University of Alberta

Coordination of the Arms and Legs during Human Locomotion

by

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Abstract

The neural control of locomotion is thought to involve a complex series of interactions between spinal interneuronal networks (i.e. central pattern generators – CPGs), afferent information, and supraspinal input. These interactions not only produce the basic gait pattern, but also enable substantial flexibility in the pattern to allow animals to interact meaningfully with the environment. The manner by which the human nervous system flexibly coordinates the limbs to generate different forms of rhythmic movement was examined in the four projects of this thesis. First, the task-dependency of cutaneous reflexes during level walking, incline walking, and stair climbing was investigated to determine whether locomotor tasks that differ in task mechanics share common neuronal components. Considerable similarities amongst tasks suggest that the underlying neural mechanisms involved in coordinating walking could be modified to also coordinate other locomotor tasks such as stair climbing. Second, the degree of autonomy between CPGs for each leg was investigated in human infants using a split-belt treadmill. It was found that CPGs for human infant stepping behaved in a remarkably similar way to other animals, and the two legs were autonomous from one another except when they needed to interact for coordination (i.e. at the beginning of swing phase). Third, an asymmetrical arm cycling task was used to examine whether neural coupling between arm CPGs is revealed when the two arms perform very different activities. While some evidence for inter-arm coupling was found, this coupling was weaker than that between the legs, which is likely reflective of the increased independence of the arms during locomotion. Forth, the functional role of interlimb reflexes in arm muscles after electrically stimulating a nerve in the foot was examined during locomotion. There was a facilitation

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of reflexes in arm muscles when a hand-rail was held, which occurred specifically in muscles that could be important in using the rail for support. This suggests a role for these interlimb reflexes in stumble correction when the arms have a larger mechanical role in restoring stability. Overall, these results shed light on contributions of reflex pathways and interlimb connections to the coordination of human gait.

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To my family for believing in me

and to Muraari for encouraging me to believe in myself.

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List of Abbreviations

3,4-dihydroxy-L-phenylalanine	L-DOPA
Analysis of variance	ANOVA
Anterior deltoid	AD
Biceps brachii	BB
Biceps femoris	BF
Central nervous system	CNS
Central pattern generator	CPG
Contralateral	c
Electromyography	EMG
Erector spinae	ES
Flexor carpi radialis	FCR
Flexor reflex afferents	FRA
Gastrocnemius-soleus (infant)	SOL
Growth associated protein	GAP
Ipsilateral	i
Medial gastrocnemius	MG
Peripheral nervous system	PNS
Posterior deltoid	PD
Rectus femoris	RF
Standard deviation	SD
Tibialis anterior	TA
Triceps brachii	TB

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Transcranial magnetic stimulation	TMS
Vastus lateralis	VL

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CHAPTER 1: GENERAL INTRODUCTION

1.1 Introduction

> *The overall picture [of the neural control of locomotion] is an interaction between subtle volitional corrections and basic central and peripheral elements, all of which in a joint effort may produce movements ranging from a ballet dance to a tightrope walk.*
>
> *~ S. Grillner (1981) from "Control of locomotion in bipeds, tetrapods and fish". In Handbook of Physiology. The Nervous System. Motor Control, ed. Brookhart JM and Mountcastle VB. pp. 1179-1236. American Physiological Society, Bethesda, MD.*

The smooth, coordinated control of the limbs during locomotion is something that we often take for granted. However, the intricacies of the neuronal circuitry controlling locomotion are still largely unknown and this subject remains one of the most studied areas in motor control neuroscience. This thesis builds upon current knowledge about the neural control of human gait and provides insight into how the limbs are coordinated during different tasks. Increasing our understanding of the neural control of locomotion will lead to more effective therapies to help people regain movement and independence after neurological injury.

The neural control of locomotion is thought to involve spinal interneuronal networks (i.e. central pattern generators – CPGs) interacting with afferent feedback and supraspinal input, which modify the motor output to meet specific task and environmental demands (reviewed in Nielsen, 2003; Zehr and Duysens, 2004). Available evidence suggests that humans, like other animals, possess spinal CPGs capable of generating the basic rhythmic

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walking pattern; however, it is not known to what extent these networks are modified to meet the functional requirements of bipedal walking. Bipedal walking poses unique challenges and offers unique opportunities for movement. Since we have a relatively high centre of mass which is balanced on only two legs, maintaining stability during gait is more challenging for us than it is for other animals. Furthermore, our arms are not constrained in the production of gait, and we are capable of independent, skilled hand movements even when our legs are occupied with walking. These fundamental differences in how we walk may have coincided with evolutionary differences in CPG networks. This thesis addresses these issues by (1) investigating to what extent human locomotor CPGs for the legs can flexibly coordinate different forms of gait and (2) investigating the coordination of the arms with each other and with the legs during rhythmic movements. In this introductory section, a general overview of the background and major concepts related to these areas will be provided.

1.2 Central Mechanisms Generating Locomotion

The spinal origin of basic locomotor movements has been recognized for some time. Observations of spinalized or decerebrate cats and dogs in the late 19th – early 20th century have revealed that these reduced animals can perform coordinated locomotor movements (Brown, 1911, Freusberg, 1874, Phillippson, 1905, Sherrington, 1910a and b). It was initially hypothesized that the alternating flexion and extension of the legs was produced by a chain of peripheral reflexes, in which the afferent signals associated with one movement would initiate the next movement, and so on (Sechenov, 1866; Sherrington, 1910a and b). However, Graham Brown (1911) later demonstrated that

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following a low thoracic spinal transection in the cat, the deafferented lumbosacral spinal cord could generate rhythmical activity in the ankle flexor and extensor muscles. Therefore, these patterned locomotor movements could be produced in the absence of afferent input to the lumbosacral spinal cord.

Neural networks that are capable of generating a rhythmic pattern of motor activity in the absence of phasic input have been termed central pattern generators or CPGs (Grillner, 1981). The earliest CPG model proposed by Graham Brown (1914) consisted of a flexor and an extensor half-centre coupled by mutually inhibitory interneurons. These inhibitory connections could account for the generation of alternating flexion and extension movements (Graham Brown, 1911, 1914). In this model, afferent feedback was proposed to have a regulatory role, rather than a causative role, in rhythmic movement. Support for the half-centre model was provided by Jankowska and colleagues (1967a and b). Using acute spinalized cats treated with L-Dihydroxyphenylalanine (L-DOPA), they found that electrical stimulation of small-diameter joint, muscle, or cutaneous afferents (flexor reflex afferents or FRA) produced long lasting excitation of ipsilateral flexors and contralateral extensors. Conversely, stimulation of the contralateral FRA caused excitation of ipsilateral extensors and contralateral flexors. Furthermore, FRA stimulation sometimes resulted in sequences of alternating flexor and extensor activity, similar to that observed during locomotion, and excitatory responses could be suppressed by a conditioning stimulus to the antagonist group of interneurons. Taken together, these findings suggest the presence of mutually inhibitory pathways between interneurons that excite flexor and extensor motoneuron

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pools, which is consistent with Graham Brown's suggestion that mutually inhibiting half-centres produce the alternating activity in flexor and extensor motoneurons.

The half-centre model has limitations in that it is too simplistic to explain the diversity and complexity of motor patterns that occur during real locomotion. The activity in flexor and extensor muscles does not simply alternate during locomotion, which is what would be predicted from the half-centre model. Rather, the timing and level of activity in different muscles, particularly in biarticular and flexor muscles, vary widely. For example, in the cat, the hip flexor (iliopsoas) contracts continuously during swing while the bifunctional knee flