotypical movements were discussed. Sensory reflexes were believed to be responsible for generation of locomotor activity according to the first hypothesis (Philippson, 1905), while according to the second hypothesis, reflexes modulated the centrally generated motor patterns

(Sherrington, 1910; Brown, 1911). On one hand, deafferentation studies in the cat provided evidence that many subtle features of muscle activity during overground locomotion are present in the centrally generated patterns. For example, it was suggested that a small burst of activity in bifunctional knee flexors at the end of the swing phase was evoked by sensory feedback during overground locomotion (Engberg & Lundberg, 1969). However, it was later shown that this double-bursting persisted in animals with transected hindlimb dorsal roots and therefore had to be generated centrally (Grillner & Zangger, 1975). On the other hand, the locomotor rhythm is generally more variable after sensory feedback is abolished by deafferentation (Grillner & Zangger, 1975; Wetzel *et al.*, 1976; Goldberger & Murray, 1980; Giuliani & Smith, 1987). Moreover, deafferented animals are less capable of compensating for changes in load and roughness of terrain (Allum *et al.*, 1998). The locomotor deficits are even more devastating in humans with sensory neuropathy than in deafferented quadrupeds (Lajoie *et al.*, 1996). Evidently, humans with large-fibre sensory loss develop a control strategy based on passive stability (increased base of support, prolonged double stance) and multisensory information on body position from vision, vestibular systems and remaining proprioceptors, including those in the neck muscles. If vision is obstructed, these people find it difficult to walk without stumbling and falling even on a flat surface.

Several studies have indicated that humans may have a spinal locomotor CPG, but the evidence is inconclusive. People with functionally complete spinal cord injury (SCI) have been capable of producing weak locomotor-like activity inadequate for weight-bearing (Calancie *et al.*, 1994; Dimitrijevic *et al.*, 1998). However, because sensory pathways were intact, the activity could have originated from reciprocating stretch reflexes. Also, steady vibration of the legs of people with SCI induces air-stepping (Gurfinkel *et al.*, 1998). However, the origin of the rhythmic activity can be

either central or peripheral in this study also, i.e. muscle contraction may lead to pendular motion of the suspended leg and cause phasic sensory drive to muscles. Indirect evidence for the existence of the human locomotor CPG comes from studies of stepping in human infants. Human infants readily produce locomotor patterns in the presence of motion-related proprioceptive feedback, even though their cortical and cerebellar control was incomplete (Lamb & Yang, 2000).

Much of what we know about the motor control strategies for locomotion comes from studying motor activity during artificial interventions in reduced preparations. Half a century ago a Moscow group (Shik *et al.*, 1966; Shik & Orlovsky, 1976) discovered that cats decerebrated rostral to the superior colliculus could locomote when a region called the midbrain locomotor region (MLR) was electrically stimulated. In this preparation sensory information can entrain and reset the centrally generated activity (Guertin *et al.*, 1995). This was confirmed in chronic spinal cats and acute spinal cats administered with clonidine (Rossignol, 1996). For example, chronic spinal kittens could rapidly adapt the rhythm of each leg to the speed of the individual belts of a split-belt treadmill (Forssberg *et al.*, 1980). In addition, state dependent changes in neural pathways were studied at rest and during either MLR- or drug-induced locomotion in decerebrate cat (Gossard *et al.*, 1994; McCrea *et al.*, 1995). Using these preparations, details about projections of sensory pathways and their contribution to motoneuron activation during locomotion were established (reviewed in Jankowska, 2001; McCrea, 2001).

Sensory feedback is not only involved in the amplitude regulation of muscle activity (see 1.4.3), but it can also modify timing of the locomotor rhythm. A number of studies have shown that proprioceptive signals entrain, enhance and reset the ongoing locomotor activity (Duysens & Pearson, 1980; Andersson & Grillner, 1983). Figure 1.2 contains Brown's classical model of flexor

and extensor half-centers controlling each limb (Brown, 1911). In this diagram, the sensory pathways affecting the CPG, e.g. from load-sensitive afferents (Duysens & Pearson, 1980; Conway *et al.*, 1987; Pearson & Collins, 1993) are shown by loop 3, which embeds intrinsic muscle properties (loops 1), stretch reflexes (loop 2) and other pathways, e.g. beta drive to muscle spindles (Emonet-Denand *et al.*, 1975). Sensory pathways projecting to the locomotor CPG may provide its autonomous adaptation to the dynamics of the mechanical system and are important for maintaining locomotor stability (Ogihara & Yamazaki, 2001).

Multisensory signals including those related to extensor muscle force and hip position (Fig. 1.2, loop 4) have been hypothesized to initiate the phase transition from stance to swing in a number of species, e.g. locusts, crayfish and cats (Prochazka, 1996). The process can be expressed as the following simple sensory rule that "fires" when its conditions are met: IF the leg is extended AND extensor force is low, THEN initiate swing. This type of controller is called a finite state system, which was initially devised to control industrial production processes. It has also proved to be effective in the control of above-knee prostheses (Prochazka, 1993), functional electrical stimulation in human gait (Popovic *et al.*, 1989) and control of movement in biomimetic robots (Nelson & Quinn, 1999). The finite state control of locomotion may provide automatic adaptation to large perturbations of the body.

To summarize, locomotor patterns are generated by complex interactions between multiple control systems. This organization provides stable and automatic load compensation as well as higher-level control based on state regulation.

1.6 Spinal cord trauma

> "Everything may die, nothing may be regenerated. It is for the science of the future to change, if possible, this harsh decree."
> — Santiago Ramon y Cajal (1852-1934)

Spinal cord injury generally presents as devastating damage to the nervous system produced by mechanical crush of the spinal cord by ruptured vertebral bones, which normally enclose and protect it. During the early stage, about 18 hours after injury, cell bodies and axons disrupted by the trauma undergo passive, or necrotic, death characterized by swelling and evidence of nuclear dissolution. The initial injury triggers cascades of pathological changes such as inflammation, local ischemia and release of free radicals. Glial cells including microglia and astrocytes proliferate to form a scar and increase production of axon growth inhibitory molecules (McKeon *et al.*, 1991; Fitch & Silver, 1997). The secondary damage spreads several spinal segments in the rostral and caudal directions during the next several weeks. Neurons die not only passively, but also actively, a process called apoptosis. Evidence of oligodendrocyte death and consequent demyelination of spared axons has been found below and above the lesion (Bresnahan *et al.*, 1976; Liu *et al.*, 1997).

Another key aspect of spinal cord injury is neuronal reorganization. Almost a century ago, Ramon y Cajal not only recognized that the glial scar represents a physical barrier for axon regeneration, but he also observed that injury to the optic nerve, spinal cord or cortex induces a sprouting response (Ramon y Cajal, 1928). This sprouting response is likely mediated by neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) secreted from activated microglia close to the edge of the injury (Batchelor *et al.*, 2002). The new connections may even be functional (Bareyre *et al.*, 2004). Other structural and physiological changes to spinal segments

below the lesion include: loss of the terminals of the descending pathways (Yakovleff *et al.*, 1995), reinnervation of vacant synapses by afferent pathways, decrease in the threshold of the postsynaptic neurons, and loss of the presynaptic inhibition (Basso, 2000).

Thus, a comprehensive spinal cord repair strategy should minimize the effect of the secondary injury, remove the physical barrier formed by the cyst and glial scar, compensate for demyelination of spared axons, promote axonal long-distance regeneration and ensure proper wiring of the regenerating axons with their targets.

A number of therapies have been developed to reduce and repair trauma to the spinal cord. Primary and secondary injury may be reduced by the administration of anti-inflammatory drugs and agents reducing apoptosis, such as selective blockers of glutamate receptors (McDonald *et al.*, 1998). A number of repair and rehabilitation strategies are the subject of current research including cell transplantation (Kawaja & Gage, 1991; Tuszynski *et al.*, 1994a; Tuszynski *et al.*, 1994b; Lazarov-Spiegler *et al.*, 1996; Nakahara *et al.*, 1996; Guest *et al.*, 1997; Li *et al.*, 1997; Diener & Bregman, 1998; Ramon-Cueto *et al.*, 1998; Tuszynski *et al.*, 1998; McDonald *et al.*, 1999; Ramon-Cueto *et al.*, 2000), gene therapy (Kawaja & Gage, 1991; Tuszynski *et al.*, 1994a; Tuszynski *et al.*, 1994b; Menei *et al.*, 1998), modulation of intracellular signalling (Liu *et al.*, 1999; Cai *et al.*, 2001), manipulation of growth inhibitory environment (Schnell & Schwab, 1990; Fouad *et al.*, 2001; Raineteau *et al.*, 2002), locomotor training (Barbeau & Rossignol, 1987; Edgerton *et al.*, 1997; De Leon *et al.*, 1998; Fouad *et al.*, 2000) and restoration of lost functions using functional electrical stimulation (Brindley *et al.*, 1986; Stein *et al.*, 1992; Mushahwar *et al.*, 2000).

1.7 Thesis objective and summary

The objective of this thesis is to study the control of locomotion by multiple systems including the musculoskeletal system, sensory pathways and the lumbosacral pattern generator.

Chapter 2 deals with a controversy around intrinsic muscle properties important for modeling of muscle behavior. The study reported in this chapter tests the following two hypotheses: 1) static isometric length-tension curves of active wrist muscles apply to continuous movements; 2) hysteresis in length-tension curves of active wrist muscles is maximal at maximal muscle lengths.

Chapter 3 contains a study of stretch reflexes during locomotion. The goal of this study was to test the following two hypotheses: 1) stretch reflexes are too weak and too delayed to contribute significantly to weight-bearing; 2) the important contributions of sensory input involve state-dependent processing.

Chapter 4 describes a study of locomotor pattern generation during normal locomotion. This study introduces a novel method of analysis of the lumbosacral motoneuron activity. It reports details of the spatiotemporal motoneuron activity pattern during normal level locomotion and the implications for the locomotor control system.

Chapter 5 contains a study of fictive locomotion. In this study, the analysis of the locomotor activity revealed a novel flexibility in the pattern generation, which may reflect general properties of the locomotor pattern generating networks.

Chapter 6 describes a study of motor function recovery after spinal cord injury. It tests the following hypothesis: the observed motor improvements after long distance axon regeneration may be explained by excitation of propriospinal neurons that relay excitation to the locomotor CPG.

Finally, Chapter 7 contains a summary, general discussion and future directions of the studies described in this thesis.

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CHAPTER 2

Isometric muscle length-tension curves do not predict angle-torque curves of human wrist in continuous active movements.

Adapted from an original publication:

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2.1 Introduction

The force generated by active muscles depends not only on activation level but also on length and velocity. The classical length-tension curve of active muscle, first described by Blix (Blix, 1894), has an ascending limb, a plateau, a descending limb and a final ascending limb at maximal physiological lengths. The initial ascending and descending limbs are attributed to increases and decreases in overlap of actin and myosin filaments as sarcomeres lengthen (Gordon *et al.*, 1966). The final ascending limb is attributed to passive stiffness (Huijing, 1985; Zajac, 1989).

Isometric length-tension curves in different cat hindlimb muscles range from the classical curve described above to monotonic curves (Gareis *et al.*, 1992). From experiments in healthy human subjects and cadavers, Lieber et al. (Lieber *et al.*, 1994), Loren et al. (Loren *et al.*, 1996) and Lieber and Frieden (Lieber & Friden, 1998) predicted that over the physiological range of motion (ROM), wrist flexors would have monotonic isometric length-tension curves while wrist extensors would show classical curves with prominent descending limbs. The data indicated that extensor carpi radialis brevis (ECRB) might operate on the descending limb over much of its functional range. As this seemed at odds with the mechanical properties of the hand during functional electrical stimulation (Prochazka *et al.*, 1992), we wondered whether the isometric data were really as predicted by Loren et al. (Loren *et al.*, 1996) and more generally, whether isometric length tension curves generalized to continuous active movements.

Though one often hears the opinion that because isometric length-tension curves represent discrete static measurements they probably do not generalize to continuous movement, few publications address the issue in detail and the evidence is fragmentary (Geffen, 1964; Baratta *et al.*, 1993; Vance *et al.*, 1994). In biomechanics, muscle is often modeled as the product of an

isometric length-tension curve and a Hill-type force-velocity relationship (e.g. Veltink *et al.*, 1992). Such models predict that for constant-velocity stretches muscle force should decline as stretch proceeds beyond the peak of the isometric length-tension curve. A decline in force with increasing length may be interpreted as negative stiffness (Stein *et al.*, 1999), a property that is difficult to deal with in feedback control systems.

Actively contracting muscle has a high short-range stiffness when stretched through an initial ~1% of rest length (Joyce *et al.*, 1969). Actin-myosin cross-bridges are thought to remain attached and deform elastically within this range and become detached when stretch continues, resulting in a lower stiffness (Rack & Westbury, 1974). Consequently, when an active muscle is cyclically stretched and shortened, short-range stiffness may produce hysteresis (a divergence of length-tension curves for shortening and lengthening not attributable to force-velocity characteristics). Another nonlinear property of muscle that could produce hysteresis is the so-called force-enhancement effect during stretch and force deficit during shortening (Julian & Morgan, 1979a; Edman *et al.*, 1982; Sugi & Tsuchiya, 1988; Morgan, 1990). This and a related phenomenon, "creep", have been attributed to the build-up of sarcomere non-uniformities at long muscle lengths (Gordon *et al.*, 1966; Julian & Morgan, 1979a; Edman & Reggiani, 1984; Morgan, 1990).

In this study we tested two hypotheses: 1) static isometric length-tension curves of active wrist muscles apply to continuous movements; 2) hysteresis in length-tension curves of active wrist muscles is maximal at maximal muscle lengths.

2.2 Methods

Angle-torque curves of wrist flexors or extensors were measured in 4 subjects, 2 males and 2 females, aged 21-52 in steady contractions produced either by electrical stimulation or constant

voluntary contraction. No subjects had any history of wrist trauma or musculoskeletal disease. All subjects gave written consent in accordance with the requirements of the University of Alberta Human Ethics committee and the Declaration of Helsinki.

The subject was seated in a comfortable chair with his/her elbow flexed to 90° and shoulder abducted by 30-40°. The elbow was held in place with a rigid molding of thermoplastic material (Sansplint) and the forearm was stabilized just distal to the elbow and just proximal to the wrist with two pairs of padded restraints (Fig. 2.1). The hand was formed into a fist and secured in a form-fitting Sansplint cast. The cast was attached to a rigid, circular arc which acted as a guide for a flexible steel cable connecting one end of the arc to a force gauge and a rotary servo motor. This allowed force to be applied at a constant distance from the wrist pivot point. Other cables suspended from the ceiling supported the cast in the horizontal plane. A pointer centered at the mid-point of the arc was positioned over a protractor for calibrating angle measurements obtained from an angular displacement transducer attached to the servo motor. The servo (stiffness 19.3 N/mm) was controlled using Simulink and Real-Time Workshop Toolbox (The Mathworks, Inc, Natick, MA) and a digital microprocessor interface (dSPACE Inc., Northville, MI).

Two fatigue tests were performed in each subject prior to the main experiment. With the wrist in neutral position, 10% MVC was maintained for 120s by 1) electrical stimulation or 2) voluntary effort. In stimulation trials, torque typically declined presumably because of fatigue in fast-fatiguable muscle fibres, reaching a plateau after about 20s. In the main experiments, 20s of stimulation was applied between trials to bring the muscles to this plateau state. The same fatigue tests were performed immediately after the experiments to test for stationarity.

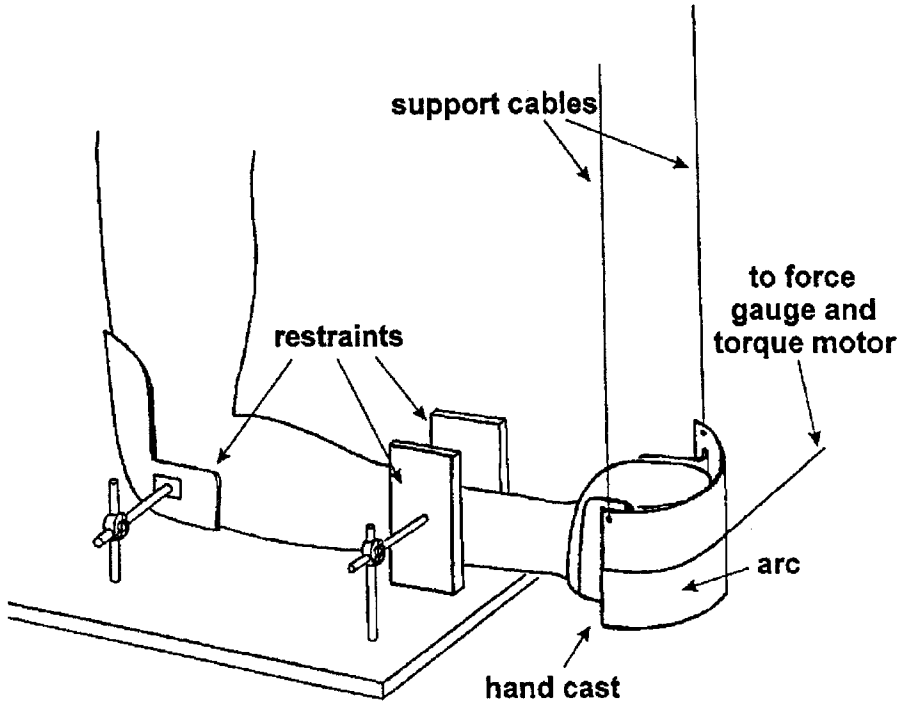

Figure 2.1. Experimental set-up. Subject's elbow and forearm were stabilized with an elbow cast and padded wrist restraints. The hand, bound into a fist, was secured inside a form-fitting cast attached to a circular arc suspended from the ceiling. The arc guided a cable connected to a rotary servo motor, allowing force to be applied at a constant distance from the wrist joint, i.e. force was proportional to torque. The torque required to move the wrist through different angles was measured while muscles were activated at a constant level, either voluntarily with EMG feedback, or by trains of electrical stimuli applied to the muscle nerves proximal to the elbow.

The servo moved the hand at a speed of $10° s^{-1}$. To avoid fatigue in full range of motion (ROM: +50°) trials with active muscle contraction, data were obtained separately for 1) muscle shortening over the whole 50° range, 2) first 25° of lengthening; 3) second 25° of lengthening. All trials were repeated 3 times. Sets of six +10° movements centered around -40°, -20°, 0°, +20°, +40° (flexion negative, extension positive) were recorded in random order. Next, torque in isometric contractions from rest at each of the above angles was measured. Finally, passive angle-torque characteristics with muscles relaxed were recorded in +50° and +10° movements.

In voluntary contraction experiments, EMG recordings were obtained from pairs of self-adhesive electrodes (Jason ElectroTrace, ET001, Huntington Beach, CA) 1cm apart over ECRB 6cm distal to the lateral epicondyle, and flexor carpi radialis (FCR) 6cm distal to the medial epicondyle. A reference electrode was placed over the ulnar olecranon process. EMG signals were high-pass-filtered (30Hz), amplified, full-wave-rectified, low-pass-filtered (1Hz) and displayed on an oscilloscope. The subject flexed or extended isometrically from the neutral wrist position to match the force trace to a target level corresponding to 10%MVC, using wrist muscles only. EMG cross-talk from finger muscles was estimated in separate strong contractions to be <10% of EMG from wrist muscles. Antagonist EMGs were monitored and played through a loudspeaker to the subject, whose job was to minimize co-contraction. Muscle activation commenced 2s before the onset of recording to give the subject time to stabilize the EMG.

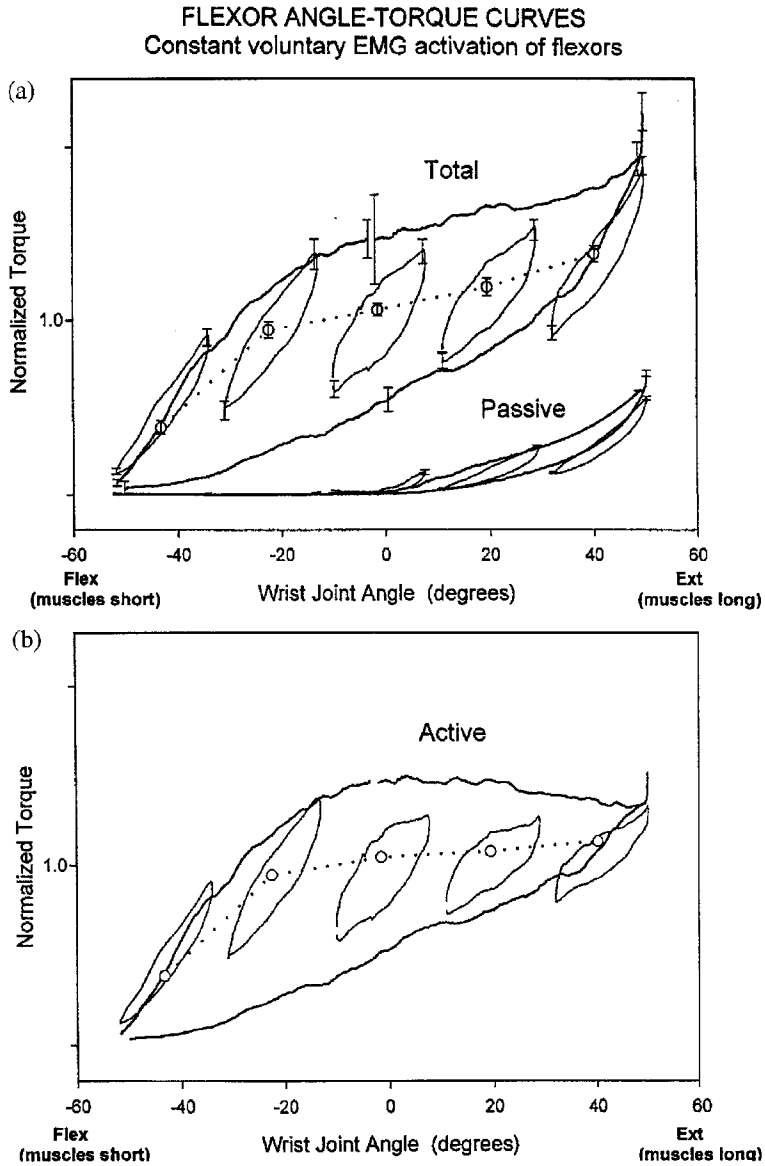

Figure 2.2. Mean angle-torque data for constant voluntary activation of wrist flexors. (A) Torque was measured in three contraction conditions: (1) isometric (dotted lines); (2) $\pm 10°$ triangular displacements centered at five different angles (small loops); (3) $\pm 50°$ triangular displacements over full range of motion (full ROM) of wrist joint (large loop). Narrow loops at bottom of figure show angle-torque profiles with muscles completely relaxed. Note that all torque values in this and subsequent figures were normalized to mean torque of $\pm 10°$ angle-torque loops at 0° wrist angle. (B) Active torque derived by subtracting passive torque values from corresponding total torque values in (A). Vertical bars: standard errors of means. Note large width of all ROM loops, even though the movements were applied very slowly to minimize velocity-dependent torque components. This suggests hysteresis rather than viscous properties. Note the positive slopes of the $\pm 10°$ ROM loops too, even in the range 0-30°, over which the isometric angle-torque curve descended slightly.

The same subjects participated in the experiments involving electrical stimulation. Self-adhesive electrodes (ConMed Versa-stim, 45x45 mm) were placed over the median nerve 8-10 cm proximal to the humeral medial epicondyle to stimulate flexors and over the radial nerve 8-10 cm proximal to the lateral epicondyle to stimulate extensors. Stimulation at motor points proximal to the origins of the muscles activated, avoided variations in stimulus efficacy as muscles changed length and shape. An indifferent electrode (45x90 mm) was placed on the skin just proximal to the wrist crease on the anterior surface of the forearm. Stimulation comprised trains of current-controlled biphasic pulses (33/s, 100ms primary pulse width). Subjects were instructed to remain relaxed and allow the stimulation to control their hand. Practice was required not to intervene. We checked for intervention periodically by turning stimulation off unexpectedly (Prochazka *et al.*, 1997).

Since electrical stimulation does not recruit motor units in physiological order (slow, fatigue-resistant to fast-fatiguable), stimulation was turned on between each set of cyclical movement trials for 20s to cause fatigue of fast-fatiguable muscle fibers. Stimulation was then adjusted to achieve 10% MVC and commenced 2s before recording started to allow for equilibration. The same number and types of movement trials were performed as in voluntary contraction experiments.

Signals from the force transducer, servo input, displacement sensor and EMGs were digitized at 100/s and averaged using a Cambridge Electronic Design (CED) 1401 laboratory interface and personal computer using SIGAVG (version 5.42), Matlab (version 4.2c) and SigmaPlot (version 4.0) software. For each subject torques were normalized to the mean torque of the $+10°$ angle-torque loops at $0°$ wrist angle.

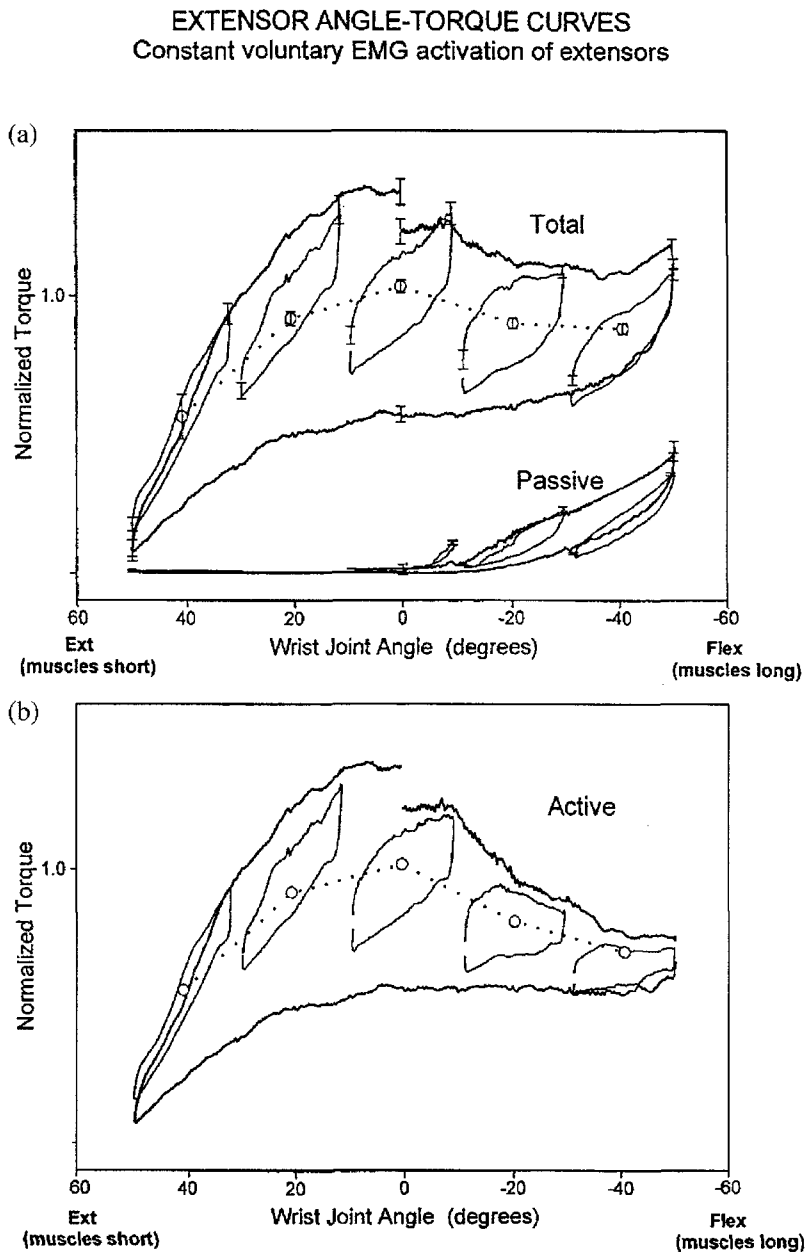

Figure 2.3. Mean angle-torque data for constant (10% maximal), voluntary activation of wrist extensors. Trials and data presentation identical to those in Figure 2.2. Compared to the flexor data, total isometric torque declined more noticeably as the wrist was flexed beyond neutral (i.e. as the extensors lengthened). There was also a decline in torque over this angular range in full ROM trials. These declines were particularly marked in Figure 2.2B, after the passive angle-torque data had been subtracted. In spite of these descending limbs, the slopes of smaller $\pm 10°$ loops remained positive.

2.3 Results

Full ROM trials involving voluntary control of flexor EMG produced monotonic angle-torque curves for active lengthening characterized by an initial steep slope, followed by a less steep slope (Fig. 2.2A). At the onset of shortening there was an initial steep drop in torque followed by a less steep decline. $\pm 10°$ trials also showed monotonic angle-torque curves, but notice the greater mean slopes, i.e. larger stiffnesses than would be predicted either from the isometric or full ROM curves. The isometric data show a classical length-tension profile: an ascending limb, a descending limb and a small increase at the longest muscle length. All angle-torque movement trials with voluntary flexor contractions showed prominent hysteresis, torque being larger during lengthening of the active muscles than during shortening. The passive angle-torque curves also exhibited some hysteresis, but far less than the active curves. Passive hysteresis is attributable to connective tissue properties (Given *et al.*, 1995).

In our first subject full ROM cycles were applied in one continuous sweep, but fatigue near the end of each trial, evidenced by mismatches between start and end coordinates of angle-torque curves, led us to split full ROM trials into three portions in the other subjects (see Methods). Good correspondence between start and end coordinates in most trials indicated that fatigue was thereby minimized. Figure 2.2B was obtained by subtracting passive angle-torque curves in Figure 2.2A from corresponding total torque curves. This standard procedure provides an estimate of the active angle-torque profile and emphasizes the descending limb in isometric and full ROM trials.

In contrast to the flexor results, in full ROM trials of wrist extensors (Fig. 2.3), the total angle-torque curve during muscle lengthening showed a clear transition from an ascending limb to a descending limb with a final small increase in torque at longest muscle length. Thus, although the

full ROM plot had a zone of negative stiffness during muscle lengthening, stiffness was always positive in the $\pm 10°$ trials. A prominent hysteresis was again evident in both the full ROM and $\pm 10°$ trials. The active angle-torque plots (Fig. 2.3B) showed similar properties to those in Figure 2.2B, though with more prominent descending limbs in isometric and full ROM curves.

Electrical stimulation of median nerve proximal to the elbow elicited reliable contractions of the wrist flexors in all subjects. Unfortunately it was much more difficult to find equivalent motor points over radial nerve proximal to the elbow. Seven people were tested but only one had an accessible and selective wrist extensor motor point. We felt it essential that the motor point be proximal to any part of the muscles being stimulated, so only this one subject was tested for this part of the experiment.

The flexor stimulation angle-torque curves (Fig. 2.4) showed the same general characteristics as those in voluntary trials (Fig. 2.2). This shows that whether the muscles are activated at a constant level by electrical stimulation or by voluntary effort, the same general conclusions may be drawn.

Since only one subject was tested with extensor stimulation, the same experiment was done on two separate occasions to obtain enough data for averaging. Again the results were comparable to the voluntary trials (Fig. 2.3), though the full ROM did not exhibit the descending limb of the angle-torque curve as clearly and maximal torque occurred at a more flexed angle than in isometric trials. As in Figure 2.3, the $\pm 10°$ trials showed little evidence of negative stiffness.

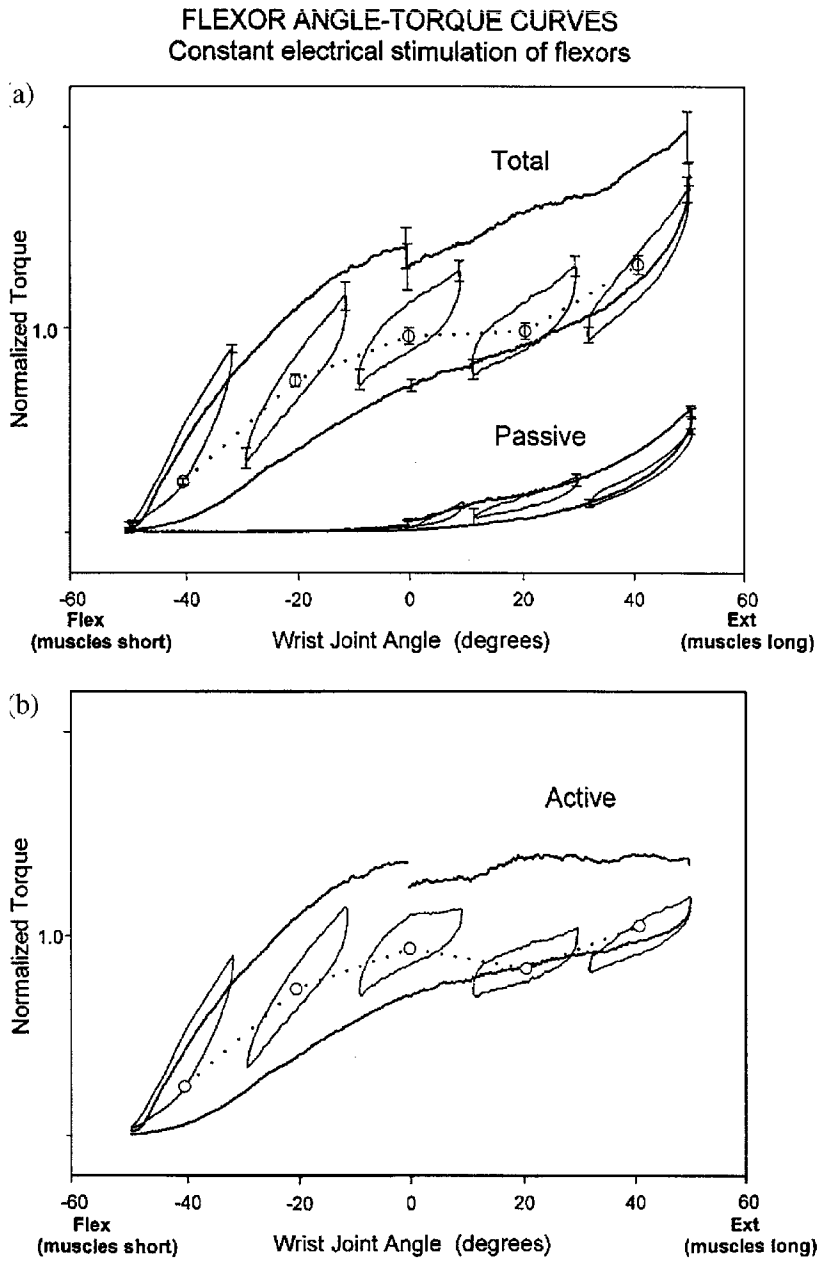

Figure 2.4. Mean angle-torque data for constant (10% maximal), electrical activation of wrist flexors. Stimulation was applied to the muscle nerve above the elbow to minimize the effect of changes in muscle length and shape on the position of the motor point. Trials and data presentation as in Figure 2.2. The properties of the angle-torque curves were basically the same as those in Figure 2.2, which indicates that the muscles were activated similarly in both situations.

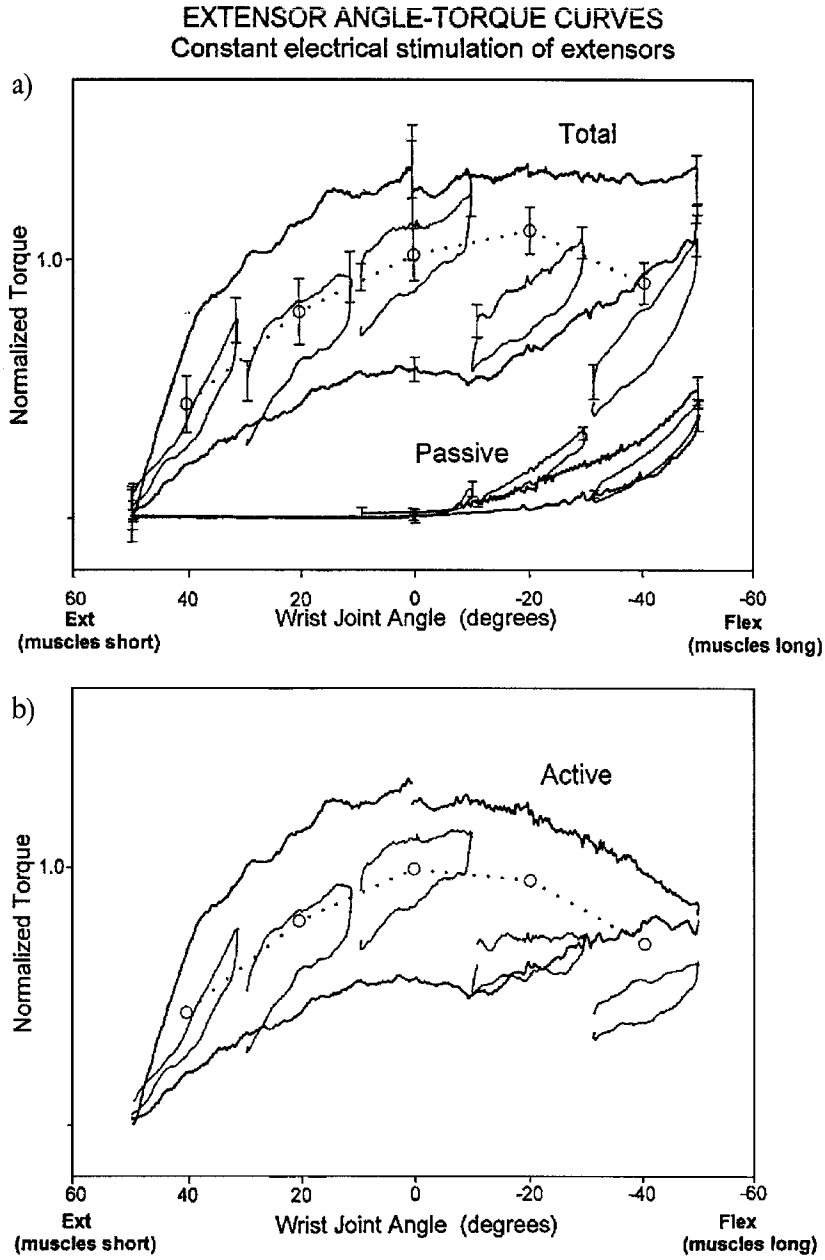

Figure 2.5. Mean angle-torque data for constant (10% maximal), electrical activation of wrist extensors Trials and data presentation as in Figure 2.3. The properties of the angle-torque curves were similar to those in Figure 2.3, indicating that the muscles were activated similarly in both situations. In this case the data could only be obtained in one subject, because only this subject had an accessible motor point for extensor stimulation proximal to the elbow.

In order to explore how much of the separation between lengthening and shortening curves in movement trials was due to static hysteresis as opposed to viscosity, we performed a series of $\pm 10°$ trials at different velocities in one subject. Figure 2.6 shows the maximal difference in torque (δT) within the angle-torque loops of averaged cycles plotted against angular velocity. δT is the sum of the components of force due to viscosity and hysteresis, so there is a steady increase in δT with velocity. The regression line, which intercepts the y-axis above zero provides a measure of the separation of the angle-torque loops that can be attributed to static hysteresis alone.

2.4 Discussion

Our study was originally motivated by Loren et al. (Loren *et al.*, 1996) who predicted that isometric angle-torque curves of active wrist extensors would differ from those of flexors. The study developed into a test of the more general hypothesis that isometric length-tension curves of active wrist muscles are also valid in continuous movements. In agreement with Lieber et al. (Lieber *et al.*, 1994; Lieber & Friden, 1998) we found that in isometric and full ROM trials, active wrist flexor torque increased monotonically with extension whereas active extensor torque followed the classical isometric length-tension curve with an ascending limb, a plateau region, a descending limb and a final ascending limb.

The striking difference was that when $\pm 10°$ cyclical movements were imposed, stiffness during extensor activation remained positive at all wrist angles, including the range in which isometric and full ROM trials showed descending limbs. Stein et al. (Stein *et al.*, 1999) reported similar observations in electrically-stimulated quadriceps muscles in humans but not in voluntary contractions.

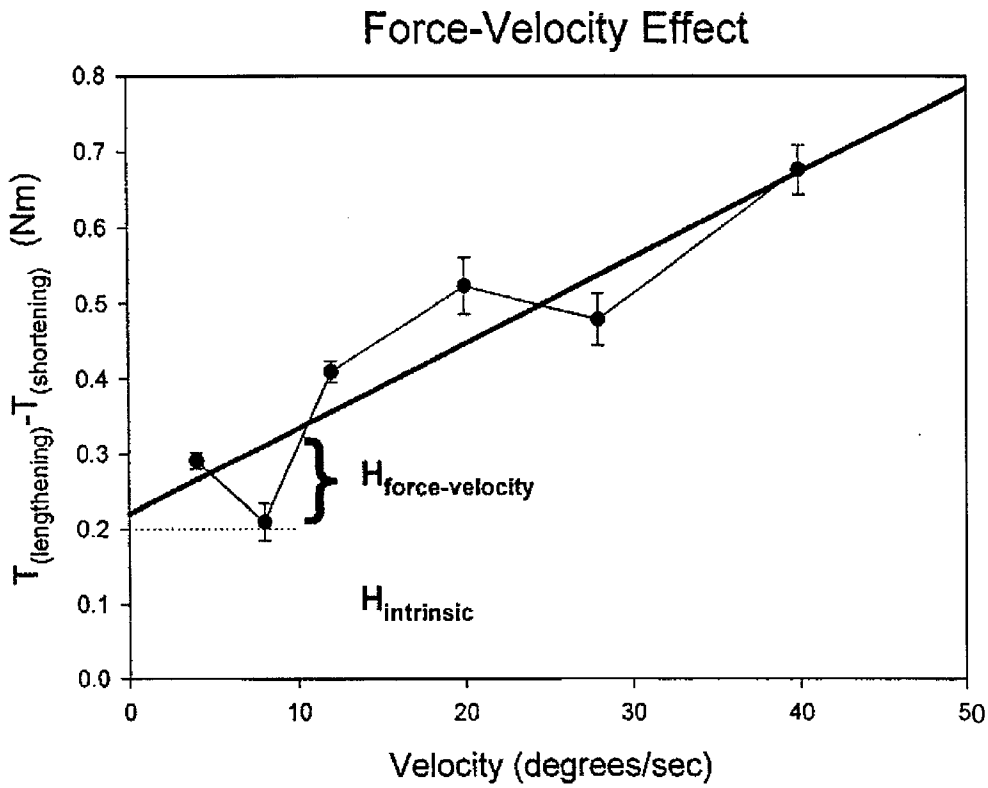

Figure 2.6. Effect of muscle force-velocity properties on the width of the ±10° ROM loops. The maximal difference in torque (δT) within the loops is plotted against angular velocity. δT is the sum of the components of force due to the force-velocity relationship and hysteresis. The force-velocity contribution to δT is reflected in the linear increase in δT with velocity. The regression line intercepts the *y*-axis above zero. This provides a measure of the separation of static hysteresis due to muscle short-range stiffness properties.

We measured angular displacement and torque about the wrist rather than muscle length and force. If angle were proportional to muscle displacement and torque were proportional to force, the angle-torque profiles would be identical to the muscle length-tension curves. However, wrist torque is the sum of torques of several muscles, each of which has a slightly different moment arm. The larger the moment arm, the greater the muscle length change for a given change in joint angle. This means that each muscle might traverse a different region of its length-tension curve. Furthermore, torque is only proportional to muscle force if the moment arms remain constant. Horii et al. (Horii *et al.*, 1993) and Loren et al. (Loren *et al.*, 1996) estimated the moment arms of the main wrist muscles: FCR, flexor carpi ulnaris (FCU), extensor carpi ulnaris (ECU), ECRB and extensor carpi radialis longus (ECRL) from the slopes of the relationship between wrist angle and muscle length in cadavers. According to Loren et al, the moment arm of FCU (generating 50-60% of flexor torque) decreases by 35% as FCU lengthens from full wrist flexion to extension, but Horii et al. concluded that it increases by ~15%. The corresponding values for FCR moment arms were a 12% decrease (Loren *et al.*, 1996) and a 20% increase (Horii *et al.*, 1993). From Loren et al., the reduction in flexor stiffness we observed as the wrist extended (Fig. 2.2) could therefore be due to declining flexor moment arms (not just muscle length-tension properties), but from Horii et al. we could conclude that active muscle force declined with increasing stretch.

Regarding extensors, according to Loren et al. ECRB (~60% of extensor torque) moment arm increases slightly (~10%) as the wrist is moved from full extension, but then declines by ~50% to full flexion. Horii et al. estimated a linear decline of ~13%. For ECRL (20-25% of extensor torque), Loren et al. estimated a large decline in moment arm, while Horii et al. found only a small (~6%) decline restricted to the last 30° of flexion. ECU (20-25% of extensor torque) moment arm

showed a bell-shaped profile peaking in the mid-range according to Loren et al., while Horii et al. maintained that it changed little from extension to mid-point, then declined 75% to full flexion.

Though these results vary in detail, the common factor is a general decline in extensor moment arm with increasing flexion, particularly beyond the mid-point of wrist angle. This could partly explain the descending limbs of isometric and full ROM angle-torque profiles in our data (Fig. 2.3). But the important point remains that when $\pm 10°$ movements were imposed within the angular range of the descending limb of the isometric and full ROM curves, mean wrist stiffness was positive rather than negative, in spite of possible declines in moment arms. The prediction from the isometric angle-torque relationship of a region of negative stiffness is therefore clearly invalid for small dynamic movements. Given that in activities of daily life, the wrist normally operates over the range 10° flexion to 35° extension (Brumfield & Champoux, 1984), isometric curves, with their descending limbs, do not represent normal muscle behavior.

The marked difference in torque during lengthening and shortening of active muscles, which resulted in the loops seen in all the continuous angle-torque plots, took us by surprise, because we had chosen very low angular velocities to minimize viscous forces. When we examined this further (Fig. 2.6), it became clear that the loops resulted more from static hysteresis than viscosity. In most of the $+10°$ loops the hysteresis was attributable to abrupt changes in torque at the onset of muscle lengthening and shortening which suggests short-range stiffness effects (Rack & Westbury, 1974). However the maintained separation of ascending and descending portions of the loops throughout the cycles is not explained by short-range stiffness. Lombardi and Piazzesi (Lombardi & Piazzesi, 1990) adduced evidence that cross-bridge cycling rates in frog muscle fibers are higher during lengthening than during shortening contractions and other work suggests that the average force produced for each attached cross-bridge is higher in lengthening than in isometric

contractions (Herzog, 1998). These mechanisms may explain the maintained separation of ascending and descending limbs of angle-torque curves. According to Nardone et al. (Nardone *et al.*, 1989), fast and slow motor units of human calf muscles are recruited selectively in voluntary lengthening and shortening contractions respectively. This is another possible cause of hysteresis, though we would then have expected to see larger differences between voluntary and electrical stimulation data.

Stein et al. (Stein *et al.*, 1999) applied velocities of 24°/s to the knee and so the component of viscous stiffness was probably quite large (see our Fig. 2.6). Nonetheless, some static hysteresis was inferred. Because this reached a maximum at maximal muscle length, sarcomere nonuniformities were suspected, the notion being that some sarcomeres "pop" to a length beyond which cross-bridges can form and in-series sarcomeres shorten to compensate (Julian & Morgan, 1979b). When muscle is allowed to shorten after a large lengthening contraction, its ability to sustain force is therefore reduced (Gordon *et al.*, 1966; Edman & Reggiani, 1984; Morgan, 1990). In our study, the fact that the $\pm 10°$ loops were actually thinner at long than at intermediate lengths detracts from this explanation. Furthermore, our hysteresis loops were symmetrical: increases in torque at the onset of lengthening were similar to decreases in torque at the onset of shortening. Sarcomere nonuniformities would operate chiefly at one end of the loop: at the onset of shortening after a large lengthening contraction. Stein et al. (Stein *et al.*, 1999) reported that the hysteresis was not present in voluntary activation. This was not the case in our study: if anything, hysteresis was more marked in voluntary trials than in stimulation trials. Gielen et al. (Gielen *et al.*, 1984) measured the elastic and viscous properties of reflexly active wrist muscles in humans. Subjects were instructed "not to intervene" when force or displacement applied to the hand during voluntary contractions against a steady load was altered. There was a prominent static hysteresis

which the authors attributed to short-range stiffness rather than reflex action. Our results support this interpretation.

In summary, although we confirmed the claim of Lieber et al. (Lieber *et al.*, 1994) and Loren et al. (Loren *et al.*, 1996) that active wrist extensors but not flexors showed descending limbs in their isometric angle-torque curves, in small continuous movements, stiffness remained positive throughout. We conclude that isometric length-tension curves of active wrist muscles are not representative of continuous movements. Hysteresis in angle-torque curves was attributed to short-range stiffness and stretch-potentiation rather than to sarcomere nonuniformities.

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CHAPTER 3

Contribution of stretch reflexes to locomotor control: a modeling study.

Adapted from an original publication:

Yakovenko S, Gritsenko V, and Prochazka A.
Biological Cybernetics 90: 146 - 155, 2004.

3.1 Introduction

At an international symposium on Movement and Sensation held in Cairns, Australia in 2001 we made the provocative suggestion that stretch reflexes do not contribute substantially to load compensation in mammalian locomotion (Prochazka *et al.*, 2002). We made this suggestion on the basis of the relatively small size and long latency of electromyogram (EMG) responses that occur after ground contact in the stance phase of the locomotor step cycle. Some initial biomechanical modeling we had done indicated that these responses may only have a modest effect on the kinematics of quadrupedal gait.

Classical studies demonstrated many years ago the ability of the spinal cord to produce the basic locomotor rhythm in the absence of sensory feedback (Brown, 1911). Brown coined the term "intrinsic factor" to describe the underlying neural mechanism and more recently Grillner renamed this the "central pattern generator" (CPG) (Grillner & Zangger, 1974). The fact that under some circumstances rudimentary weight-bearing locomotion can occur in the absence of sensory input indicates that the biomechanical properties of the limbs provide some flexibility in load compensation (Pearson *et al.*, 2003). However, studies on animal and human subjects have also shown that after sensory loss gait is far less coordinated and less able to adapt to changes in terrain and body posture (Bickel, 1897; Lajoie *et al.*, 1996; Bloem *et al.*, 2002). Thus, two main roles are usually attributed to sensory feedback: it provides control of the stiffness of individual muscles and it allows higher-level control of balance, stability and coordination.

Stretch reflexes associated with locomotion, on which we will concentrate in the following discussion, have been extensively studied with a variety of physiological techniques for over a century. Most of the studies have dwelt on the electrical responses of muscles to electrically-evoked sensory inputs or to imposed muscle stretching and shortening. The amplitude of sensory-evoked

EMG responses is modulated throughout the step cycle, (Akazawa *et al.*, 1982; Capaday & Stein, 1986; Dietz *et al.*, 1990; Stein *et al.*, 2000) so that contribution of stretch reflexes has been posited to be highest when the receptor-bearing muscle is active. Estimates of the relative contribution of stretch reflexes to overall muscle EMG during locomotion are in the range 25% -35% (Yang *et al.*, 1991; Bennett *et al.*, 1996; Stein *et al.*, 2000). But if the sensory input due to ground contact is removed at the onset of the stance phase of cat locomotion ("foot-in-hole" experiments), changes in EMG appear surprisingly late (30-40ms) (Gorassini *et al.*, 1994). In another set of experiments, stretch of the ankle extensors after ground contact was artificially exaggerated (Gritsenko *et al.*, 2001). This confirmed the timing of the stretch reflexes and further emphasized the modest size of the reflex components in unperturbed steps.

So what is the functional role of the stretch reflexes during locomotion? After recovery, de-afferented animals show little change in yield of the limb during the stance phase of level overground locomotion, though in more demanding tasks abnormally large yielding can occur (Abelew *et al.*, 2000). However, in experiments of this type it could be that compensatory changes in central drive learnt over a period of time may replace the missing stretch reflex components and thereby mask the role of these reflexes under normal conditions. The ideal experiment would be to study locomotor performance when sensory input is suddenly abolished. It is hard to see how this manipulation could be done in real animals, but it is certainly possible in biomechanical simulations. We have used these in this study to test the following two hypotheses. 1) Stretch reflexes are too weak and too delayed to contribute significantly to weight-bearing. 2) The important contributions of sensory input involve state-dependent processing.

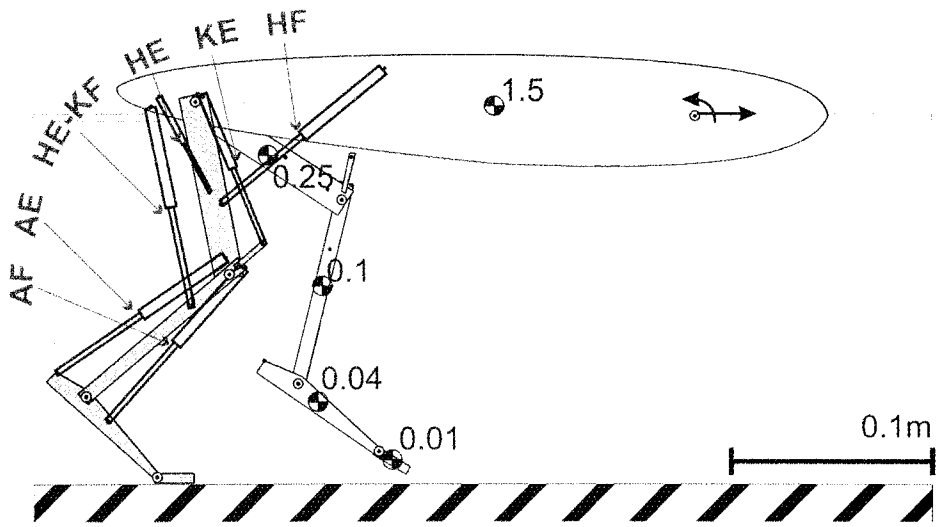

Figure 3.1. Structure of neuromuscular locomotor model. A musculo-skeletal model with segment lengths and weights (indicated next to segment centers of mass) based loosely on the hindlimbs of cat. Muscle groups and their origins and insertions are represented by the following musculotendon actuators HF (hip flexors), HE (hip extensors), KF-HE (bifunctional muscle with knee flexor and hip extensor function), KE (knee extensors), AF (ankle flexors) and AE (ankle extensors). Shoulder joint is constrained to 2 degrees of freedom, horizontal translation and rotation.

3.2 Methods

3.2.1 Structure of the locomotor model

We developed a planar locomotor model of two hind limbs attached to a horizontal torso supported at the front by a frictionless wheel (Fig. 3.1). Each hindlimb comprised 4 rigid-body segments (thigh, shank, foot and toes) which were driven by 6 musculo-tendon actuators. All joints were modeled as frictionless revolutes. The model was developed using Matlab version 6.1 software (The MathWorks, USA) coupled to Working Model 2D version 5 software (Knowledge Revolution, USA). The foot interactions with the ground were modeled with the use of Working Model software. The following parameters were chosen to minimize slipping: the coefficient of restitution was set to 0.1 and the coefficient of Coulomb friction to 10. The results were computed using the Kutta-Merson numerical integration method with 0.1 msec fixed steps and 1/100 mm error tolerance.

In the model to be presented, the dimensions, masses of the segments and moment arms of the actuators were chosen approximately to mimic those of a cat. All simulations were started just prior to foot contact, with initial velocity of the trunk and leading leg segments set to 0.7m/s. We intentionally did not strive for a rigorously accurate model as we were seeking conclusions that would generalize across a large range of parametric variation (see Discussion). Internal properties of the individual actuators were modeled by Hill-type force-velocity (Hill, 1938) and linear force-length (Gillard *et al.*, 2000) relationships. Muscle force was calculated as

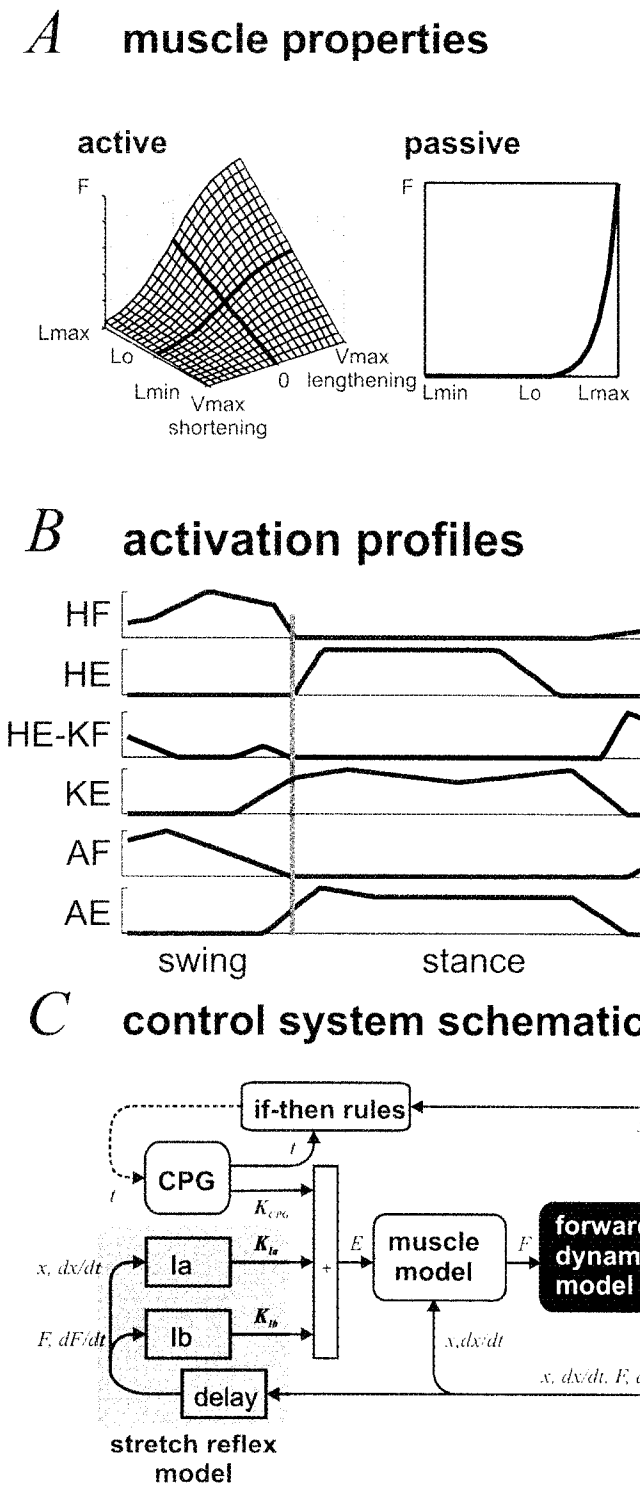

Figure 3.2. **a** Active and passive force-length-velocity relationships used to model muscle properties of actuators. **b** EMG profiles used as CPG outputs to the corresponding musculotendon actuators. **c** Schematic of the organization of control system in our model.

$$F = F_{\max} \cdot f_v \cdot f_l \cdot a_t + F_{\max}^{passive} \cdot f_l^{passive} \qquad \text{Eq. 3.1}$$

where F is muscle force, F_{max} is the maximum isometric force, f_v and f_l are the force-velocity and the force-length relations respectively, $F_{\max}^{passive}$ is the maximum passive force, $f_l^{passive}$ is the normalized passive force-length relation (Fig. 3.2a) and a_t is muscle activation, whose dynamics was described by He-Zajac-Levine excitation-contraction coupling equation (He *et al.*, 1991):

$$\dot{a}_t + \frac{1}{\tau_{act}} \cdot \left(\frac{\tau_{act}}{\tau_{deact}} + \left[1 - \frac{\tau_{act}}{\tau_{deact}}\right] \cdot u_t\right) \cdot a_t = \frac{1}{\tau_{act}} \cdot u_t \qquad \text{Eq. 3.2}$$

where τ_{act}, τ_{deact} are activation and deactivation time constants (20 msec and 40 msec, respectively) and u_t is motoneuron excitation described by Eq. 3.6.

Electromyographic (EMG) profiles of the simulated muscle groups during slow level walking (Fig. 3.2b) served as CPG outputs to the musculotendon actuators. Swing and stance durations were set to 30% and 70% of the step cycle period respectively, which is an appropriate phase relationship for slow gait with a cycle period of 600ms (Goslow *et al.*, 1973; Halbertsma, 1983). The activation profiles were based on a large number of locomotor studies and are described in Chapter 4.

3.2.2 Parametric testing

A direct dynamic approach was applied to compute kinematics and to study the contribution of different types of sensory feedback to motor control of the musculoskeletal system. Instead of using

dynamic optimization methods with set performance goals for certain types of locomotion, we chose to study performance of the model in parametric space, constructed from combinations of stiffnesses of the musculotendon actuators (Fmax in Eq. 3.1). A random search method was used to determine "stable" regions of parametric space, defined as the ability of the model to locomote for at least 12 seconds (equivalent to about 20 steps). Principal component analysis (PCA) was further performed on the "stable" sets. To quantify locomotor stability, we computed the moment of inertia for the "stable" volume defined by the sets of force parameters in Fig. 3.4 using the following formula:

$$I = \sum_{i}^{n} \frac{r_i^2}{n} \qquad \text{Eq. 3.3}$$

where r_i is the vector magnitude defining distance from the center of the "stable" volume to each individual parameter set and n is the total number of parameter sets (100 "stable" simulations). Locomotor performance was analyzed in the plane formed by the two principal component vectors that accounted for most of the variability in the data.

The analysis was repeated in the model where the length of the ankle was increased to match the relative segment lengths of a horse. The reason for repeating the analysis in the "horse" model was to test whether our basic conclusions held in the face of large changes in limb geometry (Fig. 3.1). The length of the ankle relative to the femur and tibia in the horse is double that in most other mammals.

3.2.3 Stretch reflex model

The stretch reflex model comprised simulated feedback from spindle Ia and tendon organ Ib afferents onto homonymous motor pools. Heterogeneous excitation and reciprocal inhibition components of afferent feedback, described by Lundberg, (Engberg & Lundberg, 1969) were neglected for the sake of simplicity. The contributions of Ia and Ib afferents to the output of homonymous motoneuron pools were represented by the following formulae derived from the literature (Prochazka, 1999).

Ia model:

$$f_{Ia}(l,v) = K_{Ia} \cdot (21 \cdot v^{0.5} + 200 \cdot l + 60) \qquad \text{Eq. 3.4}$$

Ib model:

$$f_{Ib}(s,F) = K_{Ib} \cdot \frac{(s+0.15)\cdot(s+1.5)\cdot(s+16)}{(s+0.2)\cdot(s+2)\cdot(s+37)} \cdot F \qquad \text{Eq. 3.5}$$

where f_{Ia} is a time function of the Ia afferent firing rate response to changes of muscle length and velocity, l is the muscle length in rest length units, v is the muscle velocity expressed in rest length per second, f_{Ib} is the tendon organ response in the frequency domain, s is a frequency domain operator and K_{Ia} and K_{Ib} are gain coefficients. The middle of the range of motion of each musculotendon actuator was chosen as its rest length. Note that the above equations are in the time and frequency domains respectively. Though equation 3.3 can be written as a differential equation in the time domain, its filtering properties are then very difficult to infer. Inspection of equation 3.3 indicates that tendon organs act as high pass filters, with 20 dB/decade increases in transmission occurring between the following pairs of frequencies: 0.15-0.2, 1.5-2, 16-37 rad/s (i.e. ~0.02-0.03,

0.2-0.3 and 2.6-5.9 Hz). Matlab Simulink allows a mixture of time and frequency domain transfer functions within the same model.

The Ia and Ib reflex feedback contributed to the muscle contractile force with a latency of 35ms (Gorassini *et al.*, 1994; Gritsenko *et al.*, 2001). This feedback was active only when the CPG EMG profile for the receptor-bearing muscle was non-zero to satisfy the known modulation of the stretch reflex within the step cycle (Capaday & Stein, 1986). The gain coefficients K_{Ia} and K_{Ib} were adjusted so that the Ia and Ib signals each added a mean of 15% to the CPG EMG component throughout a full locomotor cycle. Thus, together both signals contributed 30% to the CPG EMG profiles, which is in accordance with the suggested reflex contribution to the EMG during walking (Bennett *et al.*, 1996; Stein *et al.*, 2000). When the stretch reflex component was present, the excitation function in Eq. 3.2 was of the form:

$$u_t = CPG_t + f_{Ia} + f_{Ib} \qquad \text{Eq. 3.6}$$

3.2.4 Finite-state rules

The following IF-THEN rules were used to model higher level control of phase–switching in the step cycle (Granat *et al.*, 1993; Prochazka, 1993):

1. Stance to swing transition: IF stance AND ipsilateral hip is extended AND ipsilateral leg is unloaded THEN swing;
2. Swing to stance transition: IF swing AND ipsilateral hip is flexed AND ipsilateral knee is extended THEN stance.

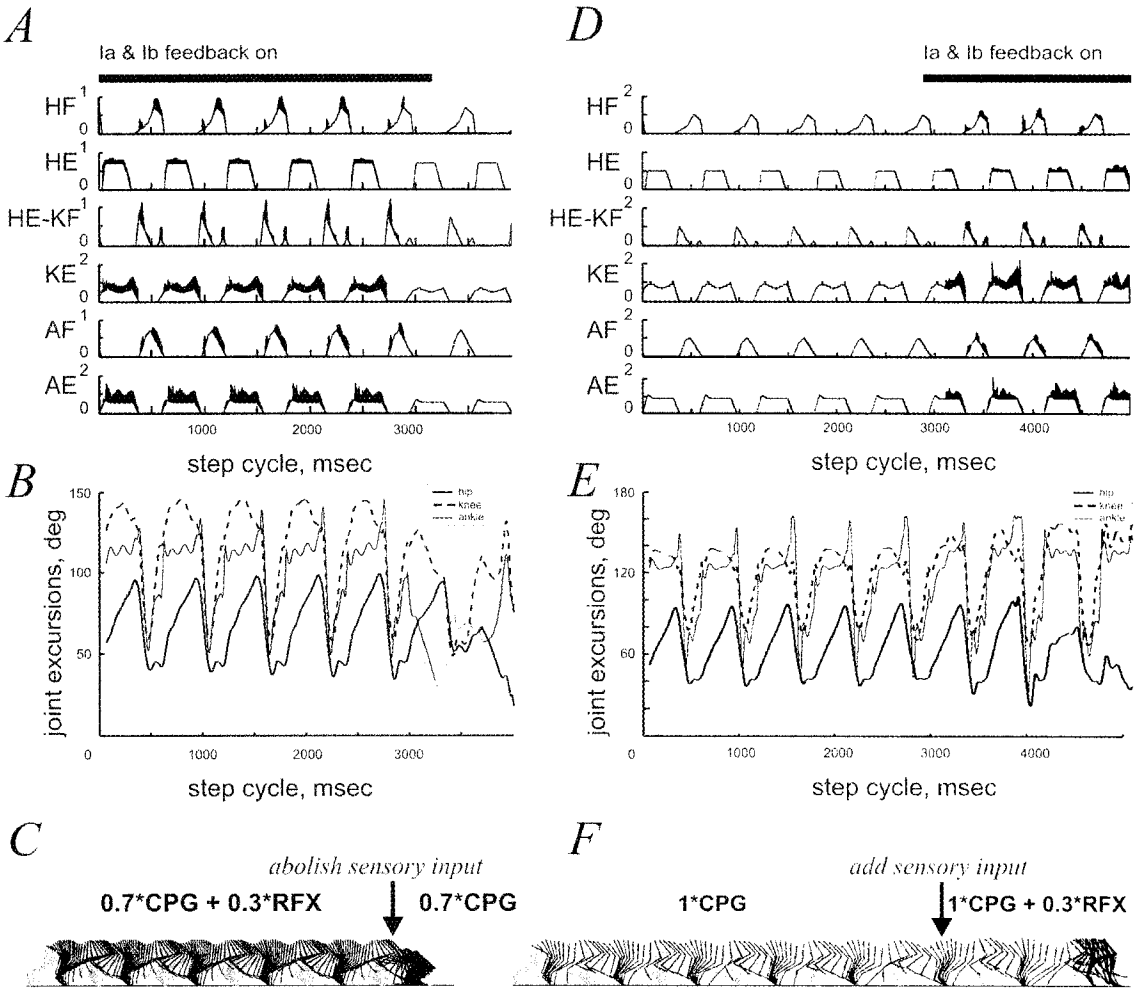

Figure 3.3. Contribution of stretch reflexes during locomotion. Two simulation results are shown in the *left* and *right* panels. **a—c** Centrally-generated levels of muscle activation were not quite high enough to support stable locomotion. Stretch reflex contributions comprising 30% of the total activation (*black* portions of the activation profiles) were initially present and then suddenly removed. **d—f** Centrally-generated levels of muscle activation were sufficient to support stable locomotion; stretch reflex contributions (*black* portions of profiles) were initially absent and then suddenly added. Stick figures of **c** indicate that the stretch reflexes are crucial to maintain stable locomotion when CPG activation alone is insufficient to support load bearing. However, in a trial with sufficient CPG activity (**f**), the addition of stretch reflexes only had the effect of slightly increasing gait velocity, which eventually led to collapse (see supplementary material).

The thresholds for the firing of these rules were determined on a trial-and-error basis. The schematic of all control systems implemented in the model are shown in Fig. 3.2c.

3.3 Results

3.3.1 Simulation of "deafferentation" experiment

Figure 3.3 illustrates two examples of the type of experiment mentioned in the Introduction, where a sudden change in sensory feedback occurs during locomotion. In the left panels (Fig. 3.3a—c) sensory feedback was suddenly withdrawn, whereas in the right panels (Fig. 3.3d—f) sensory feedback was suddenly added. In both cases the sensory inputs contributed about 30% to the overall muscle activation profiles as described above. In the example on the left the amplitudes of the centrally generated components were deliberately chosen to produce forces that alone would be insufficient for weight-bearing, whereas in the example on the right the CPG activation levels were chosen to be sufficient to produce stable locomotion. In the first case, withdrawal of the stretch reflex contribution led to a collapse of the model, from which one would conclude that stretch reflexes are crucial to maintain stable locomotion. This would refute the suggestion we made in Cairns (hypothesis 1 above) that stretch reflexes are too weak and too delayed to contribute significantly to weight-bearing. But in the second case, when the levels of the CPG muscle activation profiles were 30% higher, i.e. enough to support body weight, the sudden addition of the stretch reflexes caused only a modest increase in the rate of locomotion and often decreased stability of the model. From this we would conclude that stretch reflexes had a rather minor role, which supports hypothesis 1. Thus it seems that the importance of stretch reflexes depends on whether central drive is well matched to the anticipated loads or not.

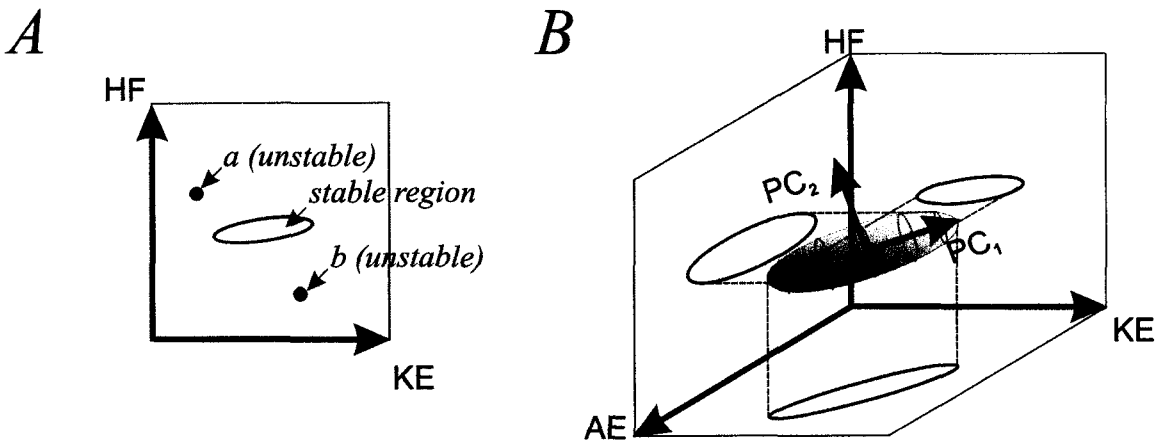

Figure 3.4. Schematic explaining how Principal Component Analysis (PCA) was used to evaluate the locomotor stability over a wide range of parameter variations. **a** Hip and knee forces must lie within the ellipse for stable locomotion to occur. The larger the ellipse, the greater the range of parameters for which locomotion is stable. **b** When another parameter is added, (in this case ankle extensor force), the stable region lies within a three-dimensional volume. The larger the volume the greater the range of stability. Orthogonal axes can be identified mathematically that point in the directions of largest variations of the coordinates within the volume. Unit vectors along these axes are called Principal Components. PCA may be extended to any number of parameters, in our case 6, corresponding to the 6 actuators in our model. PCA allowed us to compare the sizes of 6-dimensional stability "volumes" for different types of control. Since the first and second principal components described on average 90% of all variability in the stable parameters, the two-dimensional plots of Figures 3.4 provide nearly all of the information required to compare stability between control schemes over a large range of parameter variation.

To gain a better understanding of the range of central drive over which stretch reflexes would have a major versus a minor role, and also to test the validity of our conclusions in the face of parametric variations in both muscle stiffness parameters and limb geometry, we examined changes in the parametric space defined by forces of musculotendon actuators in the two models. Because six actuators were used in each limb in the models, parametric space was six-dimensional and therefore hard to represent with conventional Cartesian plots. However it is possible to simplify this representation by grouping variations in the six force parameters into "principal components." This is illustrated schematically in Fig. 3.4. First let us consider the effect on locomotion of changes in the peak forces of just two of the six actuators (hip flexors and knee extensors: Fig. 3.4a). Clearly if hip flexion is too forceful (point "a") the leg will overshoot, and locomotion will be destabilized. If there is too little hip flexion coupled with forceful knee extension (point "b"), locomotion will also be unstable. Only certain combinations of the two parameters will be compatible with stable gait. Suppose that these are contained within the shaded ellipse. Now let us add ankle extensors (Fig. 3.4b). Again there will be a restricted range of peak ankle extensor force compatible with stable locomotion. When combined with the stable combinations of the other two variables, the stable region may now be represented by the shaded volume in the 3-dimensional plot. By calculating eigen vectors and eigen values for the data autocorrelation matrix it is possible to define orthogonal axes along which the parameter combinations making up the volume are the most spread out. These axes thus "account for" the largest amount of variation in the data and are called the "principal components (PC)." Principal Component Analysis (PCA) can be extended to all six parameters.

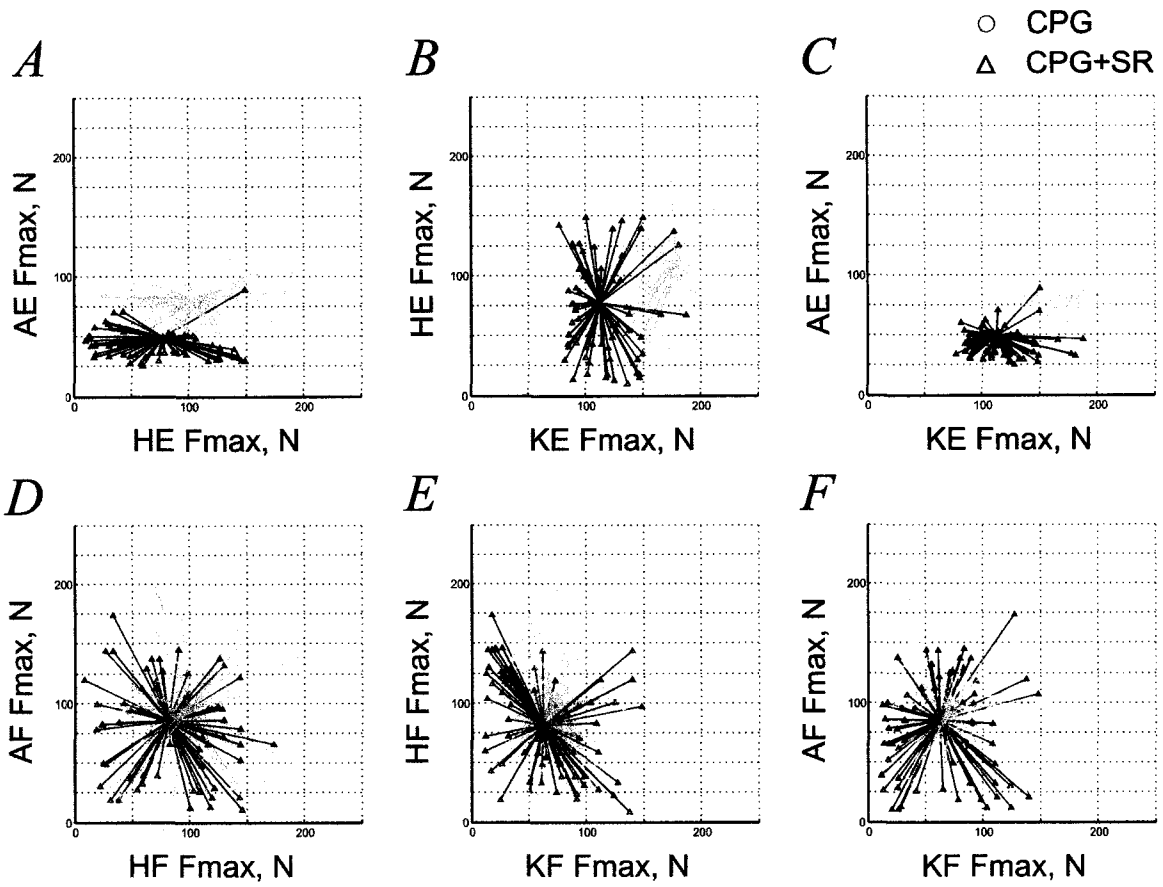

Figure 3.5. Locomotor performance plots of stable trials of models without sensory feedback (***gray circles***) and with stretch reflex (***black upward triangles***) are plotted as projections on the planes of three extensor (**a—c**) and three mainly flexor (**d—f**) musculotendon actuators. Horizontal and vertical axes represent maximum isometric force at muscle rest length (Fmax in Eq. 3.1) of the corresponding musculotendon actuator. The large region of stability of the model without sensory feedback indicates that the intrinsic stiffness of the muscles sufficed to compensate for kinetic and kinematic variations over a fairly large parametric range. Since the origins of the coordinate systems corresponds to a simulation with zero muscle stiffnesses, the minimum vector to the stable region corresponds to the minimum muscle stiffnesses required for locomotion. Region of stability of the model with stretch reflexes extends closer to the origin compared to the one without sensory contribution (**a—c**), i.e. when stretch reflexes contribute 30% of overall extensor muscle excitation, less central activation is required to maintain stable locomotion. However, largely overlapping stability regions of flexors (**d—f**) indicate that stretch reflexes are not essential for flexor activity without additional kinematic constraint.

3.3.2 Locomotor performance in the absence of sensory feedback

Locomotor performance was explored in six dimensional parameter space of musculotendon actuator forces using a gradient-descent method with random initial position. Figure 3.5 shows sets of parameters associated with stable locomotor performance of the model driven by the CPG alone (grey circles) and the CPG with the addition of stretch reflexes (black upward triangles). Figure 3.5a—c show the projections of six-dimensional data on the planes formed by the three extensor actuators. Where panels D, E and F show the projections of the same data on the planes formed by the remaining predominantly flexor actuators. The origin of the plots corresponds to zero force in all actuators. It is clear from Fig. 3.5 that stable locomotion can emerge over a fairly large range of force parameter variation, even in the absence of sensory feedback. Similar results have been previously obtained using inverse dynamics or neural networks to optimize activation patterns (Taga *et al.*, 1991; Taga, 1998; Neptune *et al.*, 2001; Ogihara & Yamazaki, 2001).

3.3.3 Effect of stretch reflexes on locomotor performance

Figure 3.5 shows a stability plot for the cat model where stretch reflexes contributed about 30% of the overall activity (see equation 3.6). In Fig. 3.5a—c the volume of stability associated with the model with sensory feedback (upward triangles) extends much closer to the origin than in the "deafferented" model (grey circles). This was also the case for the "horse" model (not illustrated). This indicates that stretch reflexes are capable of increasing extensor muscle stiffness when central drive is low and thus provide the necessary load compensation. In spite of the long latency of the reflexes and their dynamic nature, their 30% mean contribution essentially takes the place of the

missing 30% of central activation. This explains the destabilizing effect of a sudden removal of the stretch reflex contribution in Fig. 3.3a—c where the central activation levels were low. A contribution of stretch reflexes to flexor activity does not seem essential judging by the largely overlapping stable parameter spaces of both models in Fig. 3.3d—f .

In the example in Fig. 3.3d—f the addition of stretch reflexes when central activation was adequate to provide stable locomotion has a destabilizing effect. However, if we consider all the stable simulations obtained, the moment of inertia for the "stable" sets (see Eq. 3.3) of the model with stretch reflexes was 1.9 times larger than that without (I_{CPG+SR}= $5.6*10^4$ N^2, I_{CPG}=$3.0*10^4$ N^2). This indicates that stretch reflexes can contribute significantly to load compensation and taken over the entire parameter space, may even stabilize locomotion in many cases.

3.3.4 Contribution of state-dependent control to locomotor stability

In the stretch reflex modeling above, the cycle frequency of the CPG pattern was invariant. This placed constraints on the range of gait velocities consistent with stability. Adaptive control of cadence based on sensory information about actual biomechanical states might therefore be expected to increase stability. To test this hypothesis we implemented simple sensory rules to trigger the transitions between flexion and extension phases of the centrally generated pattern of muscle activation profiles. Figure 3.6 shows the extent to which If-Then sensory rules controlling step transitions (see Methods) stabilized the simulations in the cat model with CPG and stretch reflexes active. Figure 3.6a and c shows locomotor performance plots in the plane of two first principal components of the stable sets of parameters associated with the model with stretch reflexes in Fig. 3.5. The stability region is further defined by a contour plot, which shows the amount of time that elapsed in simulations before the model collapsed. In Fig. 3.6c the stable

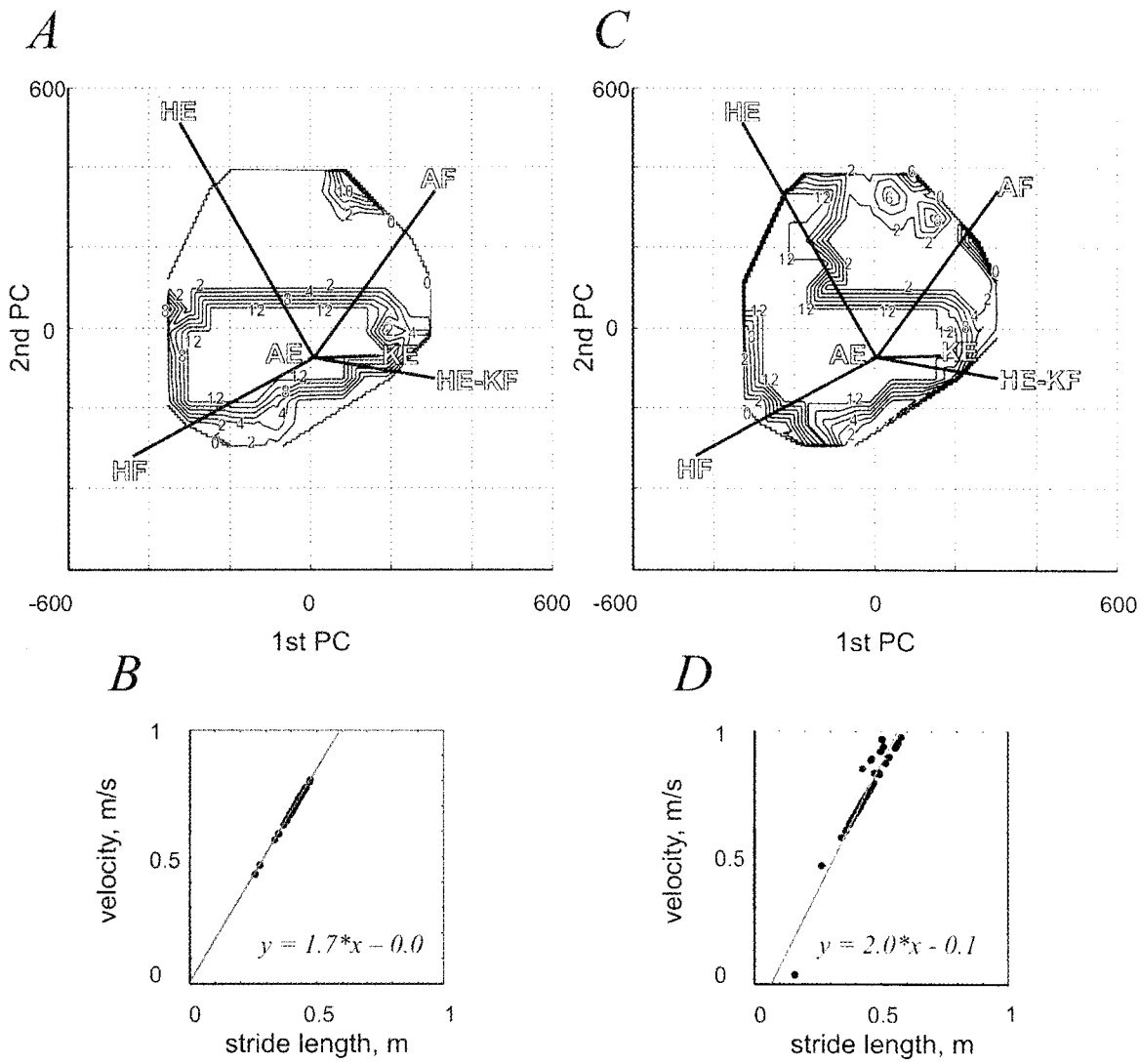

Figure 3.6. PCA locomotor performance plots and plots of mean gait velocity versus stride length of stable trials of models with CPG and stretch reflexes (**a, b**) and with CPG, stretch reflexes and If-Then rules (**c, d**). The *contour* lines connect points of equal stability measured in seconds of stable gait. Region of stability in the model with If-Then rules is about 30% bigger, particularly in the direction of increase of hip extensor force. Labeled ***straight*** lines in **a** & **c** represent projections of the six-dimensional force axes (axis vector magnitude is 250N) on the plane of two first principal components. **b** Velocity was proportional to stride length in stable locomotion because the cycle frequency of the centrally generated pattern was constant in these trials. Stability was confined to a fairly narrow band of velocities. **d** Addition of If-Then rules to the control system shown in **a** and **b** increased the range of stable gait velocities.

region was enlarged by about 30% compared with that in Fig. 3.6a. Note that the main increase of stability coincides with the direction of the increasing force of hip extensor actuator, which regularly led to an increase of forward velocity.

An analysis of the velocities of gait corresponding to the stable and unstable regions showed that stability was associated with a fairly narrow band of gait velocities. Figure 3.6b shows that gait velocity was closely related to stride length for all of the stable simulations. This is not surprising, given that the frequency of the CPG rhythm in all of these simulations was constant. If stride length increases at a constant step cycle frequency, velocity *must* increase, or stability is lost. Consider the case where hip flexor forces are large but body velocity is low. The leg swings far forward and at ground contact the ground reaction force points back, decelerating the body. If velocity drops to zero before the centre of mass moves over the point of support, further forward motion becomes impossible and the model collapses backward. As we shall see, stretch reflexes do not fundamentally change this effect because in essence they just augment the inherent load compensation mechanism provided by muscle stiffness and viscosity (Partridge, 1966). On the other hand, conditional control can increase the range of stable velocities by adapting the cadence (cycle frequency) to the actual kinematics of the limb. The range of velocities of the stable simulations shown in the plot of Fig. 3.6d was greatly increased, showing that adaptive control of cadence granted by If-Then rules was associated with the increased range of stability.

3.3.5 Closed-loop gain of stretch reflex during locomotion

Figure 3.7 shows the dependence of the response of the system on the amplitude of the feedforward commands in our model. It is clear that increases in the central command are

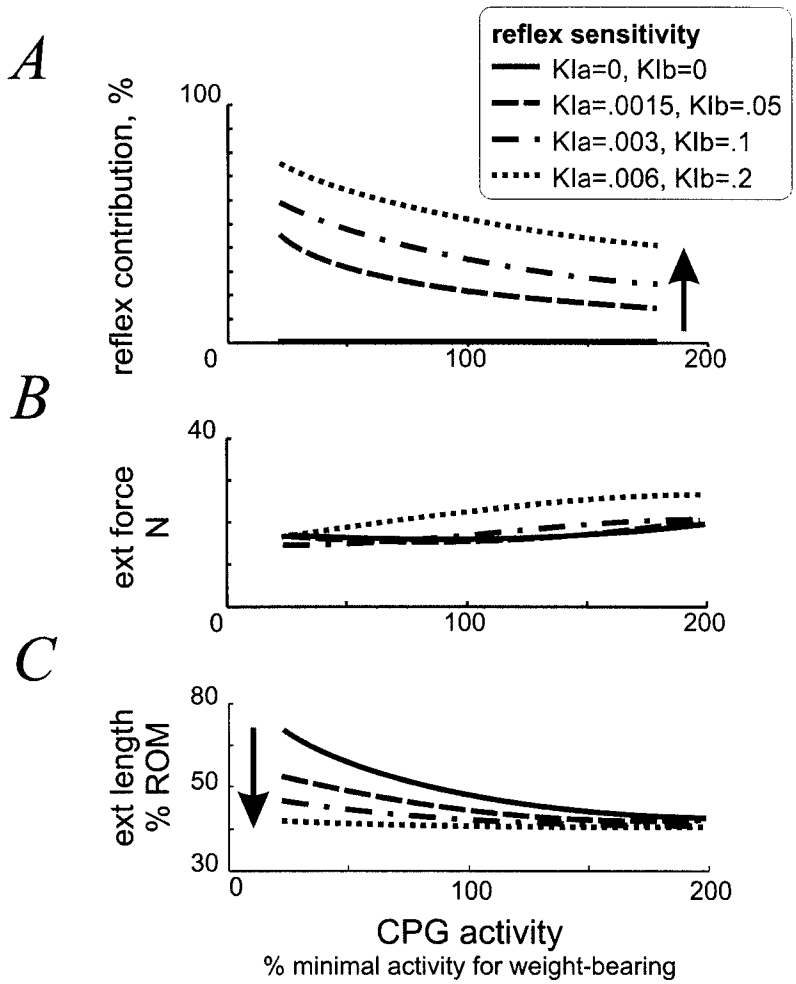

Figure 3.7. Strength of stretch reflexes is modulated by muscle length. **a** Relationship between CPG amplitudes and relative reflex contribution for four sets of reflex gains. Both parameters are normalized to the minimal activity level required to produce stable locomotion in the "deafferented" model. Notice the decrease in the reflex component with the increasing CPG amplitude. **b** Mean extensor force did not change significantly with CPG amplitude except at the highest reflex gains. **c** Mean muscle length decreases with increasing CPG amplitudes. This effectively decreases stretch reflex loop gains (see text).

associated with decreases of the contribution of the stretch reflex loops. This cannot be attributed to changes in the generalized forces because these changes are quite small (Fig. 3.7b). Rather, Figure 3.7c demonstrates that higher activation levels drive the extensor muscles to operate at substantially shorter lengths, which attenuates the closed-loop gains of the length and force feedback loops, thus reducing the relative contribution of reflexes. As has been shown previously (Prochazka *et al.*, 1997), when muscles are short, they respond with a smaller force output to a given motoneuronal input and this in turn attenuates the gains of the feedback loops of which they are a part. Thus, the relative force contribution of proprioceptive reflexes is attenuated.

This mechanism may play a particularly important role when changes in muscle length are restricted, e.g. by experimental design. For example, numerous studies have been performed to investigate control of treadmill locomotion in decerebrate cats where the hips are firmly clamped or pinned to a strong metal frame over the treadmill. This constraint abolishes vertical motion of the hips, which is normally present in locomotion, and confines extensor muscle operation to longer lengths during the stance phase of gait. One would predict that extensor muscles would therefore operate further up the force-length curves, thus increasing the loop gain of the stretch reflex pathways. Estimation of the reflex contribution under these conditions would result in overestimates compared to normal unrestrained locomotion.

Figure 3.8 shows simulation results of "unrestrained" and treadmill locomotion with "fixed hips". The moderate increases of the operating length of extensors due to hip fixation resulted in a dramatic increase of the activation level and generated force. This indicates that experiments of this type, where hip motion is prevented may distort the neural control of locomotion significantly.

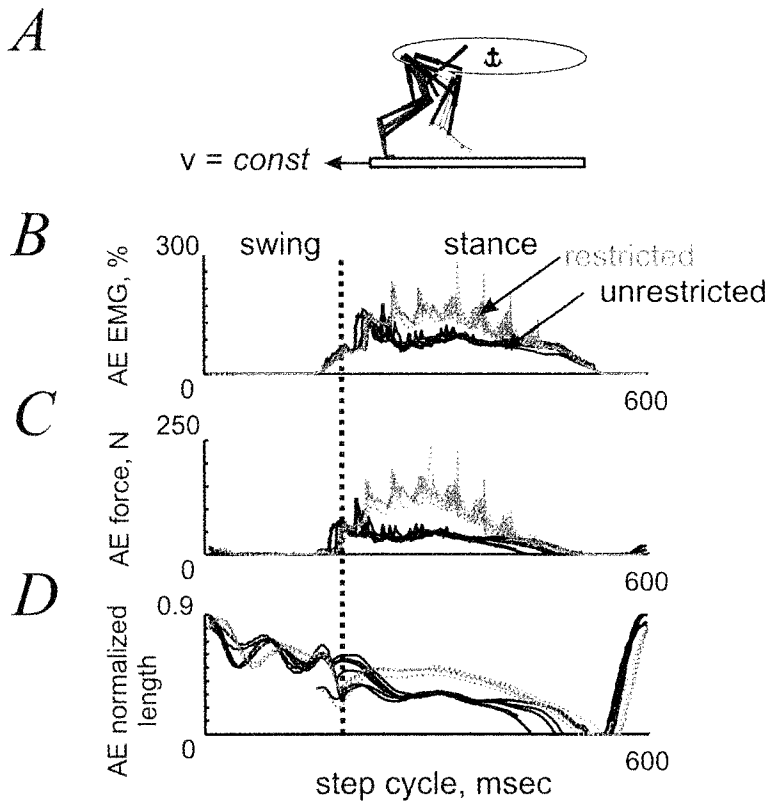

Figure 3.8. Effect of abolishing vertical hip motion on EMG, force and length. Restriction of vertical hip movement during walking increases activation levels of extensor muscles (**a**), increases extensor muscle forces (**b**), increases extensor muscle lengths during stance (**c**). This restriction, which is used in many decerebrated locomotor experiments, may lead to overestimations of the level of reflex contribution during locomotion.

3.4 Discussion

The purpose of this study was to gain insight into the contribution of sensory input to the control of locomotion. Though many experiments have been done on locomotor stretch reflexes, most of these have been limited to EMG measurements (Capaday & Stein, 1986; Gorassini *et al.*, 1994; Gritsenko *et al.*, 2001) rather than kinetic or kinematic changes of the whole limb (Sinkjaer *et al.*, 1988; Pearson *et al.*, 1999). The amount of EMG activation attributable to reflexes elicited by muscle length and force changes during the step cycle is generally less than 35% of the total (Stein *et al.*, 2000). Furthermore the reflex components have rather long latencies (Gorassini *et al.*, 1994; Gritsenko *et al.*, 2001). Finally, bursts of sensory input from stretch receptors elicited by electrical stimulation (Whelan & Pearson, 1997) or by muscle vibration (Ivanenko *et al.*, 2000) have surprisingly little impact on the kinematics of locomotion. All of these findings led us to propose that the biomechanical consequences of stretch reflexes elicited in the course of unperturbed gait did not contribute significantly to load compensation during weight-bearing locomotion.

The biomechanical modeling described in this paper was done to test the following hypotheses: 1) Stretch reflexes are too weak and too delayed to contribute significantly to weight-bearing. 2) The important contributions of sensory input involve state-dependent processing. The results did not support hypothesis 1, but they did support hypothesis 2.

Hypothesis 1 was not supported, because stretch reflexes could rescue stable locomotion in cases where the amplitudes of the CPG activation profiles were insufficient to support locomotion. On the other hand, adding stretch reflex components to CPG activation profiles that *were* adequate to support stable locomotion tended to increase body height, propulsion and speed. When these increases were inappropriately scaled between muscles, locomotion became unstable.

Thus although hypothesis 1 was not supported, nonetheless, stretch reflexes only seem to contribute significantly when central activation levels are low.

Regarding hypothesis 2, the incorporation of If-Then rules increased stability and in particular it greatly increased the range of cadences and velocities of stable locomotion, even though the time course of the actual muscle activation profiles did not change. How could gait velocity vary without the activation profiles changing? The answer is that the triggering of say the stance-swing transition terminates the reading-out of the extensor muscle activation profiles, even if they are only half completed, and initiates the reading-out of the flexor activation profiles. Similarly flexor profiles are terminated earlier in the next half cycle. The interesting point here is that cadence and gait velocity can be varied over a wide range without changing the time-course or amplitude of the activation profiles, but merely by skipping parts of these profiles and resetting to a new part of the step cycle. To our knowledge, this possibility has not been recognized until now.

It is important to mention some of the restrictions and limitations of the modeling performed. A general criticism of modeling of this type is that when the model contains many parameters, there is scope for choosing sets of parameters that fit one's favored hypothesis. To safeguard against this, we deliberately avoided making the models represent faithfully the anatomy of a particular "animal." Furthermore, our approach to the exploration of parameter space using an observer-independent stability search algorithm and PCA allowed for an unbiased representation of stability over several hundred sets of parameters in two models that represented extremes of limb geometry. The conclusions we drew regarding the two hypotheses were therefore broad-based and not model-specific, i.e. they remained valid in the face of a large range of parameter variations.

This is not to say that we were able to explore all types of parameter variations that could influence the conclusions. For example, the muscle models did not take into account the hysteretic

property of short-range stiffness (Rack & Westbury, 1973). We intend to add this characteristic in future modeling work. We did not explore all possible muscle spindle and tendon organ models (Prochazka & Gorassini, 1998), nor did we vary the latency of the stretch reflex, nor try to represent separate short-, medium- and long-latency reflex components or heterogeneous reflex connections. There is in any case considerable uncertainty in the literature regarding the relative sizes of such components. In previous modeling, we have found that varying reflex latencies within reasonable limits did not change muscle kinetic responses substantially (Prochazka *et al.*, 1997). By varying the level of the stretch reflex contribution during locomotion we found that closed-loop gain of the sensory evoked responses depended strongly on muscle length and velocity. This dependence was previously implicated in stabilizing positive force feedback (Prochazka *et al.*, 1997). Since estimation of the gain of the closed-loop reflex system during various tasks poses a considerable experimental challenge, the relative level of contribution of the stretch reflex to muscle excitation remains a contentious issue. Models such as ours may greatly clarify and assist in evaluation of components of feedforward and feedback systems of motor control.

To conclude, our model provided insight into the interplay between neural commands and biomechanical properties of the musculoskeletal actuators of the locomotor system. The main conclusion was that homonymous stretch reflexes are capable of adjusting muscle forces at several joints to achieve weight bearing during gait, and this is particularly significant when central activation is low. Simple finite state rules provide dramatic improvements in flexibility and stability of level overground locomotion in our model. Cadence is constantly adjusted to kinematic state. This also raises the possibility that gait velocity could be controlled by changing the firing thresholds of sensory rules in a state-dependent controller. Our method of testing stability by

exploring parametric space using PCA is novel and provides a systematic measure of the generality of the conclusions.

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CHAPTER 4

Spatiotemporal activation of lumbosacral motoneurons in the locomotor step cycle

Adapted from an original publication:

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4.1 Introduction

It has been known for centuries that for a short time after cervical spinalization, many animals can still generate a locomotor rhythm (Mettrie de La, 1745). T. Graham Brown coined the term "intrinsic factor" to describe the rhythm generator in the spinal cord and suggested a simple "half-center" oscillator as the underlying mechanism (Brown, 1911). Brown's intrinsic factor was renamed the "central pattern generator" (CPG): (Grillner & Zangger, 1975). Many experiments have been done in different species to localize and characterize the basic neuronal machinery of locomotor CPGs (Kiehn *et al.*, 1998). Broadly speaking, opinion is split between those who believe that the vertebrate CPG is fairly localized (Cazalets *et al.*, 1995) and those who think it is distributed (Deliagina *et al.*, 1983; Arshavsky *et al.*, 1997; Kremer & Lev-Tov, 1997; Kiehn & Kjaerulff, 1998). One version of the "distributed" hypothesis envisions a series of semi-autonomous "unit" oscillators distributed along the neuraxis and dynamically coupled by propriospinal pathways (Grillner & Wallen, 1985). A consensus seems to be emerging that rhythmogenesis is more robust in one or two spinal cord segments just rostral to the lumbosacral enlargement than in more caudal segments, and that these rostral segments may "lead" the other segments in generating the locomotor rhythm (Deliagina *et al.*, 1983; Arshavsky *et al.*, 1997; Kiehn & Kjaerulff, 1998; Marcoux & Rossignol, 2000).

It is impossible with current techniques to measure directly the activity of large ensembles of neurons of a particular functional type in the mammalian spinal cord during normal behaviour. Though this may be possible in the future by identifying neurons according to some feature such as size, molecular affinity or luminance, to our knowledge this has not been attempted to date. Consequently, all of the experiments on localization of pattern generators within the spinal cord have been performed on reduced preparations of different types, including isolated embryonic

spinal cords of the rat (Lev-Tov & O'Donovan, 1995), in vitro portions of fetal rat spinal cords (Hochman *et al.*, 1994; Kiehn & Kjaerulff, 1998), spinalized fictive locomotor cat (Andersson *et al.*, 1978; Grillner & Zangger, 1979; Deliagina *et al.*, 1983) and lamprey (Zhang *et al.*, 1996; Grillner *et al.*, 1998). In these various experimental situations the locomotor rhythm is usually initiated and maintained by the application to the spinal cord of pharmacological agents such as dihydroxy-phenylalanine (DOPA) or N-methyl-D-aspartate (NMDA) or by electrical stimulation applied to descending or peripheral sensory pathways. The rhythms generated in muscle nerves or ventral roots can often have many of the specific characteristics of those seen in normal locomotion, so the assumption is made that the patterns of central neural activity studied in this way are similar to those occurring in normal behavior. However, not only are descending inputs absent in these preparations but in most cases so are sensory inputs from actively moving limbs. Descending control originating in cerebral, cerebellar and midbrain areas has a profound influence on the amplitude and timing of muscle contractions during locomotion (Armstrong & Marple-Horvat, 1996; Rho *et al.*, 1997). Step-cycle phase transitions, as well as modulation of motoneuron (MN) activity during swing and stance phases depend heavily on sensory input during normal locomotion (Grillner & Zangger, 1975; Grillner & Rossignol, 1978; Pearson, 1995; Prochazka, 1996).

The aim of our study was to characterize the spatiotemporal migration of neuronal activity in the cat spinal cord during normal locomotion. We combined two sets of data: the known distribution of MN pools innervating different muscles within the spinal grey matter and the known EMG activity profiles of these muscles in the locomotor step cycle.

The approximate rostrocaudal distribution of MN pools in the cat spinal cord was originally deduced from the distribution of ventral roots innervating different hindlimb muscles (Sherrington, 1892). Several important studies have greatly extended the scope and accuracy of this information

(Romanes, 1951; Vanderhorst & Holstege, 1997). In addition, there is now a large body of information on the location and reflex connections of interneurons of different types (Jankowska, 1992) and on the distribution of the terminals of sensory axons (Brown, 1981).

Each action potential in a MN propagates along the efferent axon and gives rise to a motor unit action potential (MUAP) in the muscle the MN innervates. All of the MUAPs generated by the numerous active MNs sum in a slightly nonlinear manner (see Discussion) to produce the recordable signal called the electromyogram (EMG). The rectified EMG thus provides an indirect measure of the net firing of MNs of that muscle in the spinal cord at any particular moment. In numerous functional studies over the last three decades the EMG patterns of cat hindlimb muscles recorded during locomotion have been described in great detail (see Methods).

The model of MN distributions in the spinal cord and the model of spatiotemporal activity described in this paper are in digital form. This will allow them to be used in other laboratories not only to test for differences in spinal activity patterns in different preparations (e.g. fictive locomotor versus normal), but also to help predict which muscles will be activated or inactivated by local electrical stimulation of the spinal cord (Mushahwar & Horch, 1998; Mushahwar *et al.*, 2000), lesions or local excitation or inhibition with microinjections of pharmacological agents (Marcoux & Rossignol, 2000). A preliminary report on this work has been published (Yakovenko *et al.*, 2000).

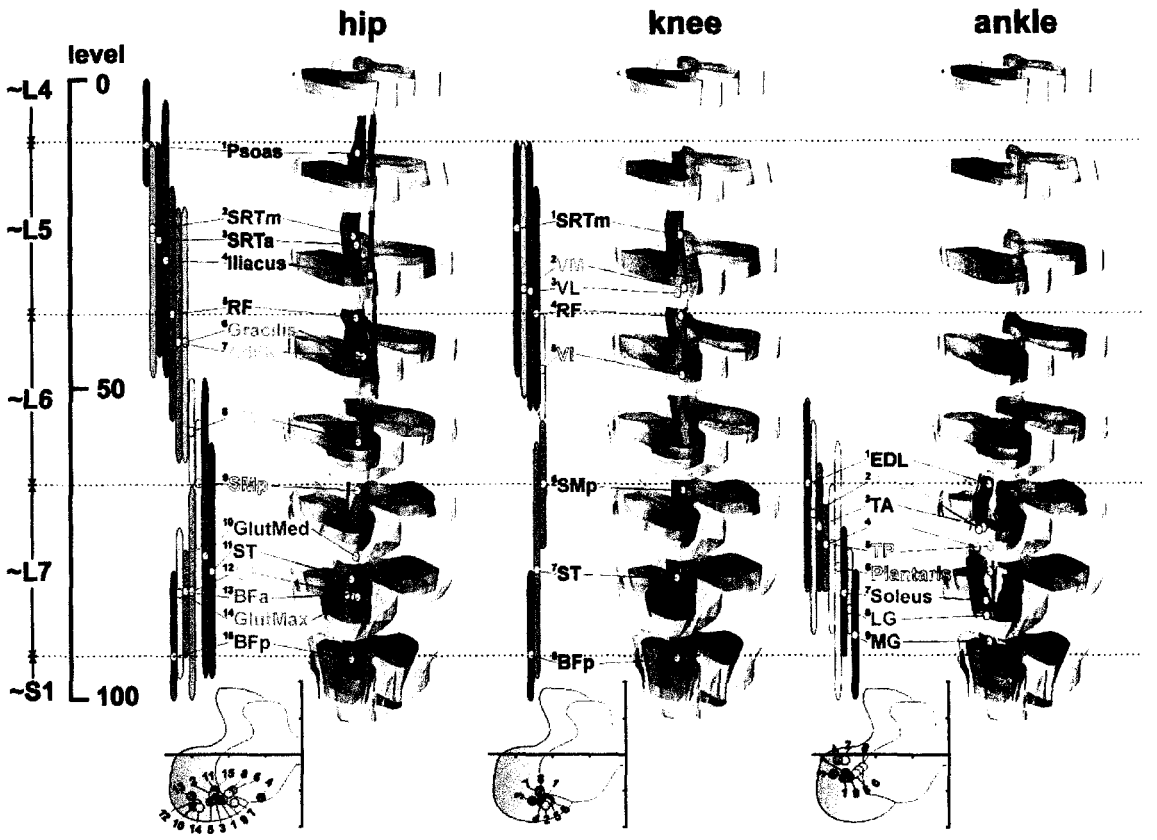

Figure 4.1. Anatomical models of the organization of the MN pools within the cat lumbar enlargement. The pools innervating muscles acting about the hip (left), knee (middle) and ankle (right) are presented separately. The anatomical data were digitized from 30 summary drawings or "slices"(Vanderhorst & Holstege, 1997). The rostrocaudal axis is normalised and divided into 100 levels. On average slices were 0.96 mm thick and corresponded to 3.3 levels. The models show a structure 29 mm long on average, spanning the caudal L_4 to rostral S1 segments of the spinal cord. The fusiform MN pools within the grey matter are long and intertwined (MN pools are identified by colour, abbreviation and a number in each of the three representations). The schematic bars on the left show their rostrocaudal extent. The centers of mass are shown as open circles on these bars and their positions in the transverse plane are shown in the bottom panels. The lines leading from the abbreviations point to the location of the corresponding center of mass. Note the rostral locations of hip flexor pools (psoas, SRT, iliacus) and more caudal locations of hip extensor pools (e.g. SMa, BFa). The reverse relationship is true of MN pools innervating knee muscles: knee flexor MN pools (e.g. BFp, ST) are more caudal and knee extensor MN pools (e.g. VL, RF) more rostral. Ankle MN pools are relatively close together in the caudal region, with flexors slightly more rostral than extensors. BFp, SMp and ST are bifunctional muscles that flex the knee and extend the hip, so their MN pools are represented in both the middle *and* left panels.

4.2 Methods

4.2.1 Digitization

In a recent study the horseradish peroxidase (HRP) staining technique was used to determine the distributions within the cat spinal cord of 50 MN pools innervating hindlimb, pelvic floor and lower back muscles (Vanderhorst & Holstege, 1997). The entire lumbosacral enlargement of the frozen spinal cord (mean length 28.8 mm) was cut into 40 μm sections. Every second section was collected. Groups of 12 such sections were used to construct composite digital drawings showing the positions of all stained MNs in the group (i.e. each drawing corresponded to a 12 x 2 x 0.04 = 0.96-mm thick slice of spinal cord). The rostral and caudal ends of the enlargement were identified on the basis of distinctive transitions in shape of the ventral horn. Because the enlargements varied in length between animals, a normalization procedure was used to divide each enlargement into 100 levels. A summary figure of 30 slices was presented (Vanderhorst & Holstege, 1997) (Fig. 28), corresponding to a mean length of the enlargement of 30 x 0.96 = 28.8 mm.

Based on the EMG profiles available to us (see following text), we digitized the positions of MNs of the following 27 hindlimb muscles: adductor femoris magnus (AdFM), biceps femoris anterior and posterior (BFa, BFp), caudofemoralis (CF), extensor digitorum longus (EDL), flexor digitorum longus (FDL), flexor hallucis longus (FHL), gluteus maximus and medius (GlutMax, GlutMed), gastrocnemius lateralis and medialis (LG, MG), gracilis, iliacus, plantaris, psoas, rectus femoris (RF), sartorius anterior and sartorius medialis (SRTa, SRTm), soleus, semimembranosus anterior and posterior (SMa, SMp), semitendinosus (ST), tibialis anterior (TA), tibialis posterior (TP), vastus intermedius, vastus lateralis and vastus medialis (VI, VL, VM).

Initially we classified MNs as innervating either flexors or extensors, according to the predominant biomechanical action of the corresponding muscles or parts of muscles activated in the flexor and crossed extensor reflexes (Sherrington, 1910; Eccles & Lundberg, 1958; Carrasco & English, 1999). Flexors: BFp, EDL, gracilis, iliacus, psoas, RF, SMp, SRTa, SRTm, ST, TA; extensors: AdFM, BFa, FDL, FHL, GlutMax, GlutMed, LG, MG, plantaris, soleus, SMa, TP, VI, VL, VM. However, we will argue later that in locomotion BFp, SMp and ST muscles and possibly Gracilis and FDL form a separate functional group and their MNs produce a distinct locus of activation in the spinal cord.

In the anatomical study (Vanderhorst & Holstege, 1997) two characteristics of the spinal cord were recognised when determining the spatial relation between lumbosacral MN pools. First, there were major differences in the organization of spinal rootlets between cats and so segmental localisation varied. Second, the length of the lumbar enlargement varied considerably (mean: 28.8 mm $\pm$ 2.4 mm (SD), range: 23.0 – 34.1mm (n = 120)). To normalise the data across animals, two easily recognizable landmarks were chosen to identify the rostral and caudal ends of the enlargement. The rostral landmark (defined as level 0) was a sudden lateral extension or bulging of the ventrolateral corner of the ventral horn into the white matter in the midlumbar cord. The caudal landmark (defined as level 100) was in the sacral cord where the border of the ventral horn changes from a rounded profile to a straight line joining the ventromedial and dorsolateral corners of the horn. These changes in shape at levels 0 and 100 took place over a distance of 0.5 – 1.0 mm (~1.5 to 3 levels). Locating them accurately required careful comparisons of several sections in the vicinity of the transitions. The 0- to 100-level scale allowed the relative distributions of many MN pools to be compared. In the numerous case studies illustrated (Vanderhorst & Holstege, 1997) the rostrocaudal locations of the centers of mass of individual MN pools ranged over maximally 10 levels (i.e., localisation was reliable to within 10% of the length of the enlargement). It should be

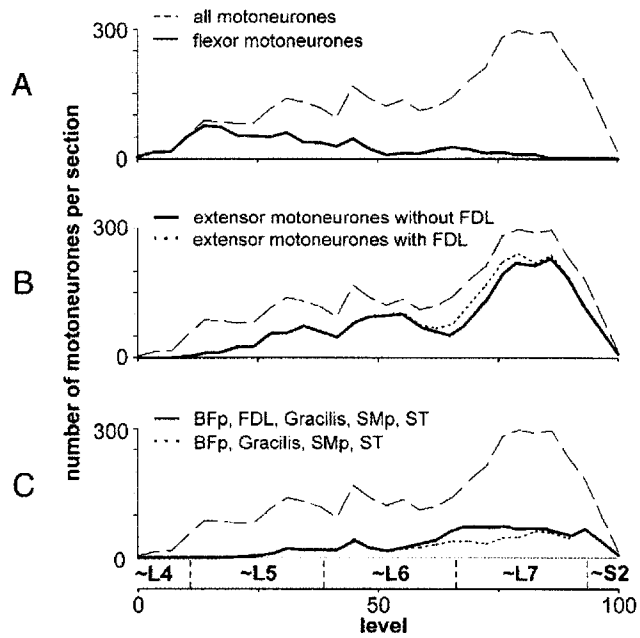

Figure 4.2. MN distribution along the rostrocaudal axis of the lumbar enlargement of the cat spinal cord. The enlargement was divided into 30 slices as described in the text. The number of MNs digitized from the source data in each slice is plotted. As no distinction was made between alpha and gamma MNs, the number of alpha MNs is probably overestimated (see text). The total population distribution is shown as a dashed line in each panel, and reveals a significant imbalance between the rostral and caudal halves of the lumbosacral enlargement. ***A.*** flexor MNs are concentrated mainly in the rostral part of the spinal cord (levels 0 to 50). ***B.*** Extensor MNs are more caudal, with peak density in the L_7 segment. Two distributions are shown, one that includes FDL MNs (dotted) and one that does not (solid: see text). ***C.*** Distribution of MNs innervating BFp, gracilis, SMp and ST (with and without FDL). We argue in the text that these muscles, normally classified as flexors, have a biomechanical role in the step cycle that is best described as retraction.

Table 4.1. Rostral (L_5-L_6) and caudal (L_7-S_1) populations of extensor, BFp-FDL-gracilis-SMp-ST and flexor MNs in the cat lumbar enlargement.

	extensor motoneurons		BFp, FDL, gracilis, SMp,ST motoneurons		flexor motoneurons		all motoneurons	
rostral half (L_4-L_6)	549	23%	155	17%	620	81%	1324	33%
caudal half (L_7-S_1)	1817	77%	752	83%	141	19%	2710	67%
full (L_4-S_1)	2366		907		761		4034	

noted that this normalisation procedure was seen as an important advance over previous methods, in which relative positions of MN pools could only be studied accurately in individual animals.

To enter the positions of MNs into the model we scanned Figure 28 of (Vanderhorst & Holstege, 1997), at high resolution (600 x 600 dpi). A software program written in Matlab (version 5.3, The MathWorks) was used to display each scanned slice on a 17 inch computer monitor and to digitize the coordinates of individual MNs together with the outline of the grey matter. The coordinates, stored in ASCII format, were imported into a three-dimensional (3-D) model constructed with a second Matlab software program. In the model, the slices were all represented as being 0.96-mm thick and accordingly the length of the enlargement was represented as 28.8 mm. It should be stressed that in the original study lumbar enlargements varied in length over the range 23.0 – 34.1 mm (Vanderhorst & Holstege, 1997). In this paper we will therefore usually refer to the 100 normalized levels, however certain calculations such as that of the center of activation require absolute positions. The slices were aligned using the central canal for lateral centering and the midline between dorsal and ventral horns for dorsoventral centering. As the dimensions of individual cross-sections varied, the absolute positions of MNs and the boundaries of MN pools in the slices can only be considered accurate to within the variance of the original dimensions. In unpublished data from five cats, one of the authors (V. Mushahwar) made measurements of the width and thickness of the spinal cord at different segmental levels. The mean distance from the midline to edge of the white matter at L_7 level was 3.6 $\pm$ 0.04 mm and the corresponding mean dorso-ventral thickness of the spinal cord was 5.5 $\pm$ 0.1 mm. As most MNs were located about halfway from the midline to the edge of the white matter and less than half of the thickness of the cord from the central canal, we estimate SDs of the absolute positions of MNs to be no more than 0.02 mm anterolaterally and 0.05 mm dorsoventrally.

The Matlab functions for visualization of 3-D structures were used to generate a 3-D model of the lumbar enlargement. Figure 4.1 shows graphical displays of this model in which surfaces have been "rendered" with SURFdriver version 3 software (Surfdriver.com).

EMG patterns of cat limb muscles have been studied extensively by many researchers. In a previous paper from this laboratory we reviewed the available data and constructed a chart of standardised EMG profiles of a variety of muscles in the cat step cycle (Prochazka *et al.*, 1989). In the present study we again reviewed all the currently available literature and collated as many examples as possible of segments of EMG locomotor recordings from hindlimb muscles. From this collection we constructed EMG profiles representing the activity in one step cycle for each of 27 muscles. We considered doing this mathematically by digitizing and processing the individual traces from the different studies to produce averages aligned to foot contact and normalised to one cycle period, however we decided against this for the following reasons. The EMG recordings were not of uniform quality or format. Some EMG signals were rectified and smoothed while others were unrectified. Some were averages of several steps while others were raw traces from single cycles. Some recordings included reliable measurements of ground contact and foot lift while others did not. Cycle durations varied and as a result so did the relative durations of swing and stance phases. Even when EMG recordings are made in the same laboratory with the same techniques there is some variation in the time course of EMG profiles from a given muscle that appears to depend on electrode dimensions and placement as well as possible differences in gait between animals. Taking all of these factors into consideration, we therefore opted to construct each profile from linear segments after carefully comparing the available records in the collection and using our judgment on critical features such as the timing of onset and duration of the EMG signal, the timing of the peaks and the overall shape of the signal. Profiles were constructed from a number of piecewise

linear segments, the onset, duration and slope of which were entered digitally in a standardized spreadsheet of the step cycle in which the landmarks were the moments of foot lift and foot contact. The swing phase occurring between these landmarks was standardized to 30% of the cycle, a proportion associated with gait velocities in the range 0.6 – 0.8 m/s and cycle periods of about 700ms (Goslow *et al.*, 1973; Halbertsma, 1983). Particular attention was paid to the time course of EMG signals at and around these critical points in the cycle. When possible, concurrent EMG signals from ankle extensors MG, LG or soleus were used as a reference for foot contact, as the mean delay between the onset of EMG in these muscles in the E1 phase and the moment of foot contact is 60ms, with a range of $\pm$5 ms (Trend, 1987; Prochazka & Gorassini, 1998). Muscle length or joint angle traces, when available, also helped us to identify timing landmarks. Each profile was normalized, i.e., it reached a peak of 100% at least once in the cycle. The assumption implicit in this normalization is that all muscles are fully recruited at least once in the step cycle. Clearly this does not occur in slow locomotion (Walmsley *et al.*, 1978). However we viewed this simply as a matter of scaling; the important point was that all MN pools, whether flexor, extensor or retractor, reached the same maximal level of recruitment when their EMG signals were maximal. Different relative levels of recruitment of flexors and extensors were then explored to address the issue of parametric sensitivity.

The step cycle was divided into 100 equal intervals corresponding to about 7ms each for gait of moderate speed. For each of these intervals the normalized level of EMG of each hindlimb muscle was obtained from the corresponding profile in the chart. The EMG levels from each muscle were used to scale the number of dots displayed within the volume of the corresponding MN pool in the digital model. This assumes a linear relationship between EMG and MN firing rate (Hoffer *et al.*, 1987), an assumption we discuss in detail later. To simplify the simulation, we also assumed a

random topographical distribution of active MNs within each MN pool. The 100 consecutive images or frames were saved in bitmap format and when displayed in rapid sequence with Corel Photo-Paint software they formed a continuous-loop movie showing the spatiotemporal activity of hindlimb MN pools. The effect of our simplifying assumptions on the results and conclusions will be dealt with in more detail in the Discussion. However, to gain an overall idea of the parametric sensitivity of our main conclusions to details of EMG profiles, we did a set of simulations in which the profiles were converted to all-or-nothing step functions such that all non-zero values were assigned the value 100%.

4.2.2 Calculation of the center of MN activity

The locus of MN activity was analyzed by calculating the center of mass (r_{cm}) of the n active MNs (the "center of activity") at each of the 100 moments in the step cycle, using the following formula:

$$r_{cm} = \frac{\sum_{i=1}^{n} r_i}{n} \qquad \text{Eq. 4.1}$$

where r_i is the vector from the origin to the *i*th MN (The origin being defined as the rostral-most point of the neuraxis). As mentioned above, the center of activity was expressed in terms of absolute position within the standard 28.8 -mm long enlargement.

4.3 Results

4.3.1 Anatomical distribution of MN pools in the lumbosacral spinal cord

Figure 4.1 shows three views of the 3-D model of the lumbar enlargement of the cat spinal cord, rendered from the digitized transverse slices of HRP-stained MNs described above. Each of the three displays shows the outlines of the gray and white matter as well as MN pools of muscles acting about the hip, knee and ankle respectively. The Figure illustrates some interesting general features of MN pool distributions. The pools are long, spindle-shaped and intertwined. The bars on the left of each model were included to show the rostrocaudal extent and the center of mass of each pool.

As is well known from classical myotomal charts (Romanes, 1951; Sharrard, 1955), there is a correspondence between the rostrocaudal position of MN pools and the anatomical position of their respective muscles in the leg. It follows that there must be a correlation between the position of MN pools and the biomechanical action with which they are associated. Thus starting at level 0 at the rostral end of the enlargement and moving caudally to level 80 we find the MN pools of muscles distributed in proximal to distal order in the anterior compartment of the leg: hip flexors (psoas, SRTm, SRTa, iliacus) knee extensors (VL, VM, RF, VI) and ankle flexors (EDL, TA). When the leg is pendant, these muscles all act to move the foot forward. Starting from level 40 and moving caudally to level 100 (S_1 segment), we find the hip extensor pools (AdFM, Gracilis, SMa, SMp), followed by toe plantarflexors (FDL, FHL) and ankle extensors (MG, LG, Pl, soleus). The most caudal part of the enlargement also contains MN pools of proximal muscles with complex actions about hip and knee (GlutMed, ST, CF, BFa, BFp). When the leg is pendant these muscles all assist in moving the foot and toe back. Note that in terrestrial locomotion, some of the muscles assume a load-compensating role which can no longer be described in terms of a simple forward-backward dichotomy (see Discussion).

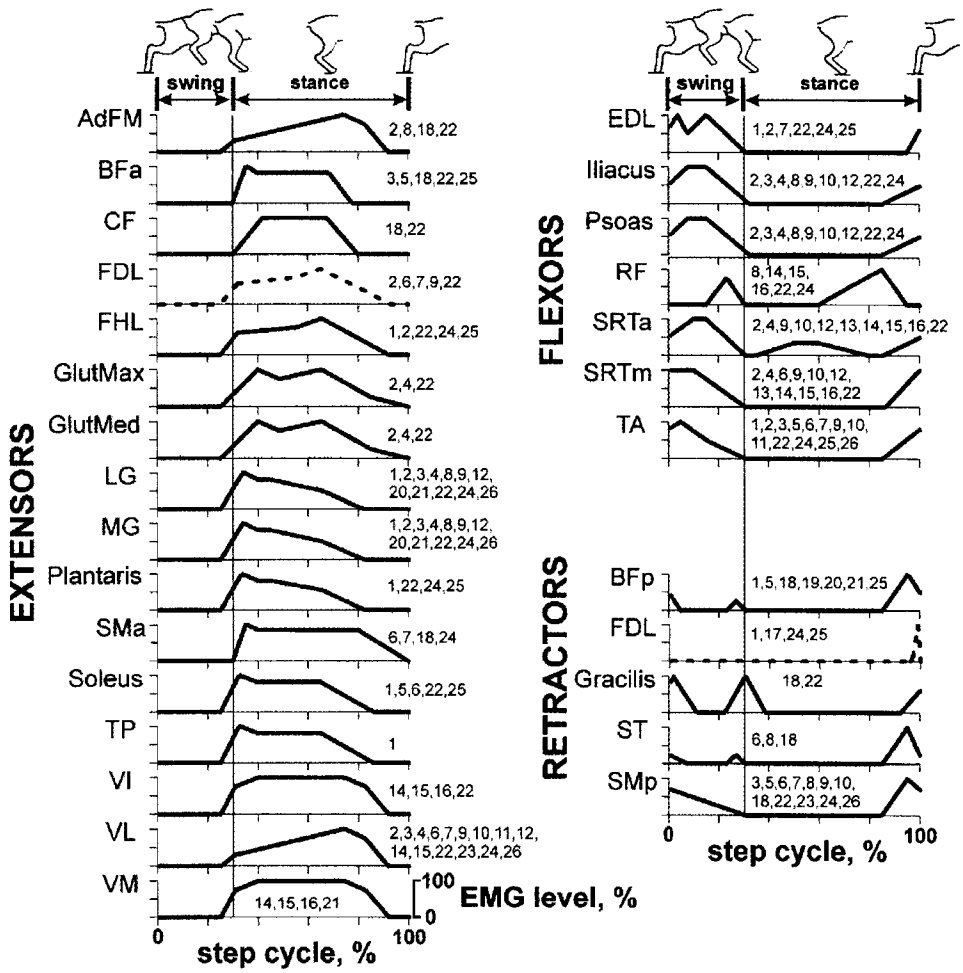

Figure 4.3. EMG patterns of hindlimb muscles during forward level locomotion at moderate speeds (~0.6m/s). The normalized patterns are plotted for a full step cycle, starting from the onset of swing. The left and right panels show respectively the EMG profiles of extensors, flexors and the muscles we classify as retractors. FDL, which has a variable EMG pattern, is shown dashed in both extensor and retractor groups. These EMG profiles were constructed from piece-wise linear segments from numerous sources in the literature, the numbers in each panel referring to the papers listed below. The profiles were used in the subsequent figures to modulate the number of MNs shown as being active in the spatiotemporal model.

References: 1. (Abraham et al. 1985), 2. (Belanger et al. 1996), 3. (Buford and Smith 1990a), 4. (Carrier et al. 1997), 5. (Chanaud et al. 1991a), 6. (Duysens and Loeb 1980), 7. (Engberg 1964), 8. (Engberg and Lundberg 1969), 9. (Forssberg 1979b), 10. (Forssberg et al. 1980), 11. (Gritsenko et al. 2000), 12. (Halbertsma 1983), 13. (Hoffer et al. 1987a), 14. (Hoffer et al. 1987b), 15. (Loeb and Hoffer 1985), 16. (Loeb et al. 1985), 17. (O'Donovan et al. 1982), 18. (Pratt et al. 1996), 19. (Pratt et al. 1991), 20. (Prochazka et al. 1976), 21. (Prochazka et al. 1989), 22. (Rasmussen et al. 1978), 23. (Rossignol et al. 1986), 24. (Smith et al. 1998), 25. (Trank and Smith 1996), 26. (Wand et al. 1980).

The numerical distributions of the digitized MNs are illustrated in Fig. 4.2. There is a significant imbalance between the sizes of rostral and caudal populations of MNs. The caudal half of the enlargement (level 50 – 100) contains far more MNs than the rostral half. Indeed it is well known that the caudal ventral horns have lateral extensions that accommodate the increased numbers of MNs (Vanderhorst & Holstege, 1997). Most of the MNs in this region innervate extensor muscles (Fig. 2B). Over 75% of all hindlimb extensor MNs are in this region (Table 1) and their density reaches a peak at about level 80. Flexor MNs (Fig. 4.2A) are less tightly clumped but even so, over 80% of them are found in the rostral half. The distributions of BFp, FDL, gracilis, SMp and ST MNs are illustrated separately in Fig. 4.2C. These MNs are mainly in the caudal half of the enlargement. We will argue that the function of these muscles in locomotion is to provide the final extensor thrust during the stance phase, and then to provide ground clearance for the swing phase by extending the hip while at the same time flexing the knee and the toes. For this reason we have borrowed the term "retractors" from the invertebrate literature to describe them (see Discussion).

4.3.2 Analysis of the spatiotemporal pattern of MN activity

Figure 4.3 summarizes the EMG profiles of all the muscles we included in the model during level forward walking, starting from the onset of swing (see Methods for details on how the profiles were constructed). The muscles in each group behave rather uniformly during locomotion. The flexors are active mainly in the early and mid-swing phase, when the foot is off the ground. The extensors become active during the last 100 ms of swing. In fact this part of the step cycle is usually designated E1, the first extensor phase of the step cycle (Philippson, 1905). The bifunctional muscles BFp, gracilis and ST show two EMG bursts in the step cycle, at the transitions from stance to swing and from swing to stance respectively (SMp does not always show this burst clearly). These muscles produce a backward and upward motion of the foot at the end of the stance phase, a

function we will argue below is best described as *retraction.* FDL may contribute to this action by plantarflexing the paw. FDL was originally considered an extensor, along with its synergist FHL (Sherrington 1910). Several EMG studies supported this view, showing FHL and FDL to have extensor bursts similar to those of the gastronemii and soleus. However, in a study that focussed specifically on the biomechanical actions and EMG of FDL and FHL, it was concluded that unlike FHL, FDL acted predominantly as a toe flexor at the stance-swing transition with a brief, intense burst of EMG (O'Donovan *et al.*, 1982). It was suggested that the EMG during the stance phase recorded by others may be due to cross-talk. The flexor burst pattern of FDL is supported in neurograms recorded during fictive locomotion (Fleshman *et al.*, 1984) and in more recent EMG recordings in normal cats (Loeb, 1993; Trank & Smith, 1996; Smith *et al.*, 1998), though Loeb's group reported variable amounts of stance phase activity that was unlikely to be due to cross-talk. Given the ambiguity regarding FDL in the literature, we performed two separate analyses of the data, in one case assuming a flexor (retractor) burst EMG profile for FDL and in the other an extensor profile (see dashed profiles in Fig. 4.3).

We were unable to include some muscles with MNs in the lumbar enlargement for the following reasons. There were no recordings from intrinsic hip muscles such as obturator, pyriformis and gemellus in the literature. Tensor fascia latae, a thin, flat muscle overlying the vasti, has anterior and posterior portions which flex or extend the hip respectively (Chanaud *et al.*, 1991). These portions were not separated in the anatomical study (Vanderhorst & Holstege, 1997), and so EMG profiles could not be duly assigned. For the same reason, the small intrinsic muscles of the foot are omitted from our analysis. A case in point is extensor digitorum brevis. This muscle is innervated by branches of the deep peroneal nerve and so its MNs are probably close to those of EDL, in caudal L_6 and rostral L_7 segments. EDB EMG shows a burst of activity at the onset of the

stance phase (Trank & Smith, 1996), so this might slightly alter the activity in our model. Given the likely small number of MNs involved, this is unlikely to be a major effect.

Conversely, though the distribution of MNs of the minor portions of adductor femoris (brevis and longus) were available, the only EMG recordings in the literature were from the major portion (magnus), so the minor portions are not represented in our model.

The MN distributions of the axial muscles multifidi and longissimus dorsi showed a sparse population between L_6 and S_1 and included some tail MNs at the caudal end (Vanderhorst & Holstege, 1997). The only EMG recordings from these two muscle groups we could find in the literature were made at L_4 level in the normal cat (Carlson *et al.*, 1979) or at L_4 and L_7 levels in the decerebrate or spinal cat (Zomlefer *et al.*, 1984). In the normal cats, for locomotion at about 1 m/s these muscles showed biphasic, low-level EMG in both swing and stance phases of the step cycle. In the decerebrate cats, for locomotion slower than 0.7 – 0.8 m/s, there was virtually no detectable EMG signal in the vertebral muscles (Zomlefer *et al.*, 1984). To gain some perspective on this issue, we obtained EMG recordings from the longissimus muscle between the L_5 and L_6 spinous processes in one cat with the use of percutaneous electrodes. The EMG pattern was low level and fairly constant over the step cycle, which agrees with the above observations. In light of the incomplete nature of the data on vertebral muscles, it did not seem appropriate to include these in the analysis. Given their small numbers and apparently low level of recruitment in slow stepping, their omission is unlikely to affect our main conclusions, though it would of course be desirable to confirm this if and when more EMG and anatomical data become available.

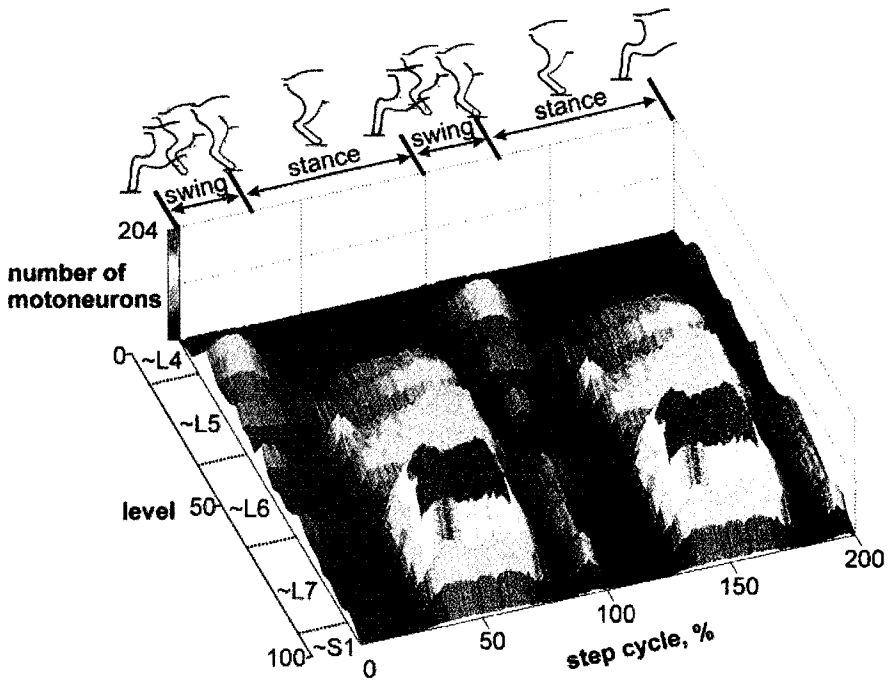

Figure 4.4. Modelled spinal MN activity in the lumbosacral enlargement during locomotion. The digitized MN distributions shown in Fig. 4.1 were combined with the EMG profiles shown in Fig. 3 to produce modelled activation patterns of MNs during two step cycles of forward, level locomotion (in this figure and the next, FDL was assigned the flexor (retractor) profile of Fig. 4.3). The phases of the step cycle are indicated above the 3-D plot. Each step cycle was divided into 100 time intervals corresponding to about 7 ms for slow walking. The plot shows the time course of activity of MNs in 30 transverse slices of the cord (level 0 most rostral, level 100 most caudal, each slice corresponds to ~3 levels or ~1mm (Vanderhorst & Holstege, 1997)). Note the dominant peaks of activity in the caudal slices in the stance phases and the relatively small rostral peaks during the swing phases. Note too the rapid migration of activity in the transition from swing to stance and a period between stance and swing where activity dwells in the caudal half of the enlargement.

The main focus of this study, the spatiotemporal pattern of the MN activity during locomotion, is illustrated in Fig. 4.4. The plot shows the distribution of the active MNs along the rostrocaudal axis in two full step cycles. As described in Methods, the step cycle was divided into 100 equal intervals and for each interval the normalized EMG level of each muscle in Fig. 4.3 was used to compute the number of active MNs per slice (a slice corresponds to approximately 1 mm of spinal cord or 3.3 normalized levels (Vanderhorst & Holstege, 1997)). Figure 4.4 reveals a large imbalance in MN activity in swing and stance phases, the latter associated with the large number of extensor MNs in the caudal half of the enlargement. Note the relatively small size of the rostral peak of activity centered between L_4 and L_5 in the swing phase.

The movie animation of Fig. 4.4 (which may be obtained in digital form from the authors) created the impression of a rostrocaudal oscillation during the step cycle. This was confirmed by computing and plotting the rostrocaudal movement of the center of activity in a plan view of the same data (Fig. 4.5A). The locus of the center of MN activity (open circles) was calculated in equal 1% increments of the cycle period, (corresponding to 7-ms intervals for a 700-ms cycle period typical of medium-speed locomotion). The distance between the open circles is proportional to the velocity of the center of activity; the velocity is maximal at the transition from swing to stance. During the stance phase there is a period in which the center of activity dwells in the region about level 70. Note that the vertical scales in the panels of Fig. 4.4 and 4.5 vary because we normalized to the peak number of active MNs per slice in each case so as to use the full range of the color spectrum.

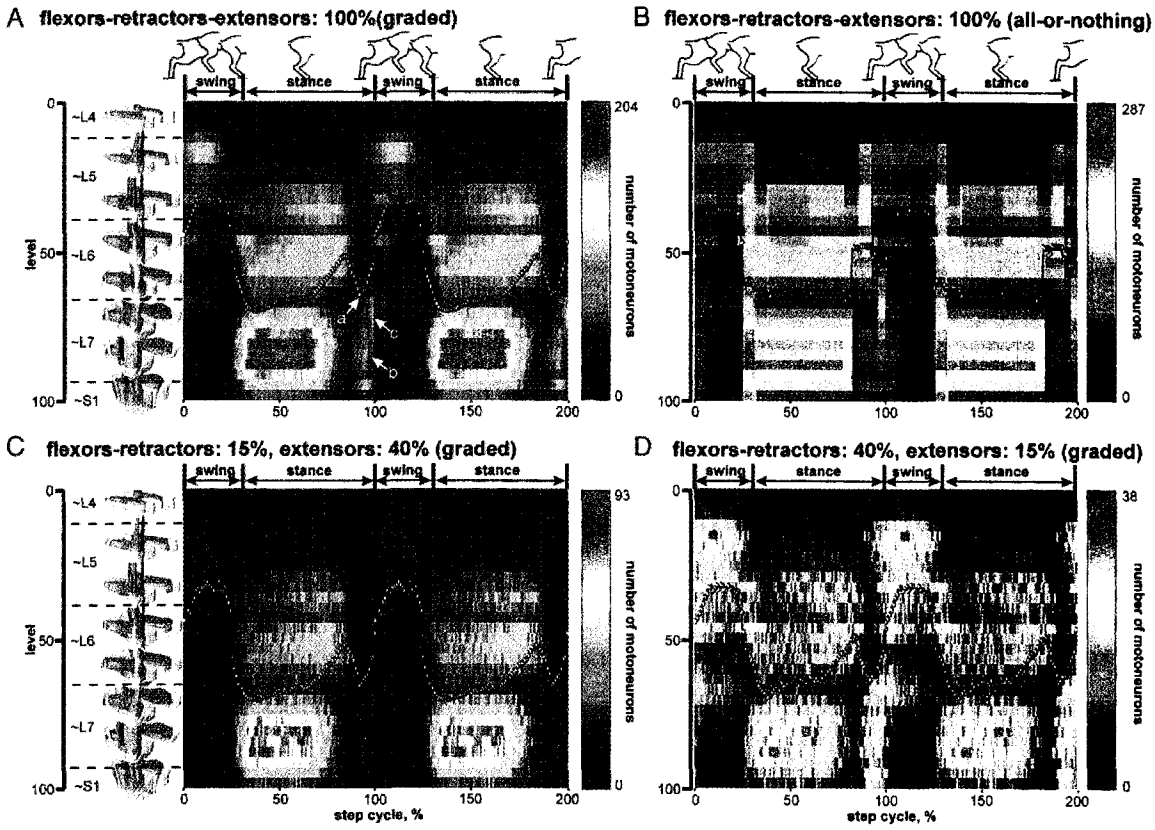

Figure 4.5. Variations in assumed activation patterns to assess parametric sensitivity. ***A. graded recruitment reaching a maximum of 100% in all MN pools.*** This plot is a plan view of the plot in Fig. 4.4. The positions of the center of mass of net MN activity were calculated at each time increment of the step cycle and displayed as open circles. The migration of these centers of activity was most rapid at the swing-stance transition. At the stance-swing transition the center of activation moves caudally (arrow a), then rostrally again. This caudal detour is associated with activity in BFp, gracilis, SMp and ST (arrow b) and FDL (arrow c). ***B. All-or-nothing recruitment:*** the EMG profiles of Fig. 4.3 were converted to step functions such that all non-zero values were assigned the value 100%. These step functions were then used to modulate the numbers of active MNs in a given pool between zero and 100%. This resulted in activity plots that had similar general features to those in A but transitions were more abrupt and the spatiotemporal migration of activity lost some of its subtleties. The caudal detour at the stance-swing transition was present, though attenuated. ***C. graded recruitment reaching a maximum of 15% in flexor and retractor MN pools and 40% in extensor MN pools.*** This is probably the case that most closely mimics real-life locomotion. (retractor profile used for FDL). ***D. graded recruitment reaching a maximum of 40% in flexor and retractor MN pools and 15% in extensor MN pools.*** The general features of the rostrocaudal movement of the center of activity shown in ***A*** were preserved in ***B*** and ***C***. The brief caudal detours of activity at the stance-swing transition are attenuated in ***C*** and exaggerated in ***D***. Note that because the total number of active MNs varied according to the assumptions, the colour scales were adjusted to preserve contrast and detail in each plot.

The large stance phase peak centered between L_6 and L_7 in Fig. 4.4 is followed by a smaller peak of activation in the L_7 segment associated with activity of FDL, BFp, gracilis, SMp and ST MN pools at the transition from stance to swing. This peak also shows up clearly in the plan view in Figure 4.5A. For a brief instant in this phase of the step cycle this is virtually the only part of the whole lumbosacral enlargement with active MNs. The transitions from swing to stance and retraction to swing are abrupt events, in which the focus of activity jumps from one end of the enlargement to the other: the computed center of activity moves between levels 36 and 74, i.e., 38 levels, equivalent to 10.9 mm in the average-sized enlargement. As the centers of activation of the most proximal and distal MN pools (Psoas and BFp) are at 4.3 and 25.8 mm respectively in the average enlargement, the maximal possible displacement of the center of activity is on average 21.5mm, i.e., 75 levels. Thus the center of activity moves 51% of its maximal possible range. The swing-stance transition is a little faster than the transition from retraction to stance: the center of activity reaches a peak velocity of 361 mm/s in the swing-stance transition and 309 mm/s during the retraction-swing transition. The figure also shows up a transient spread of activity rostrally to slice 11-15 about two-thirds of the way through the stance phase. This transient is associated with EMG activity of VL and RF muscles. Like VL, RF extends the knee but it also flexes the hip. Its activation is followed immediately by that of its antagonists, BFp, gracilis, SMp and ST, with an associated caudal shift of the center of activation in the spinal cord.

The EMG profiles on which Fig. 4.4 and 4.5A were based were derived from many studies and their construction involved qualitative judgments and approximations. How sensitive are such data to parametric variations in the EMG profiles? Three parametric variations are illustrated in Fig. 4.5, B – D. In Fig. 4.5B we eliminated incremental modulations in the amplitudes of EMG profiles and used only the timing information (i.e., the profiles of Fig. 4.3 were converted to all-or-nothing

step functions such that all non-zero values were assigned the value 100%). As may be seen, the main effect of this was to reduce the net displacements of activity and to create more abrupt changes at phase transitions. The caudal detour of activity at the stance-swing transition was reduced. However the main conclusions drawn from Fig. 4.4 and 4.5A still held.

In Fig. 4.5, C and D we assessed the sensitivity of the plots in Fig. 4.4 and 4.5A to variations in the relative level of MN recruitment in flexor, extensor and retractor muscles. Fig. 4.5C shows the data of Fig. 4.4 recalculated on the assumption that extensor MNs were recruited to a maximum of 40% at peak EMG and flexor and retractor MNs were recruited to a maximum of 15% (40% and 15% were chosen on the basis of data comparing locomotion with more forceful movements such as paw-shakes in which recruitment approaches 100% (Chanaud *et al.*, 1991)). These values were switched in Fig. 4.5D: flexor and retractor MNs were assumed to reach 40% recruitment at peak EMG and extensor MNs were assumed to reach a maximum of 15% recruitment. In all cases the general features of the rostrocaudal movement of the center of activity shown in Fig. 4.5A are preserved.

In a separate analysis, we replotted Fig. 4.5A on the assumption that FDL had the extensor activity profile shown in Fig. 4.3 (*left*). This was performed in case the inclusion of FDL as a retractor may have given undue weight to the caudal detour of activity at the stance-swing transition. The plot obtained was hard to distinguish from that of Fig. 4.5A. We conclude that the stance-swing transition does indeed represent a distinct phase of migration of MN activity associated with retraction.

4.4 Discussion

In this study two sets of data were brought together from the literature to generate a spatiotemporal model of spinal MN activation during the locomotor step cycle. Several interesting

aspects of the functional anatomy of the spinal cord and the migration of MN activity during locomotion emerged. Though some of these were known in vague terms or could have been deduced from the literature, the model presents them in a precise form.

First, it is important to identify the assumptions and limitations in our analysis. First, the anatomical study on which the work depends (Vanderhorst & Holstege, 1997) did not distinguish between alpha and gamma MNs, so the number of alpha MNs in a given pool is probably overestimated. We assume that the same bias occurred in all the MN pools and so although the absolute numbers of alpha MNs is overestimated, the data provides an accurate picture of *relative* numbers of alpha MNs across pools.

Second, by making the number of active MNs in the spinal cord proportional to the amplitude of the EMG profile of a muscle, we tacitly assumed that EMG level is linearly related to net MUAP rate (and by extension to the net firing rate of corresponding spinal MNs). This is an important issue because if the relationship were highly nonlinear, the MN activity shown in the model could be significantly distorted. In an early human study, MU firing rates were found to increase with muscle force over the whole physiological range (Bigland & Lippold, 1954). In separate studies muscle force was shown to be fairly linearly related to EMG (Basmajian & De Luca, 1985). Taking these results together, it is safe to infer that MU firing rates increase with increasing EMG, but no firm conclusions can be made regarding the linearity of the relationship. In chronic recordings from single MNs during treadmill locomotion in cats, (Hoffer *et al.*, 1987) found that MN firing rates in most muscles were linearly related to the corresponding mass EMG, at least over the range of gait velocities studied.

The relationship between EMG and net MN firing rate has also been analyzed in two theoretical studies. In the first (Person & Libkind, 1970) EMG was modeled as the sum of triangular

MUAP waveforms. The integrated EMG was roughly proportional to the square root of net MUAP rate when MUAPs were assumed to be asynchronous. The relationship became more linear with increasing synchronization. Rate modulation of MUAPs was neglected in this study, but in a more recent simulation using large numbers of realistic MUAP waveforms derived from single unit EMG recordings (Day & Hulliger, 2001), this and other more physiological activation patterns were modeled. In most cases EMG was more linearly related to the number of MUAPs than in the Person study, e.g., in the range 0-50% MU recruitment, corresponding to medium-speed walking (Walmsley *et al.*, 1978), mean EMG increased fairly linearly with the net MU firing rate (deviations from linearity $<10\%$). Taking all this evidence together, the assumption of a linear relationship between EMG and net MUAP rate, though it is a simplification, is unlikely to produce major distortions in the estimates of net activity of MNs in the simulation.

Third, our EMG profiles were normalized, so the assumption implicit in Figs. 4.4 and 4.5A was that MN recruitment reaches the same maximal level in all muscles. This is also an oversimplification, and so we added two simulations in which the relative recruitment in flexors and extensors was varied. These parametric variations showed that although some of the subtleties of the migration of activity were either attenuated or exaggerated, overall, the main features of spatiotemporal migration of activity were present in all the tests. The modeling would of course be better focused if it were possible to record from a large number of muscles in the same animal, with similarly placed electrodes and identical signal processing.

Finally, some MN pools were not included in our analysis, either because EMG profiles were unavailable or because the anatomical data did not include them in enough detail. Generally these MN pools were much smaller than those we did include, the only notable exceptions being the large paravertebral muscles. Further data would be needed to fill in these gaps. However we do

not think that their inclusion would change our basic conclusions: first, the paravertebral muscles and their MN pools are distributed evenly over the whole lumbosacral region, so their activation profiles would add uniformly to those of the other pools and not significantly change the rostrocaudal migration of the center of activation. Second, back muscles are in any case not very active electromyographically during medium-paced locomotion at speeds less than 0.8 m/s in the cat (Zomlefer *et al.*, 1984).

Regarding our results, the first remarkable feature that emerges is the imbalance between the numbers of flexor and extensor MNs in the cat spinal cord. This leads to an imbalance in net MN activity during swing and stance phases, with a dominant seat of activity at a surprisingly caudal location (levels 80-85). If anything our simplifying assumption that all MN pools reach the same maximal recruitment level underestimated the imbalance, as flexors such as TA and EDL are not strongly activated during swing phase compared to their activity in powerful voluntary withdrawal movements (Prochazka, 1986), whereas MN recruitment in soleus, for example, may be virtually complete in locomotion(Walmsley *et al.*, 1978). The actual recruitment balance during medium-paced locomotion is therefore probably most closely represented in Fig. 4.5C, where it is assumed that extensors are recruited to a maximum of 40% and flexors to a maximum of 15%.

The preponderance of extensor MNs in both numbers and activity has a bearing on ideas about the structure of the locomotor CPG. Though Graham Brown probably did not mean his term "half-center" to be taken literally, the term does imply an equal weighting of flexor and extensor subdivisions (Brown, 1911). The familiar schematic of the CPG, a circle bisected into flexor and extensor halves, continues this tradition. Our model does not of course give any information about the distributions of interneurons, of which the CPG may be largely composed (Tresch & Kiehn, 1999), but at least at the MN level, it puts a different slant on flexor-extensor weighting. Note that it

has been suggested that MNs are in fact integral elements of CPGs (Marder, 1991; O'Donovan *et al.*, 1998). Furthermore, recent studies have shown that interneurons and MNs in the same spinal segment share the same phase of activity in the step cycle (Tresch & Kiehn, 1999), suggesting that the foci of MN activity in our model may also describe the activity of many associated interneurons, but of course not necessarily all. Finally one should remember that MN firing rate profiles do not necessarily correspond completely to the time course of their locomotor drive potentials, e.g., some MNs may depolarize in two phases of the locomotor cycle but only fire action potentials in one phase (Perret & Cabelguen, 1980).

The next feature to emerge is the rostrocaudal oscillation of foci of activity during the step cycle. As MN pools innervating muscles normally thought of as flexors (including BFp and ST) are distributed through the whole length of the lumbosacral enlargement, the alternation between a caudal focus of MN activity in the stance phase and a rostral focus in late swing was not a given. Our first impressions from the movie were of a wave of activation propagating smoothly back and forth along the spinal cord. Initially we felt that this was supported by the smoothness of the trajectories of the centers of activation we published in abstract form (Yakovenko *et al.*, 2000). Propagation of activity along the neuraxis has been implicated in the orderly sequencing of activation of segmented musculature in different species (Orlovsky *et al.*, 1999). However, the rostrocaudal transitions shown in Figs. 4.4 and 4.5 are in fact quite abrupt, so if smooth propagation from one set of neurons to the next is involved, it is rapid. Our model seems more consistent with the idea of abrupt switching between three groups of MNs: extensor MNs active during late swing and most of stance, retractor MNs being active in late stance and early swing and ankle and hip flexor MNs being active in swing only.

In the intact animal, stance-swing and swing-stance transitions in normal over-ground locomotion are under sensory control: when certain sensory conditions are met, phase switching is triggered (Prochazka, 1993). It will therefore be interesting to compare the present results with those obtained when sensory input is absent (e.g., in fictive locomotion). In Results we pointed out a possible link between MN distribution and biomechanical action: in the pendent limb, rostral MNs would evoke forward movements of the foot and caudal MNs would evoke backward movements of the foot. However in real-life locomotion, movements are affected by weight-bearing so the activity of some muscles no longer fits this simple dichotomy. The spatiotemporal migration of activity in the spinal cord may then differ significantly from that in unloaded movements such as air-stepping and paw-shakes.

Regarding BFp, gracilis, SMp, ST and FDL, these muscles extend the hip and flex the knee and toes (gracilis also has an adductor role). Their main EMG bursts occur when the foot lifts off at the end of the stance phase (Engberg & Lundberg, 1969; Halbertsma, 1983), though FDL may also exhibit some activity earlier in stance (O'Donovan *et al.*, 1982; Loeb, 1993). In mid-stance the moment arms of the posterior portions of BFp, gracilis, SMp and ST about the knee are larger than those about the hip (Goslow *et al.*, 1973). But in late stance and early swing, if the knee becomes very extended, the moment arms about hip and knee become similar. The biomechanical action at foot lift-off is therefore a backward and upward movement of the foot and toes with respect to the hip, this motion being crucial for ground clearance. Thus BFp, gracilis, SMp, ST and FDL complete the backward movement of the foot that develops in the stance phase and initiate the upward movement that is then maintained by flexor muscles during swing. Sherrington listed gracilis, BFp and ST muscles as being engaged in the flexion reflex of decerebrate cats, yet in his Table 2 he lists BFp, FDL and SM as being inhibited along with pure extensors when the flexion reflex was re-

applied during a rebound extensor contraction (Sherrington, 1910). During reflex stepping and standing, BFp, gracilis and ST behaved as pure flexors while SM and FDL were classified as extensors. Short-latency (presumed group Ia) reflex connections measured between various hip and knee muscles in lightly anesthetized cats added to the uncertainty of classifying BFp, gracilis and ST as flexors (Eccles & Lundberg, 1958).

In view of these various anomalies, as well as the results of our spinal cord modeling we suggest that in locomotion BFp, gracilis, SMp, ST and FDL muscles be classified as *retractors*, their role being to complete the backward movement of the foot and toes with respect to the hip (i.e., to complete the extensor thrust of the stance phase) and then to lift the foot in preparation for forward swing. This is not to deny that they can act differently in other tasks: e.g., BFp is strongly activated during flexion phases of withdrawal movements of the limb (Prochazka, 1986) and gracilis has a significant adductor role (Sherrington, 1910). From the point of view of the spatiotemporal flow of activity in the spinal cord the activation of the BFp, gracilis, SMp and ST MN pools involves a small transient shift caudal from the focus of extensor activity in the stance phase. Activity then switches rapidly to the rostral flexor centers.

Regarding the hypothesis of discrete "unit" oscillators controlling hip, knee and ankle (Grillner & Wallen, 1985; Kiehn & Kjaerulff, 1998), Fig. 4.1 shows the actual anatomical positions of the three sets of MN pools that would be involved. If such unit oscillators exist and MNs are part of them (Marder, 1991; O'Donovan *et al.*, 1998), they are evidently widely spaced rostrocaudally and so they would require long and overlapping propriospinal connections. For example, if we equate the centers of the unit oscillators with the centers of mass of their respective MN pools, the two components of the hip oscillator would be separated by 61 levels (17.5 mm in an average-sized spinal cord), those of the knee oscillator by 58 levels (16.7mm) and those of the ankle oscillator by

only 20 levels (approximately 5.6 mm). The rostrocaudal order of the knee flexor and extensor half-centers would be the reverse of that of the hip and ankle half-centers.

The approach introduced in this paper opens up a variety of interesting possibilities. Spatiotemporal patterns of spinal cord activation could be modeled and compared in various preparations (e.g., normal, decerebrate and spinal locomotion), in different types of cyclical movement (e.g., paw-shake response and scratching) and using different representations of MN activity, e.g., intracellular recording. Models of this type could be extremely useful if and when spinal cord neuroprostheses are developed for people with spinal cord injury. The locomotor system of the cat spinal cord is not the only pattern generator amenable to this form of analysis. Any system in which the CNS distribution of neurons and the activity profiles of their target organs in the periphery are reasonably well characterized could be analyzed in this way. The dynamic models and the spinal cord movie described in this paper may be obtained in digital form from the authors.

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CHAPTER 5

The adjustments of phase and cycle durations in MLR-induced fictive cat locomotion. Implications for organization of the pattern generating circuitry.

5.1 Introduction

The study of locomotion dates back to La Mettrie's observations of rhythm generation in animals with cervical spinalization (Mettrie, 1745) and the first cinematic recordings of Muybridge (Muybridge, 1887). Apart from the neurophysiological interest, a detailed knowledge about the control of locomotion is useful in devising medical strategies to relieve locomotor disabilities in people due to trauma or debilitating diseases. The work of T. Graham Brown and Sir Charles Sherrington in the early 20th century was very influential in establishing the basic neural circuitry involved in the generation of locomotor activity. Even in the absence of supraspinal control the locomotor rhythm can be generated by the spinal cord and is subject to modification by sensory feedback. This spinal pattern generating apparatus with at least two antagonistically organized groups of neurons named half-centers was first called "the intrinsic factor" (Brown, 1911) and was later renamed the "central pattern generator" (CPG) (Grillner, 1975). A number of experiments have localized and studid the locomotor pattern generating circuitry in reduced animal preparations (Deliagina *et al.*, 1983; Ho & O'Donovan, 1993; Kjaerulff & Kiehn, 1996; Arshavsky *et al.*, 1997; Cowley & Schmidt, 1997; Kremer & Lev-Tov, 1997). In such studies, the locomotor rhythm was usually evoked by the application of pharmacological agents such as L-DOPA (L-3,4-dihydroxy-phenylalanine), mechanical stimulation of the perineum or electrical stimulation of brainstem structures or dorsal roots. Studies have shown that the control of timing and amplitude of motoneuron discharge is mediated not only by the descending pathways (Shik *et al.*, 1966; Shik & Orlovsky, 1976; Armstrong & Drew, 1984; Drew, 1988; Beloozerova & Sirota, 1993), but also by movement-evoked sensory feedback (Conway *et al.*, 1987; Guertin *et al.*, 1995; Hiebert *et al.*, 1996; Prochazka, 1996; Whelan & Pearson, 1997; Lam & Pearson, 2001). Though the location and neural structure of the locomotor network in vertebrates are poorly understood, some details of neural

connectivity, membrane properties and correlation with function are available for invertebrate species (Kristan & Weeks, 1983; Kopell, 1988; Marder & Bucher, 2001; Cymbalyuk *et al.*, 2002).

Attempts have been made to deduce the general organization of the CPG on the basis of the mutability of the patterns of muscle activation during different locomotor behaviors, e.g. comparing forward, backward, uphill and downhill walking (Grillner, 1981; Buford & Smith, 1993; Trank *et al.*, 1996; Smith *et al.*, 1998). In these studies, Brown's simple model of half-center oscillators is usually subdivided further into oscillators controlling synergies of muscles around each joint. Recently, a spatio-temporal analysis of the hindlimb motoneuron activity underlying cat locomotion was performed, which combined information about EMG activity in hindlimb muscles with the known distribution of motoneuron pools within the lumbosacral spinal cord (Yakovenko *et al.*, 2002). This revealed a rostro-caudal oscillation of motoneuron activity in the spinal cord, which has intriguing implications for the organization of the controlling pattern generating circuitry. If some or all of the interneuronal network of the CPG is distributed and co-localized with corresponding motoneurons (Ho & O'Donovan, 1993; Kjaerulff & Kiehn, 1996; Raastad & Kiehn, 2000; Tresch & Kiehn, 2000) the locus of activity of the motoneurons may also represent that of the entire controlling CPG network. The results of the analysis of motoneuron activity during normal gait are consistent with the hypothesis that flexor and extensor activity is spatio-temporally separated in the step cycle and that phase transitions are switch-like and not wave-like, as suggested elsewhere (Bonnot *et al.*, 2002; Kaske *et al.*, 2003; Patel *et al.*, 2003).

In a separate modeling study we became aware that the timing of transitions between the locomotor phases is important for adaptation of the CPG rhythm to relevant behavioral demands, e.g. an increase in the speed of locomotion. During normal overground gait an increase in the speed of locomotion is usually accompanied by a decrease of the step cycle duration, mostly due to

the shortening of the stance phase. The duration of the swing phase remains relatively constant (Goslow *et al.*, 1973; Halbertsma, 1983). The same modulation strategy of flexor and extensor phases was reported 1) in the locomotor activity of spinalized cats elicited by perineal stimulation and the administration of clonidine (Forssberg & Grillner, 1973; Pearson & Rossignol, 1991), and 2) in fictive locomotion of decerebrate cats evoked either by stimulation of the mesencephalic locomotor region (MLR) (Jordan, 1998), intravenous administration of L-DOPA (Pearson & Rossignol, 1991; Leblond & Gossard, 1997) or occurring spontaneously (Dubuc *et al.*, 1985). These results suggest that the CPG is hard-wired to produce adjustments of step cycle duration by modulating extensor, rather than flexor bursts. Here we report a novel observation that during fictive MLR-induced locomotion, cycle duration is modulated by mainly adjusting the duration of flexor bursts.

5.2 Methods

5.2.1 Surgical procedures

All data reported in this paper originated from experiments done for other reasons in David McCrea's laboratory, University of Manitoba (Gosgnach *et al.*, 2000; Quevedo *et al.*, 2000). All surgical and experimental protocols were in accordance with the guidelines set out by the Canadian Council for Animal Care. Experiments were performed on 31 cats (weight 2.2 – 3.2 kg). The animals were anesthetized with halothane delivered in a mixture of 30% oxygen and 70% nitrous oxide for the duration of the surgery. Surgical procedures consisted of a tracheotomy, cannulation of arteries and veins, dissections of various hindlimb nerves and a craniotomy. Mechanical, precollicular-postmamillary decerebration was performed by removing all the tissue

rostral to the transection. This procedure makes animals insentient (legally dead) and allows discontinuation of anesthetic. The neuromuscular blocker gallamine triethiodide (Flaxedil, 2-3 mg kg-1 h-1) was used to abolish movement and the animals were artificially ventilated. Fictive locomotion was elicited by unilateral or bilateral monopolar electrical stimulation of the mesencephalic locomotor region (MLR, 30-200 μA, 0.5-1 ms pulses, 7-30 Hz) with tungsten microelectrodes. Selected hindlimb nerves were placed on conventional bipolar hook electrodes for recording. The following nerves were included: biceps femoris posterior (BFp) or sometimes BFp and semitendinosus together (PBST), extensor digitorum longus (EDL), flexor digitorum longus (FDL), flexor hallucis longus (FHL), FDL and FHL (FDHL), gastrocnemius and soleus (LGS), peroneus longus (PerL), plantaris (Plan), rectus femoris (RF), sartorius (Sart), semimembranosus and biceps femoris anterior (SMAB), tibialis anterior (TA), and vasti. In some experiments vasti and RF were mounted on the same recording electrode.

Up to 12 nerves were recorded simultaneously in each cat. Since these preparations were intended for different studies, a laminectomy was performed usually from the L4 to the S1 regions in some animals but in others it was more extensive including thoracic and cervical segments. A lethal injection of pentobarbital anesthetic was administered at the end of the experiment.

5.2.2 Data recording and analysis

Electroneurogram (ENG) activity in hindlimb muscle nerves listed above was filtered (30 Hz - 3 KHz), rectified and digitized at 500 Hz. The smoothed waveforms were then analyzed to determine onsets and terminations of bursts using an adaptive threshold crossing method described below. Recordings with a signal-to-noise ratio less than 5 were rejected. Cycle period was determined as the time between two onsets of the ENG envelope. We intentionally restricted the

analysis to cycles with periods between 0.5 and 1.5 sec corresponding to speeds of overground locomotion between 0.2 and 1.2 m/sec (Halbertsma, 1983). Regression lines were fitted to scatter plots of burst duration and cycle period for individual nerves. One-way analysis of variance (ANOVA) and multiple comparison of regression line slopes with Tukey's honestly significant difference criterion was performed to determine significant differences ($\alpha=0.05$).

5.2.3 Adaptive threshold crossing method

Consider a portion of a noisy, time-varying signal. Now consider a horizontal line representing a threshold level, moving upward through the signal (Fig. 5.1A). Within the noise band, there are many cross-overs of the noisy signal and the threshold line. This number reaches a peak somewhere within the noise band. As the threshold line moves above the noise band and into the region of the "real" signal, the number of cross-overs drops and then remains fairly constant. We called the minimal threshold above the noise as an optimal threshold (dotted line *c* in Fig.5.2). Finally, as the line moves up into the region of signal peaks and beyond, the number of crossovers first increases and then declines to zero. Thus, we can use a simple algorithm to detect the optimal threshold and identify periods of noise from periods of signal. Then we can quantify the mean and the standard deviation of the noise and signal periods to calculate the signal-to-noise ratio. If the quality of the recording is satisfactory, we can identify onsets and terminations of bursts by calculating the sign of the slope at the intersection points (positive slope corresponds to onset, negative slope corresponds to termination). Finally, the mean and the standard deviation of the noise periods can be used to adjust the time of the onsets and the terminations to the smallest values of the signal statistically different from the noise. The detection quality of the method was similar to that of manual selection with the obvious advantages of automation and objectivity.

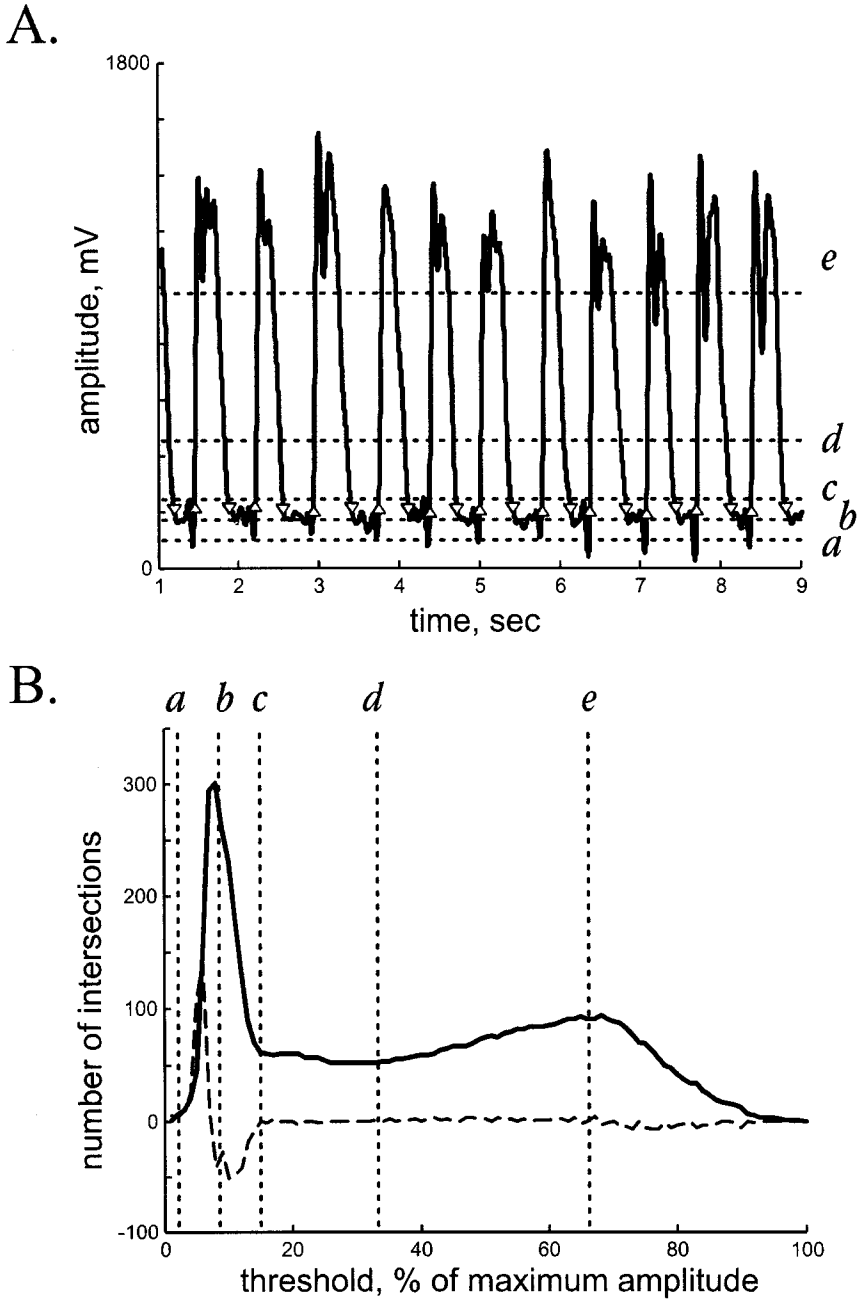


Figure 5.1. An adaptive optimal threshold method for detection of onsets and terminations of bursts. Horizontal and vertical *dotted* lines represent various threshold levels (*c* is the optimal threshold) ***A.*** A section of ENG recording analyzed with the optimal threshold method. Upward and downward triangles correspond respectively to the adjusted positions of onsets and terminations for each burst (see Methods for details). ***B.*** The number of intersections (*solid* line) and its differential (*dashed* line) are plotted as functions of the threshold level. The optimal threshold level (*c*), which corresponds to the minimum level above noise, can be found as a minimum threshold following a positive and then a negative slope of the function of intersections (*solid* line).

5.3 Results

As discussed above previous studies of the variations of swing and stance durations during locomotion have demonstrated that changes in cycle period are due less to changes in swing phase and corresponding flexor bursts than to the duration of stance phase and extensor bursts. Our study originated from a chance observation that this did not always hold in MLR-induced fictive locomotion.

In any given cat, we found that either the extensor bursts or the flexor bursts occupied more than 50% of the step cycle period in nearly all cycles (i.e. a given cat showed extensor-dominated or flexor-dominated behavior). Furthermore, the *amount of variation* in burst duration *also* seemed to depend on the cycle occupied. We therefore initially decided to divide the data into two groups, corresponding to extensor-dominated and flexor-dominated behavior.

Figure 5.1A shows a typical recording of nerve activity during fictive locomotion evoked by the stimulation of MLR in a paralyzed preparation. Note that the burst duration in this recording dominated the duration of the locomotor cycle, it occupied more than 50% of the cycle duration. During overground locomotion in the normal cat this relationship is characteristic of extensor-related activity. However, in this case the depicted activity is that of a flexor nerve (Sart). This intriguing relationship prompted us to examine the phase vs. cycle relationship in this preparation. Using the adaptive threshold crossing analysis described in *Methods* we were able to identify onsets and terminations of bursts during fictive locomotion in 31 animals. The locomotor patterns were divided into groups based on the percentage of the cycle occupied by bursts of nerves defined as flexors: Sart, TA, EDL, PerL and the following as extensors: GS, FHL, Plan, SMAB. This is in accord with the generally accepted classification of nerves, based on the predominant biomechanical

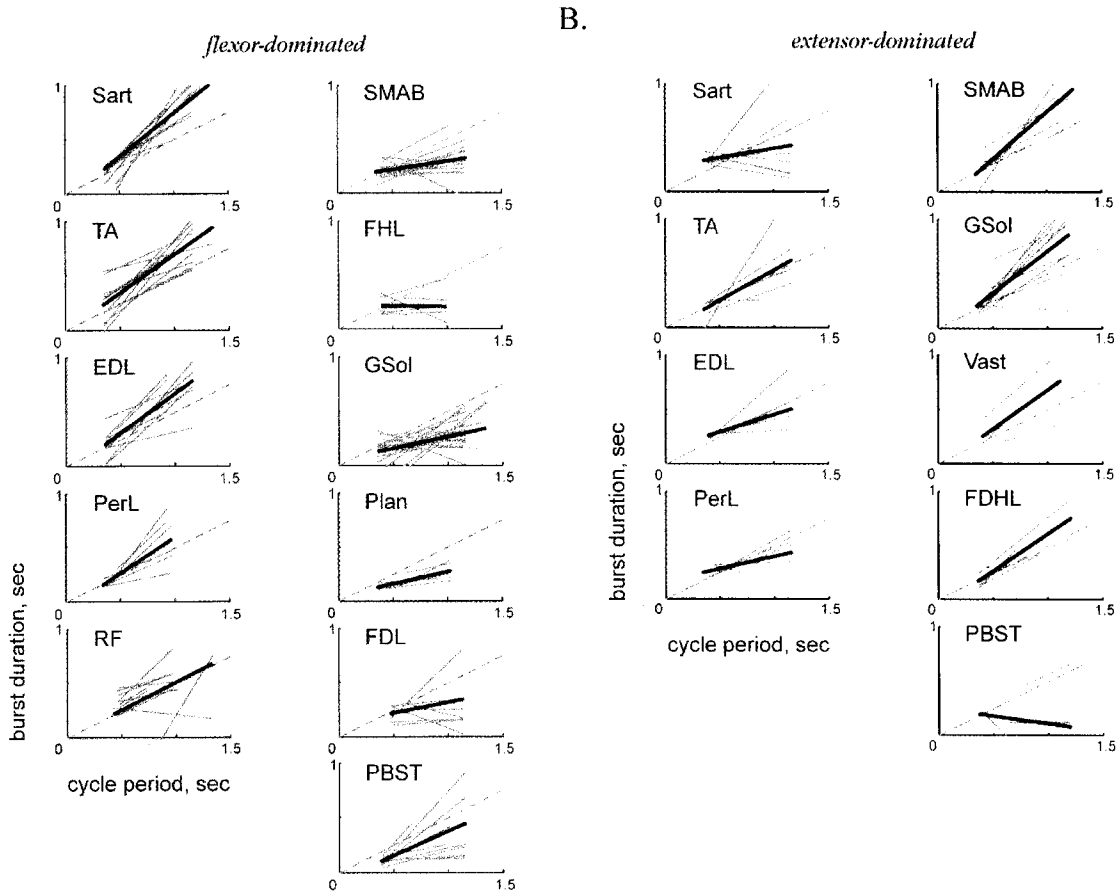
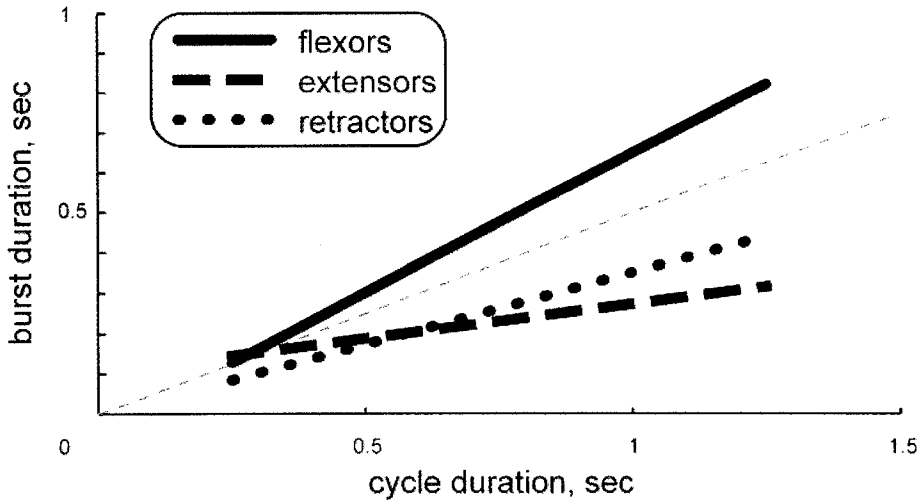


Figure 5.2. The relationship between phase and cycle duration for the hindlimb nerve activity when the cycle duration is dominated by either flexors (*A*) or extensors (*B*). The *dashed* line represents a slope of 0.5, which corresponds to the burst duration equal to 50% of the cycle duration. Thick line is the median of the sample data.

A. *flexor-dominated*



B. *extensor-dominated*

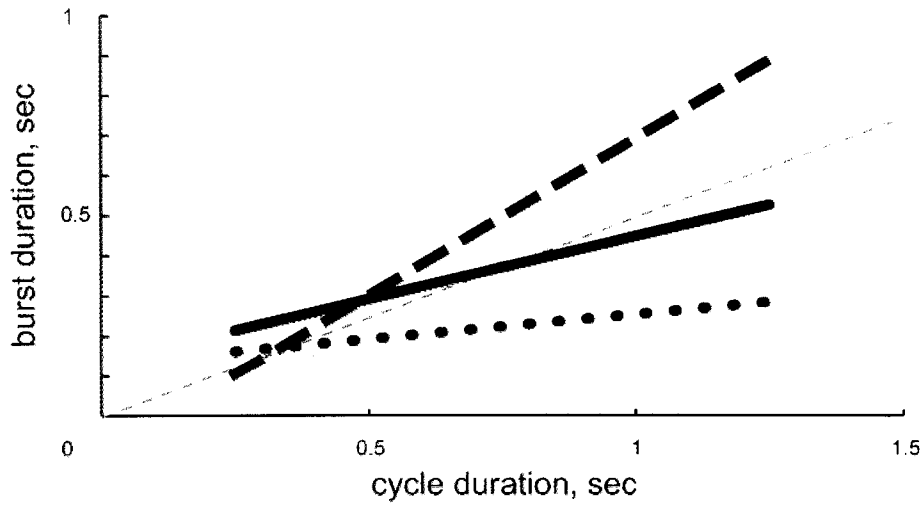


Figure 5.3. The relationship between phase and cycle duration for flexor (*solid* line), extensor (*dashed* line) and retractor (*dotted* line) hindlimb nerve activity when the step cycle duration is dominated by either flexors (*A*) or extensors (*B*).

action of the corresponding muscles in flexor and crossed extensor reflexes (Sherrington, 1910; Eccles & Lundbert, 1958) and the spatio-temporal activation of the corresponding motoneurons in the spinal cord during overground locomotion (Yakovenko *et al.*, 2002).

We found that our sample contained locomotor patterns dominated by flexor activity in 22 experiments and by extensor activity in 9 experiments. Figure 5.2 shows the lines of best fit in the relationship between burst duration and cycle duration of various hindlimb nerves. Notice that during the flexor-dominated fictive locomotion in Figure 5.2A the lines of best fit of flexor activity are mostly above the dashed line. The dashed lines represent a slope of 0.5 or 50% of cycle duration, while those of extensor activity are below this line. The opposite relationship is demonstrated in Figure 5.2B. Notice that the slopes of flexor lines of best fit in Figure 5.2A are steeper than those in Figure 5.2B; the opposite is true for the extensors. The significance of this is discussed later.

Figure 5.3 shows the mean lines of best fit in Figure 5.2 now combined into *three* groups: flexors, extensors and retractors. The latter group contains the data from FDL and PBST nerves, which showed more variability than other nerves (Fig.5.4), and have elsewhere been distinguished as a separate "retractor" group based on their spatio-temporal activity during normal gait and their biomechanical action (Yakovenko *et al.*, 2002). The slopes of the extensor and flexor lines of best fit are similar to those found in normal cats during overground gait (see Fig.6 in Halbertsma, 1983). In contrast, in the flexor-dominated group in Figure 5.3A, flexor slopes were greater than extensor slopes. Taking Fig.5.3A and 5.3B together, a more general statement can be made. The variation in burst duration depends on the percentage of the cycle occupied. Thus, extensors vary the most if they are dominant; flexors vary the most if they are dominant. During flexor-dominated locomotion

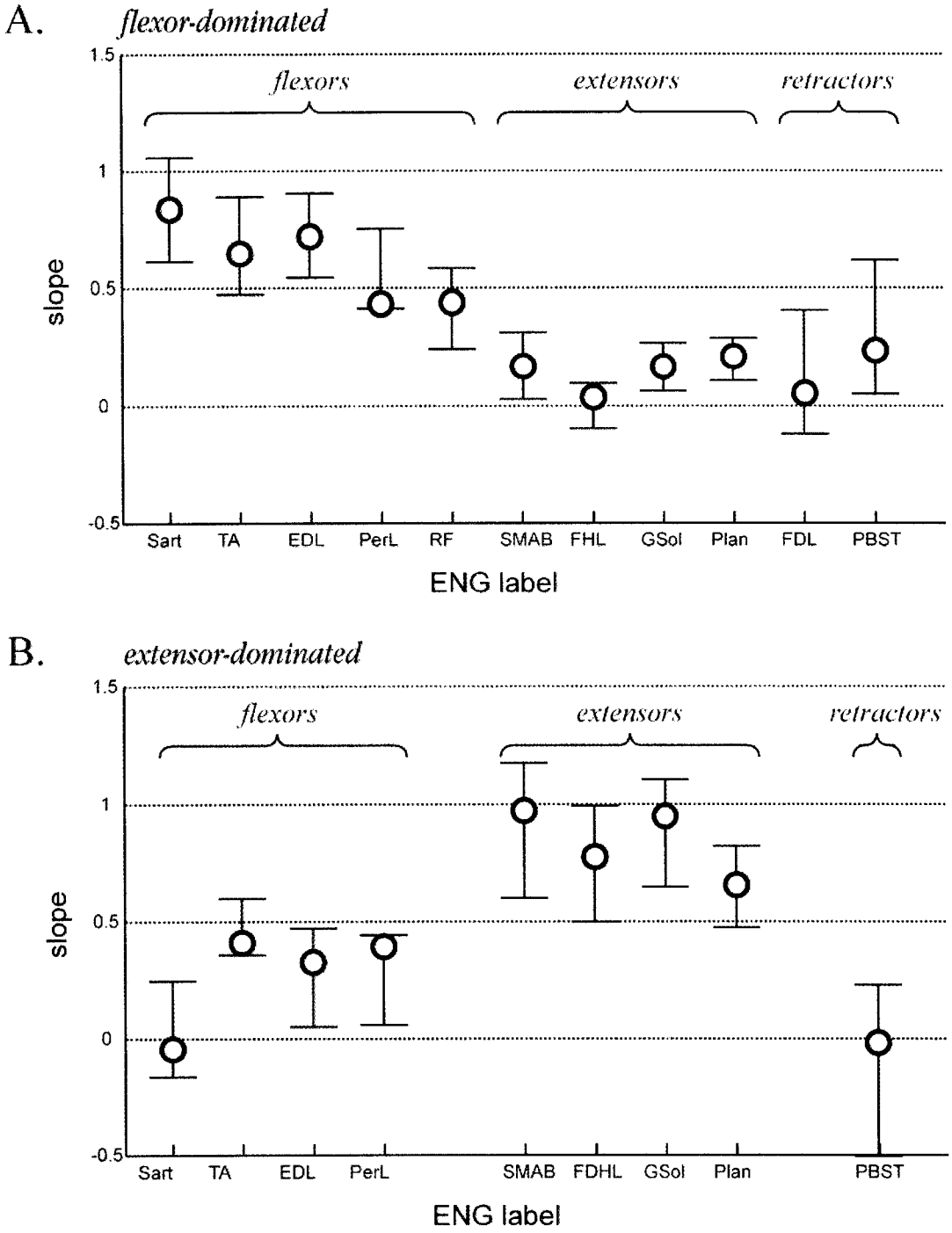


Figure 5.4. The result of a one-way ANOVA of the slopes of the relationship between phase and cycle duration of hindlimb nerve activity when the step cycle duration was dominated by either flexors (*A*) or extensors (*B*). The "whiskers" show the 95% confidence interval about the median (*circle*) of a sample.

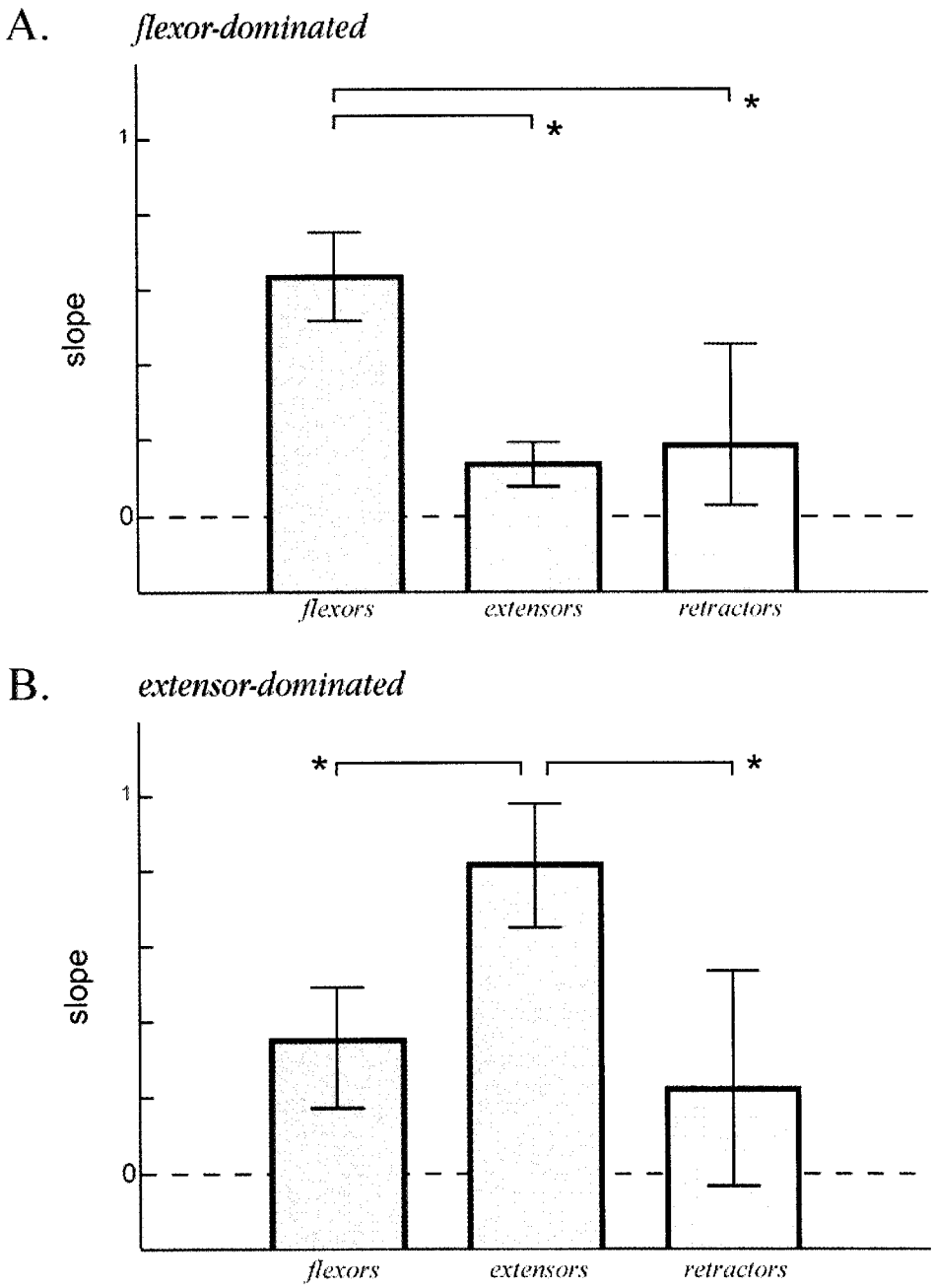


Figure 5.5. The result of the one-way ANOVA and multiple comparisons of the slopes of the phase modulation with Tukey's honestly significant difference criterion in flexor (***A***) and extensor (***B***) dominated fictive locomotor activity. The "whiskers" show the 95% confidence interval about the median of a sample.

(Fig. 5.3A) the mean slope of flexors (solid line) was 0.69, while that of the extensors was 0.17. Though the burst duration of the retractor (bifunctional) group was less than 50% of the step cycle for both the flexor and the extensor -dominated cycles, the slope of the retractors was close to that of extensors during flexor-dominated locomotion (0.36) and it was closer to flexors during extensor-dominated locomotion (0.12) (Fig. 5.3B). This result is in agreement with a study (Perret & Cabelguen, 1980), which demonstrated that activity of this group was shaped by converging inputs that excite flexors and extensors.

Fig. 5.4 and 5.5 deal with the statistical analysis. Figure 5.4 shows the results of a one-way ANOVA of the slopes of the phase adjustments during flexor and extensor -dominated fictive locomotion. It is worth noting the large variability in the slopes of FDL and PBST nerves, which again could be an indication of the convergent influences from the flexor and the extensor pattern generating networks. The mean RF slope was not significantly different from those of flexors or extensors. This might indicate a mixed functional role, or it may simply have been due to the RF nerve having been mounted together with the nerve of vasti in some experiments. Figure 5.5 shows a comparison of the means in the groups of Fig.5.3. In flexor-dominated (Fig. 5.5A) and extensor-dominated (Fig.5.5B) fictive locomotion, the slopes of the flexor and extensor groups of nerves were significantly different. The slopes of the retractor and flexor groups were significantly different from each other during flexor-dominated locomotion. During extensor-dominated locomotion, the slopes of the retractors were significantly different from those of the extensors.

5.4 Discussion

During normal overground level locomotion, adjustments of step cycle duration are mainly due to variations in stance duration (Goslow *et al.*, 1973; Halbertsma, 1983). Several features of

overground locomotion show a bias toward extensor-related activity (Fig. 5.6A). During slow walking the duration of stance dominates the step cycle period (Prochazka *et al.*, 1989; Yakovenko *et al.*, 2002). Because of the anti-gravity role of the extensor muscles, they exert proportionally higher forces than flexors during locomotion (Chanaud *et al.*, 1991). In addition, sensory pathways reinforce extensor activity during stance (Duysens & Pearson, 1980; Whelan *et al.*, 1995). Does this imply that the pattern generating circuitry is hard-wired to favor extensor-related activity?

We addressed this question by analyzing the variability of nerve activity during MLR-induced fictive locomotion. When the duration of the extensor phase dominated the duration of the step cycle (>50%) in fictive locomotion, the phase adjustments during spontaneous changes in cycle duration were similar to those during normal gait. The novel finding of this study is that the reverse behavior was also observed during MLR-induced fictive locomotion. When cycle duration was dominated by flexor activity, the phase adjustments were mainly due to variations in flexor phase duration. Grillner and Zangger noted similar behavior in one of the fourteen spinalized animals during DOPA-induced fictive locomotor activity (see Fig.6 in Grillner & Zangger, 1979). This result suggests that the locomotor pattern generating networks can be equally biased towards either extensor or flexor activity. This agrees with Brown's prediction that the locomotor pattern generating networks for each limb contain at least a "pair of antagonistic opposites", or analogous antagonistic half-centers (Brown, 1911).

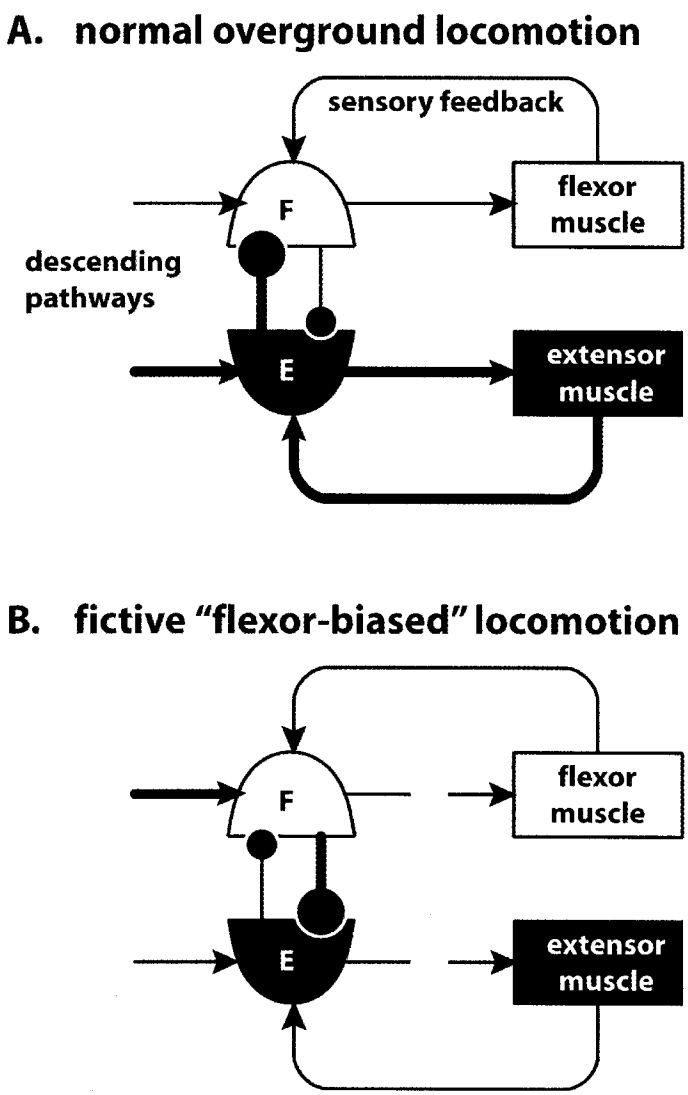


Figure 5.6. Simple schematic of the locomotor pattern generator. *A.* During normal overground locomotion, activity of extensor half-center is biased by descending and sensory pathways (*thick lines*) to cope with weight-bearing. *B.* During MLR-induced fictive locomotion, motion-related feedback is lost and descending pathways are possibly biased towards flexors.

We also found a relationship between phase duration and the variation in burst duration during spontaneous changes in cycle period. From this we propose the following rule: the variation in burst duration depends on the percent of the cycle occupied. This rule describes phase adjustments during normal overground locomotion (Goslow *et al.*, 1973; Halbertsma, 1983), spontaneous locomotion in decorticate preparation (Grillner & Dubuc, 1988), locomotion induced either by perineal stimulation (Pearson & Rossignol, 1991) or by noradrenergic agonists in chronically spinalized preparation (Grillner & Zangger, 1979).

Clearly, the speed of overground gait can be adjusted using two distinct strategies, by changing either the duration of the step cycle or the stride length. From Halbertsma's regression Eq. 1-4 (Halbertsma, 1983) for slow and fast walking, it is clear that the step cycle duration strategy contributes more than the stride length strategy to the adjustment of speed during overground locomotion. This agrees with the finding that an increase of the stride length leads to a higher loss of mechanical energy due to the collisions with the support surface (Donelan *et al.*, 2002). The locomotor system may try to minimize this energy loss. For this reason the step cycle duration strategy may be the preferred solution.

Why does the nervous system avoid adjusting the duration of swing to the same degree as that of stance during normal gait (Goslow *et al.*, 1973; Halbertsma, 1983)? Perhaps, the interactions of the locomotor system with the ground impose mechanical constraints that prevent the use of flexor phase adjustments of speed. To illustrate this suggestion, consider the situation in which an increase in flexor muscle activity and swing duration allows a more forward placement of the foot relative to the body. Then, more kinetic energy is lost due to ground contact and still more work is required to bring the centre of mass over the more distant point of support. Without an additional increase in extensor muscle thrust and / or prolongation of the stance phase the body would lose

all its forward momentum and stall. On the other hand, an increase in the stride length due to increased extensor thrust imparts a higher mechanical energy to the body, which is sufficient to counteract collision losses and allows stable locomotion at a higher speed. The locomotor system is therefore predisposed in favor of extensor phase adjustments strategy during overground locomotion. The alternative flexor phase adjustment strategy might be observed only when interactions with the ground are abolished. This hypothesis was supported by our results and by the increased flexor phase adjustments during air-stepping (Smith *et al.*, 1986).

We propose that the pattern generated by the locomotor CPG depends on the distribution of excitation between flexor and extensor half-centers, which are analogous and antagonistic. In this model, the level of excitation of each half-center can then be modified by inputs from descending and sensory pathways. A shift in the excitatory bias to one of the half-centers may lead to an increase in the duration of the corresponding phase of the cycle and a decrease in the antagonistic phase through reciprocal neural interactions (Cymbalyuk *et al.*, 2002). In our study, 22 out of 31 cats were predisposed to produce flexor dominated fictive locomotion, which may be explained by inputs to the flexor half-center through the descending pathways evoked by stimulation of MLR in decerebrated cats (Orlovsky, 1970; Shimamura *et al.*, 1982) and the absence of motion-related feedback, which would normally provide excitatory input to the extensor half-center (Fig. 5.6B).

The proposed model can describe a number of reported observations of phase modulation. Unloading in stance decreases stance phase duration in cats (Duysens & Pearson, 1980) and human infants (Pang & Yang, 2000). Electrical stimulation of extensor group I afferents increases the extensor phase duration in decerebrate (Whelan *et al.*, 1995) and fictive (Guertin *et al.*, 1995) locomotor preparations. During fictive locomotion of cats a few days after spinalization, progressive

extension of the hindlimb, which increases proprioceptive feedback to flexors, increased the rate of flexor phase modulation (Pearson & Rossignol, 1991). The flexor phase duration can be also increased by electrical stimulation of flexor group II afferents during stance (Perreault *et al.*, 1995; Hiebert *et al.*, 1996) and flexor group I afferents during swing (flexor) phase (Perreault *et al.*, 1995; Hiebert *et al.*, 1996; Lam & Pearson, 2001) in decerebrate and fictive cat locomotor preparations.

The functional relevance of the proposed balance of activity between flexor and extensor half-centers may also explain the phase changes observed during upslope and downslope locomotion (Carlson-Kuhta *et al.*, 1998; Smith *et al.*, 1998). For example, during downslope locomotion, progressive increase in slope decreases loading of the hindlimb extensors and requires higher activation of flexors. In this case, the excitatory motion-related sensory feedback to the extensor half-center would be decreased and that to the flexor half-centers would be increased. Our model would then predict that the duration of the extensor (stance) phase would decrease with the increasing downward slope, a result observed in intact cats (Smith *et al.*, 1998). During upslope locomotion, loading of both extensors and flexors is increased. However, a small increase in slope changes extensor loading only slightly, but requires a significant increase in flexor activity most likely to prevent tripping. Thus, we can predict that the duration of swing will increase for locomotion on shallow and moderate upward slopes and decrease for steep slopes, because of the increased extensor load. This pattern of phase modulation is observed experimentally in cats walking up variable slopes (Carlson-Kuhta *et al.*, 1998).

To conclude, in this paper we report a previously unreported flexibility of the locomotor pattern generating circuitry, which can be explained by changes in excitation bias from descending and sensory pathways in the half-center model of the locomotor pattern generator. The proposed

mechanism provides a new way of looking at the role of descending inputs and proprioceptive feedback in regulation of stereotypical behaviors.

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CHAPTER 6

The propriospinal hypothesis of spinal cord regeneration

6.1 Introduction

Many recovery strategies are currently under development to minimize extent of the spinal cord injury and to restore lost functions. Unlike in the peripheral nervous system (PNS), damaged neurons in the central nervous system (CNS) fail to regenerate because of the prohibitive environment attributed to the presence of growth inhibitory molecules associated mostly with proteins in myelin (Schwab & Thoenen, 1985; Caroni & Schwab, 1988) and formation of scar tissue. To overcome this non-permissive environment, Aguayo and colleagues conducted a pioneering study, where long distance axon regeneration was achieved in to a graft of peripheral nerves in the CNS (Aguayo *et al.*, 1981; Bray *et al.*, 1987).

Recently, several groups reported successful long distance regeneration across the spinal cord lesion in completely spinalized rats, which exhibited significant functional improvements, i.e. restoration of weight-bearing and coordinated locomotion (Cheng *et al.*, 1996; Ramon-Cueto *et al.*, 2000; Coumans *et al.*, 2001). These results are particularly crucial because rats do not recover spontaneously (Basso *et al.*, 1996) without extensive locomotor training (Edgerton *et al.*, 2001). Another surprising feature of these results is that significant behavioural improvements were achieved with a limited amount of regeneration. While the circuitry controlling locomotion is located in lumbar spinal segments (Cazalets *et al.*, 1995; Cowley & Schmidt, 1997; Kiehn & Kjaerulff, 1998), the regenerated axons extend only one or two spinal segments below a thoracic lesion (T8-T9), in most cases about 20 mm short of the lumbosacral locomotor region (Ramon-Cueto *et al.*, 1998; Rapalino *et al.*, 1998; Bamber *et al.*, 2001).

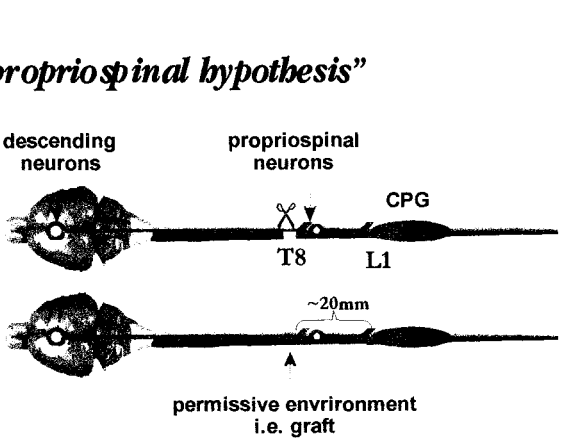
What is the mechanism of the functional recovery? One likely explanation is that regenerating axons make connections with propriospinal neurons, which extend caudally and activate hindlimb locomotor circuitry in the lumbar spinal cord (Figure 6.1A). Recently, new evidence in support of this hypothesis has demonstrated that injured axons of the corticospinal tract make spontaneous connections with propriospinal neurons (Bareyre *et al.*, 2004). If this were also the case in the studies with long axon regeneration through a complete spinal cord lesion, non-specific activation of descending propriospinal pathways immediately below the lesion might explain the observed improvements in motor performance of completely spinalized animals (Figure 6.1B). The goal of this study was to test this hypothesis.

6.2 Methods

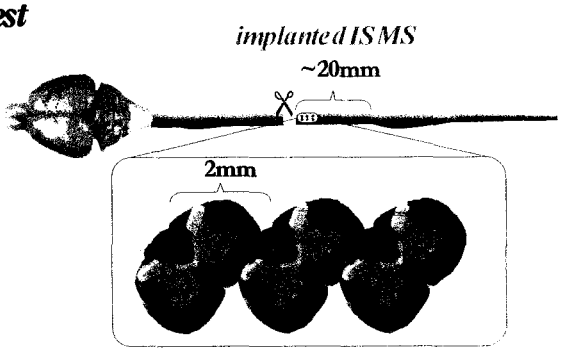
Animals. The experiments were performed on 9 adult Sprague-Dawley female rats weighing 200-250g. Animal treatment and surgical procedures were approved by the University of Alberta Health Sciences Animal Welfare Committee. They conformed to the guidelines of the Canadian Council on Animal Care.

Spinal cord transection. Animals were deeply anesthetised with isoflurane and underwent an aseptic laminectomy at thoracic T8 spinal level. Complete spinal cord transections 2-3 mm wide were produced with the use of fine suction pipettes at T8 spinal segment until no neural tissue remained and the inside of the dura could be visualized over the entire circumference. The musculature and skin were suture closed, analgesic (Buprenorphine, 5 μg/kg every 8 h) was administered and animals were placed in a heated environment until full postoperative recovery.

A.



B.



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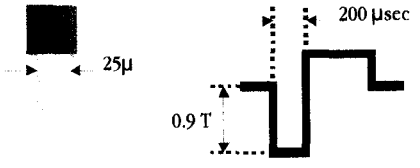


Figure 6.1. Regeneration hypothesis and methods. A. The "propriospinal hypothesis" attributes observed behavioural improvements of the axon regeneration through nerve grafts to nonspecific innervation of propriospinal neurons, which activate lumbosacral locomotor circuitry several segments below the lesion. B. Completely spinalized animals were implanted the ISMS arrays below the lesion to activate intermediate and ventral grey matter. C. Tips of microwires (diameter 25μm) were deinsulated and cut at a ~30° angle.

The bladder was emptied manually 3-4 times daily for the next two weeks until reflex voiding was established.

Implantation of electrode array. Approximately 2-3 weeks after complete spinal cord transection, when bladder and bowel functions had recovered, the animals were anesthetised again and underwent a laminectomy at thoracic T9-T12 spinal segments. An array of 6 platinum-iridium microwires (diameter - 25μm), insulated except for the bevelled tip, arranged in two rows and spaced 2 mm apart was implanted to stimulate intermediate and ventral grey matter one segment below the lesion (Figure 6.1 B). The implantation and stabilization procedures were based on a technique developed for intraspinal microstimulation (ISMS) (Mushahwar *et al.*, 2003). The electrode leads were spot-glued to the dura with cyanoacrylate and covered by a thin piece of plastic film to prevent connective tissue from dislodging the implant. An elastic tube carrying the microwires was secured to the T11 spinous process with dental acrylic. The microwires led percutaneously to a connector, which was secured to the skin with sutures. Post-operative recovery and subsequent bladder and bowel management was as described above.

Behavioural testing. Following full postoperative recovery, we filmed and rated locomotor performance before and during microstimulation using the standard BBB open-field locomotor rating scale (Basso *et al.*, 1995). Animals were rated without intraspinal stimulation for 4 minutes in the first session, which was immediately followed by another full session with intraspinal stimulation. Trains of stimulus pulses (biphasic, 200μsec, $50s^{-1}$) with amplitudes 0.8-0.9 times threshold for activation of trunk and abdominal muscles (20-200μA) were delivered through the ISMS array (Figure 6.1C).

Histology. In order to determine the location of the implanted microwires, the animals were deeply anesthetised with sodium pentobarbital and perfused through the heart with a 3.7% formaldehyde solution. The thoracic segments of the spinal cord with implanted electrodes were extracted and stored in formaldehyde. The spinal cord was subsequently sectioned and the relative positions of electrode tips were established visually.

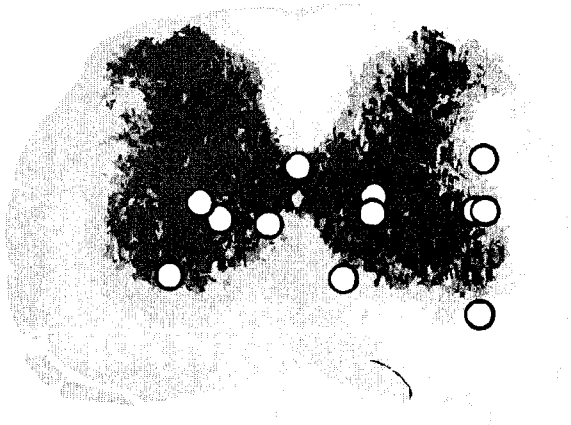
6.3 Results

To test the "propriospinal hypothesis" nine rats were implanted with the ISMS arrays about one spinal segment below the T8 lesion. Since the length of the electrode array was 4mm, the implant physically occupied just over one thoracic segment (Waibl, 1973). Using thoracic vertebrae as reference, the position of the arrays were confirmed histologically to be within the T9-T11 spinal segments. Figure 6.2A shows location of the electrode tips in the transverse plane relative to the grey matter outline. Generally, the electrodes were positioned in ventral and intermediate grey matter (Fig. 6.2A).

Motor thresholds of each of the implanted electrodes were determined 30 minutes prior to the experimental session. Stimulation elicited small contractions of abdominal and back muscles, whose motoneurons are located in the implanted segments (Nicolopoulos-Stournaras & Iles, 1983). None of the electrodes evoked directly contractions of individual hindlimb muscles or rhythmic leg movements. However, it is possible in some cases that rostral areas of thoracic spinal segment T12 were stimulated due to stimulus spread from the most caudal microwires.

The experimental sessions consisted of two 4 min trials. The first trial without ISMS was followed immediately by the second (treatment) trial with ISMS. The stimulus amplitudes for each

A.



B.

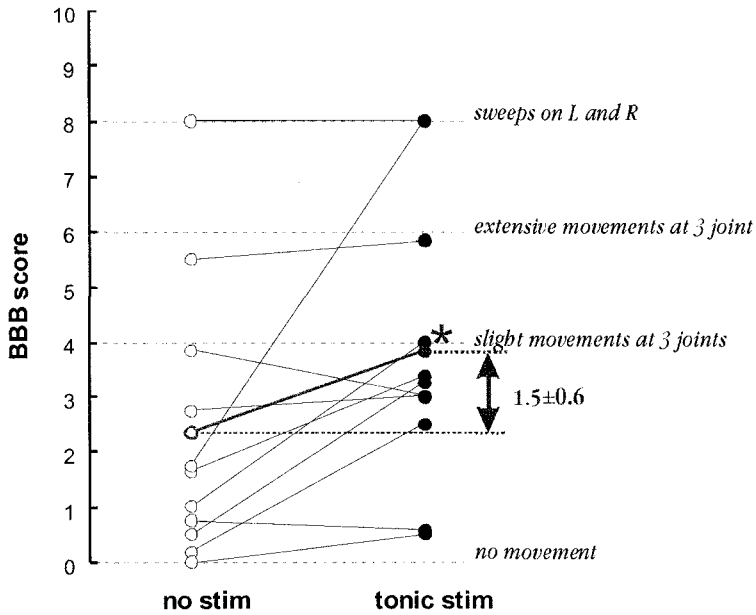


Figure 6.2. Results. A. Location of the implanted electrode tips in thoracic T10-T11 spinal segments. B. Locomotor performance of individual animals before (*open circles*) and during (*filled circles*) ISMS. The mean values for both groups are plotted in red.

electrode were set to 0.8-0.9T with respect to direct muscle activation. ISMS was delivered through all active electrodes simultaneously for the duration of the treatment trial.

Figure 6.2B shows the locomotor performance of individual animals before and during intraspinal stimulation measured with the BBB scale (Basso *et al.*, 1995). A modest, but significant ($\alpha=0.05$) improvement in the BBB score (mean 1.5 ± 0.6 s.e.m.) was observed during the stimulation. The null hypothesis (treatment had no effect) was rejected ($p<0.02$). None of the tested animals showed an adequate level of coordination and weight-bearing for walking.

Instead, they used their forelimbs to propel themselves forward before and during ISMS. Generally, most of the animals with ISMS exhibited some hindlimb rhythmicity and coordination between left and right limbs, only explicitly considered in the BBB score. Two animals scored high on the BBB scale before and during the stimulation (>5). These were the only animals with persistent bladder infections. Another feature frequently observed in several animals during the stimulation trials was tonic hip flexion of one leg and hip and knee extension of the contralateral leg. During this state, episodic movements as rated by BBB scale were more likely to be observed on the extended side, while there were rarely any episodic movements on the flexed side.

These results indicate that non-specific electrical activation of the high thoracic spinal segments can improve performance of rats with complete spinal transections assessed by the BBB locomotor rating scale.

6.4 Discussion

Overall, our results support the "propriospinal hypothesis". Though the improvement in motor performance was marginal, it is similar to that reported in recent studies of various

intervention strategies after spinal cord injury (McDonald *et al.*, 1999; GrandPre *et al.*, 2002; Hausmann *et al.*, 2002; Tuszynski *et al.*, 2003; Verdu *et al.*, 2003). In fact, because these studies tested recovery after incomplete spinal cord injuries, the rate of the spontaneous recovery in control animals was higher than that after complete spinal cord transections. Thus, it could be argued that the relative amount of recovery reported in the studies with incomplete transections was actually lower than that reported in this paper.

Propriospinal pathways have been recognized as potential targets of spinal cord repair treatments (Jordan & Schmidt, 2002). Propriospinal neurons are located in ventral as well as in dorsal laminae of the spinal cord and in fact are likely to represent the majority of spinal neurons (Skinner *et al.*, 1979; Chung *et al.*, 1984; Menetrey *et al.*, 1985; Chung *et al.*, 1987). Our choice of intermediate and ventral areas as targets of stimulation was based on evidence that propriospinal neurons in these areas are more involved in the control of locomotion than those in more dorsal areas (Kiehn & Kjaerulff, 1998; Mrowczynski *et al.*, 2001; You *et al.*, 2003). However, this does not rule out the potential importance of dorsal propriospinal pathways, which may also be involved in the activation of the hindlimb locomotor region (Gerasimenko *et al.*, 2003).

Motor improvements observed in graft regeneration studies can be alternatively explained by the possible mechanical coupling between muscles of trunk and hindlimbs (Giszter *et al.*, 1998). For example, strong contractions of abdominal muscles could stretch hip flexors. This may evoke a proprioceptive feedback to the lumbosacral pattern generator and improve the performance of rats on the open-field locomotor scale. In this case, axons regenerating through a graft need only to activate segmental pathways to the local thoracic motoneurons. We cannot dissociate possible contribution of this pathway in this study. However, a recent study of corticospinal tract behaviour

in response to injury supports the "propriospinal hypothesis" by demonstrating that axons start sprouting and form spontaneously functional circuits with short and long propriospinal neurons (Bareyre *et al.*, 2004).

A recent study of the effect of ISMS (Mushahwar *et al.*, 2003) indicates that sensory axons have a lower threshold than cell bodies. This implies that the stimulation in our study is not limited to activation of propriospinal neurons and may also involve activation of segmental afferent pathways. The current experimental approach cannot dissociate the contribution of this pathway to the observed improvement.

The limited amount of functional improvement in our results points to possible limitations of the recovery strategy based only on non-specific activation of thoracic descending systems. In our experiments, none of the animals before or during ISMS developed sufficient weight-bearing or intra- and inter- limb coordination necessary for locomotion. On the other hand, mechanical stimulation applied during bladder expression often produced long-lasting bouts of air-stepping, not observed in the same animal during ISMS. This observation supports the recent finding of pathways from sacrocaudal afferents to the lumbosacral locomotor pattern generator in neonatal rats (Strauss & Lev-Tov, 2003). However, it is possible that the efficiency of input from the thoracic propriospinal system could be increased if combined with other recovery strategies, e.g. locomotor training (Edgerton *et al.*, 2001).

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CHAPTER 7

General discussion and future directions

7.1 Summary

The control of locomotion is a complex problem of controlling the movement of a mechanical body with multiple degrees of freedom in a variable and often unpredictable environment. This problem is solved in animals by integrated neuro-musculo-skeletal mechanisms for propulsion and stabilization. Chapter 1 of this thesis reviews elements of the locomotor control system and discusses the role of passive dynamic properties of the skeletal system and the contributions of sensory feedback and spinal rhythmogenic neural networks to the control of muscle recruitment and coordination. It also describes challenges and rehabilitation approaches to restoration of motor function after spinal cord trauma. The projects presented in this thesis address questions on integration and contribution of the reviewed systems to locomotion.

The study presented in Chapter 2 addresses a question of whether isometric force-length curves describe muscle during continuous movement. If these static relationships were adequate for the description of muscle behaviour during cyclic changes in muscle length, as in overground locomotion, a region of instability would exist when the muscle is stretched beyond its optimal length. The data from this study showed that isometric force-length curves do not predict muscle behaviour during continuous movement influenced by history-dependent intrinsic muscle properties.

The study of locomotor stability in Chapter 3 takes into account findings in Chapter 2 in the development of an adequate muscle model. This study showed that spring-like muscle properties can be responsible for locomotor stability in the absence of sensory feedback. The data indicated that locomotor stretch reflexes can play a crucial role for weight-bearing when the excitation from higher centers is low. However, the sensory contribution decreases with the

increase in the centrally generated signal and may play a secondary stabilizing role of load redistribution between muscles. These results led to examining the involvement of spring-like muscle properties (Chapter 2) in the regulation of reflex contributions. It was found that muscles could adjust the gain of the sensory feedback loop (loops 2 in Fig. 1.2) during locomotion.

The next system in the hierarchical organization of locomotor control described in Chapter 1 is the central pattern generator (CPG). Its properties were investigated in Chapters 3-6. The study in Chapter 3 used a simple model of the locomotor CPG based on clock-like activation patterns of hindlimb muscles during locomotion. The study in Chapter 4 addressed a question of spatio-temporal activation of hindlimb motoneurons (MNs) controlled by the locomotor CPG during walking. The results support the hypothesis that activation of the lumbosacral MNs during walking is not wave-like, but it is a switch-like oscillation of activity. Because the CPG is thought to span the entire lumbosacral enlargement and consist of interneurons, which may be active in phase with co-localized MNs, these results suggest that activation of the CPG is switch-like as well.

The finding of the switch-like activation of the lumbosacral MNs during walking led to a study of the patterns of MN activity during fictive locomotion, which are not influenced by motion-related sensory feedback controlling transitions between locomotor phases investigated in Chapter 3 (see loop 4 in Fig. 1.2). The analysis of the fictive locomotor patterns in the study described in Chapter 5 revealed that the CPG is capable of adjusting cycle duration by modifying duration of the flexor phase primarily and not the extensor phase. This is opposite of the extensor dominated adjustments during overground locomotion. The data is consistent with the following rule: the variation in the burst duration during spontaneous changes in cycle duration depends on the percent of cycle occupied. These results are explained by a conceptual model of the CPG with two half-centers receiving excitatory bias from descending inputs and proprioceptive feedback

pathways. This model can generalize recent data in the literature on primary and secondary afferent stimulation during normal and fictive locomotion.

Finally, the study in Chapter 6 tests a "propriospinal hypothesis". It is thought that thoracic propriospinal pathways are involved in the improvements of the locomotor performance in animals with complete spinal cord transections bridged by grafts, which allow regeneration of transected descending pathways below the lesion. To test this hypothesis, intraspinal microstimulation (ISMS) was used for non-specific activation of thoracic propriospinal pathways while the locomotor performance was assessed. ISMS produced significant improvements in the locomotor performance. Thus, the results of this study support the propriospinal hypothesis.

7.2 Discussion and future directions

7.2.1 Intrinsic muscle properties

Chapter 2 investigates the dependence of muscle force generation on muscle length during continuous movements. This study was initially designed to test a prediction that isometric angle-torque curves of active wrist extensors would differ from those of flexors (Loren *et al.*, 1996). To address this issue, we measured the angle-torque relationship about the wrist. This relationship would be identical to the muscle force-length curve if angle and torque changes were proportional to muscle displacement and force. It is known that the moment arms of the wrist muscles vary with wrist angle. Using torque-length-angle relationship in cadavers, two groups (Horii *et al.*, 1993; Loren *et al.*, 1996) estimated muscle moment arms from full flexion to full extension of the wrist. Both groups reported that the extensor moment arms decrease when wrist is moved to full flexion. Therefore, when the wrist is flexed, the extensor muscles are supposed to operate on the descending limb of their force-length curve and their moment arm is small. Thus, the extensor

angle-torque curves were expected to exhibit decreasing torque production when the wrist is moved in flexion. However, in our study extensor torque decreased only in isometric trials and it increased with angle during continuous slow $\pm 10^{\circ}$ angular wrist excursions throughout the physiological range of motion (ROM). Thus, in this study we showed that isometric length-tension curves of active wrist muscles do not apply to continuous movements. This result raises a general question of applicability of isometric length-tension curves used in biomechanical studies to describe continuous movement (e.g. Gerritsen *et al.*, 1998).

During small continuous wrist movements, the torque-angle curves exhibited prominent hysteresis. In these trials, the torque increased sharply with stretch and remained high during further lengthening. During shortening the torque declined sharply and remained depressed until lengthening. The observations of sudden changes in muscle stiffness in response to displacement were most consistent with a history-dependent muscle property called short-range stiffness (SRS) (Rack & Westbury, 1974). Because the torque did not return to its isometric level throughout small continuous wrist movements, we suggested that other history-dependent muscle properties, such as force enhancement (Edman *et al.*, 1978; Herzog & Leonard, 2002), force depression (Herzog *et al.*, 2000), yielding and sarcomere length non-uniformities (Abbot & Aubert, 1951; Edman *et al.*, 1978, 1982) may contribute to the force production in our experiment. However, formation of sarcomere length non-uniformities should increase at longer muscle length (Stein *et al.*, 1999). Nevertheless, in our experiment, the torque-angle curves at long muscle lengths were in fact smaller than those at shorter lengths. Therefore, we concluded that the observed hysteresis could be attributed to SRS, force enhancement and depression but not to the sarcomere length non-uniformities.

In future studies, contribution of SRS to force generation will be investigated in humans by comparing muscle resistance to stretch before and after initial pre-stretch displacement, which exceeds the range of SRS. It would be intriguing to investigate the contribution of this hysteretic muscle property to control of locomotion and balance in a type of model described in Chapter 3.

7.2.2 Contribution of stretch reflexes during locomotion

This study in Chapter 3 investigated the contribution of stretch reflexes during locomotion. It is known that the spring-like properties of muscles provide automatic load compensation during weight-bearing. Stretch reflexes also contribute to load compensation. So the question is how crucial is the role of sensory feedback during locomotion? To address this question a neuromuscular model of the hindlimbs of a quadruped was used to test the following two hypotheses: 1) the stretch reflexes are too weak and too delayed to contribute significantly to weight-bearing; 2) the important contributions of sensory input involve state-dependent processing. We used the monotonic positive stiffness property of muscles described in Chapter 2 in the muscle models developed for this biomechanical study.

Numerous simulations of gait were performed with varying combinations of muscle activation derived from pre-set ("centrally-generated") patterns and sensory reflexes. In addition, the sensitivity of our conclusions to parametric variations in the model was studied by varying the leg anatomy and the peak forces of the different muscles. Locomotor stability in these wide-ranging simulations was assessed during locomotion in open-loop ("de-afferented") trials and in trials with feedback control based on either sensory-evoked stretch reflexes or finite-state rules.

The results led us to the following conclusions. 1) In the absence of sensory control, the intrinsic stiffness of limb muscles driven by a stereotyped rhythmical pattern can produce

surprisingly stable gait. This corroborates the conclusions of a previous locomotor modeling study (Gerritsen *et al.*, 1998). 2) When the level of central activation of muscles is low, the contribution of stretch reflexes to load compensation can be crucial. However, when the central drive is strong enough to provide adequate load compensation, the contribution of stretch reflexes diminishes. Indeed, the biological controller of locomotion is in general terms a hybrid of feedforward and feedback systems. Animals in which proprioceptive feedback has been abolished can adjust the feedforward command based on visual input, so that their limb kinematics during level walking is practically indistinguishable from normal (Abelew *et al.*, 2000). In addition, regulation of the relative gains of the feedforward and feedback systems may modify an animal's ability to resist perturbations (Kuo, 2002). The relative gains may also be adjusted continuously in intact animals to cope with rough terrain. The regulation of relative gains of the central command and sensory feedback pathways during locomotion remains a question for future studies. 3) Finite-state control can greatly extend the adaptive capability of the locomotor system. It would be intriguing to use the neuro-musculo-skeletal models to investigate the dynamic requirements of coupling between the intrinsic cycling of the locomotor central pattern generator (CPG) and mechanical oscillations due to the interactions of limbs with the ground. The analysis of gait modifications required to preserve stability may single out sensory signals involved in the regulation of phase switching during locomotion.

7.2.3 Patterns of spatiotemporal activation of the lumbosacral motoneurons

At the time of the development of the biomechanical locomotor model, we used a novel technique to analyse the spatiotemporal activity of lumbosacral motoneurons during normal level locomotion. This study is presented in Chapter 4. The aim of the study was to produce a dynamic

model of the spatiotemporal activation of ensembles of alpha motoneurons (MNs) in the cat lumbosacral spinal cord during the locomotor step cycle. The coordinates of MNs of 27 hindlimb muscles of the cat were digitized from transverse sections of spinal cord (Vanderhorst & Holstege, 1997) spanning the entire lumbosacral enlargement from the caudal part of L4 to the rostral part of S1 segments to generate a three dimensional model. We compiled a chart of EMG profiles of the same 27 muscles during the cat step cycle from previous studies. The level of EMG of each muscle was used to scale the activation level of the corresponding MN pool in the digital model. For the full step cycle, activity of the lumbosacral MNs was reconstructed as a sequence of frames that could be played as a continuous movie. A rostrocaudal oscillation of activity in hindlimb MN pools emerged. This was confirmed by computing the locus of the center of activation of the MNs in the consecutive frames of the movie. The caudal third of the lumbosacral enlargement showed intense MN activity during the stance phase of locomotion. During the swing phase, the focus of activation shifted abruptly to the rostral part of the enlargement. At the stance-swing transition, a transient focus of activity formed in the most caudal part of the lumbosacral enlargement. This was associated with activation of gracilis, posterior biceps, posterior semimembranosus, and semitendinosus muscles. These muscles move the foot back and up to clear the ground during locomotion, a role that could be described as retraction. The spatiotemporal distribution of neuronal activity in the spinal cord during normal locomotion with descending control and intact sensory inputs has not been visualized before.

Can dynamics of the lumbosacral MNs give insight into the structure of the pattern generating network? It is well known that MNs receive inputs from multiple pathways, some of which may not be involved in pattern generation. In addition, the pattern generating networks, which could be fairly localized (Cazalets *et al.*, 1995), may use propriospinal projections to activate

MNs in different spinal segments. Indeed, it is challenging to dissociate pattern generating elements from those merely following input commands. Even MNs may be considered (Marder, 1991; O'Donovan *et al.*, 1998) an integral part of the pattern generator. Because of the intrinsic dynamic properties such as plateau potentials, MNs respond nonlinearly to the input command and may continue to fire after cessation of stimulation (Conway *et al.*, 1988). An increasing number of studies support the hypothesis that the locomotor CPG is distributed throughout the lumbosacral enlargement (Kiehn & Kjaerulff, 1998). Of course, our MN model does not give any information about the distributions of interneurons, of which the CPG may be largely composed, but it may reflect general activity of the interneuronal populations segmentally co-localized with the MNs (Tresch & Kiehn, 1999).

The finding of rostrocaudal oscillation within the lumbosacral enlargement during locomotion raised a question whether the dynamics of this activity corresponded to a "wave" or it was "switch-like". An experimental study used calcium-imaging to visualize activity of MNs during locomotion in neonatal rats (Bonnot *et al.*, 2002). It showed that activation of several motoneuron pools in L1-L2 and S1-S3 spinal segments supports the "wave" hypothesis. However, it remains to be seen whether this conclusion describes activation of all lumbosacral MN pools. Also, the mode of locomotion has to be established in fictive preparations, i.e. the observed behaviour may correspond to swimming rather than to walking. Two recent modeling studies also supported the "wave" hypothesis (Kaske *et al.*, 2003; Patel *et al.*, 2003). However, unrealistic manipulation of either the somatotopic organization of MNs or their activation profiles was required to fit the conclusion. For example, Kaske et al. assumed that the knee extensor MNs were located caudally in the lumbosacral enlargement, when Patel et al. showed co-activation of ankle flexors with other extensors throughout the stance phase of quadrupedal locomotion. The results of our study show

clear spatiotemoral separation of activity during swing and stance. This pattern is consistent with the "switch-like" activation of the hindlimb MNs during walking.

From the differences between the spatiotemporal patterns of various stereotypic movements of intact and reduced preparations, information on the locomotor pattern generating circuitry may be deduced. Future studies will focus on the classification of the MN activity during stereotypical movements in intact and reduced preparations. The analysis of the spatiotemporal activity of the hindlimb MNs during fictive locomotion elicited by electrical stimulation of the mesencephalic locomotor region (MLR) is currently in progress (Yakovenko *et al.*, 2001).

7.2.4 Adjustments of the locomotor phases with changes in cycle duration

Chapter 5 includes a study of variability in the locomotor patterns during fictive locomotion, when motion-related feedback is absent. In this study, we discovered a novel flexibility of the pattern generating circuitry. Previous studies (Goslow *et al.*, 1973; Halbertsma, 1983) have shown that during intact cat locomotion the duration of the extensor phase (Te) varies more than the flexor phase (Tf) with changes in cycle period (Tc). The cycle duration is also dominated by the extensor phase (see Chapter 4). We now report that this relationship can be reversed in MLR-induced fictive locomotion when flexor phase duration dominates the step cycle. Cyclical bursts of nerve activity during fictive locomotion were analyzed using a threshold crossing method to identify onsets and cessations of activity. Regression analysis of bursting of individual nerves showed that Te and Tf increased linearly with Tc. Similarly to the intact cat, when the extensor phase was longer than the flexor phase, Te line of best fit had a steeper slope, than Tf. However, when the locomotor cycles were dominated by the flexor activity, the slope was steeper for flexors than for extensors.

Based on this finding, we proposed that the pattern generated by the locomotor CPG depends on the distribution of excitation between flexor and extensor half-centers, which are analogous and antagonistic. In this model, descending and sensory pathways can increase the level of excitation of each half-center. A shift in the excitatory bias to one of the half-centers may lead to an increase in the duration of the corresponding phase of the cycle and a decrease in the antagonistic phase through reciprocal neural interactions (Cymbalyuk *et al.*, 2002).

The proposed model can describe a number of reported observations of phase adjustments in response to unloading (Duysens & Pearson, 1980; Pang & Yang, 2000), electrical stimulation of group I afferents of extensors (Guertin *et al.*, 1995; Whelan *et al.*, 1995) and group I & II afferents of flexors (Perreault *et al.*, 1995; Hiebert *et al.*, 1996) and progressive extension of the hindlimb during fictive locomotion of cats a few days after spinalization (Pearson & Rossignol, 1991). The functional relevance of the proposed balance of activity between flexor and extensor half-centers may also explain the phase changes observed during upslope and downslope locomotion (Carlson-Kuhta *et al.*, 1998; Smith *et al.*, 1998) and air-stepping (Smith *et al.*, 1986).

Overall, the observed behavior is consistent with a theory that the CPG, controlling locomotion of each limb, consists of at least two antagonistic analogous oscillators, each of which is capable of dominating the activity in the step cycle (Brown, 1911).

7.2.5 The "propriospinal hypothesis"

Finally, Chapter 6 investigates a hypothesis, which may explain improvements of motor functions observed in spinalized rats after long distance axon regeneration. Many laboratories have reported the successful regeneration of neurons across damaged portions of the spinal cord (Cheng *et al.*, 1996; Ramon-Cueto *et al.*, 2000; Coumans *et al.*, 2001). Functional connections have been

inferred from improvements in locomotor movements. But regenerated neurons generally extend only about 10mm beyond the lesion site, about 20mm short of the lumbosacral locomotor region. The motor improvements have been explained by possible excitation of propriospinal neurons, which in turn relay this excitation to the hindlimb CPG. However, the observed motor improvements in graft regeneration studies can be alternatively explained by the possible mechanical coupling between muscles of the trunk and hindlimbs (Giszter *et al.*, 1998). For example, sensory excitation may be generated by strong contractions of abdominal muscles, which in turn stretch hip flexors. In this case, axons regenerating through a graft need only to activate segmental pathways to the local thoracic motoneurons. If trunk muscles were activated at a low level insufficient for stretching hip flexors, the contribution of this pathway would be diminished.

We tested the "propriospinal hypothesis" by using intraspinal microstimulation (ISMS) of the thoracic spinal cord to activate descending propriospinal neurons. The level of the ISMS was adjusted to reduce possible contribution of the mechanical coupling between trunk and hindlimb segments. We rated locomotion before, during and immediately after ISMS using a standard open field locomotor rating scale, the BBB scale. Stimulation significantly increased the BBB scores and increased rhythmicity of hindlimb movements. We conclude that electrically evoked activity in thoracic spinal segments isolated from supraspinal control may propagate caudally and activate lumbar pattern generating networks. Thus, this study supports the propriospinal mediation hypothesis of regeneration.

Future directions of this study will address questions about pathways activated by ISMS of thoracic spinal cord. A recent study of the effect of ISMS (Mushahwar *et al.*, 2003) indicates that sensory axons have a lower threshold than cell bodies. This implies that ISMS in our study is not limited to activation of propriospinal neurons and may also involve activation of segmental afferent

pathways. In addition, ISMS may activate sympathetic and parasympathetic preganglionic efferent pathways, which may affect activity of the spinal cord below the lesion. The current experimental approach cannot dissociate the contribution of these pathways to the observed improvement. Another future direction of this study may address a question of whether or not the efficiency of input evoked by ISMS of the thoracic propriospinal system could be increased if combined with other recovery strategies, e.g. locomotor training (Edgerton *et al.*, 2001).

7.2.6 Synthesis and impact of the presented studies

The studies completed during my Ph.D. program address a number of questions in the field of Motor Control. The common goal of these studies was to investigate integration of skeletal, muscular and neural systems for the control of locomotion. Some of the main results presented above have led to research in other laboratories around the world. The movies generated as part of the work on spatiotemporal activity of lumbosacral motoneurons (Chapter 4) are now used for neuroscience teaching in universities in North America and Europe.

The studies in my thesis have opened up the following questions for further research. What is the contribution of muscle history-dependent properties, e.g. short-range stiffness, to modulation of sensory feedback and locomotor stability? How is the contribution of locomotor stretch reflexes modulated during different modes of locomotion, e.g. walking vs. running? Is the finite control system described in Chapter 3 modified to stabilize walking over uneven terrain? Does the lumbar enlargement contain long propriospinal connections coordinating activity of rostral hip flexors to caudal hip extensors? What are the dynamics of the spatiotemporal activity during different types of locomotion? Can two coupled generic oscillators describe the behaviour of the CPG model with excitatory bias presented in Chapter 5? Does ISMS of the thoracic sensory pathways improve motor

performance in animals with complete spinal cord transection? Studies presented in Chapters 4 & 5 have raised question about the workings of the CPG. This has led to the initiation of at least two studies in other laboratories interested in fictive and decerebrate locomotion.

In conclusion, this thesis describes how the problem of locomotor control is solved by a hierarchically organized, neuro-musculo-skeletal system with dynamic interactions between its parts.

7.3 References

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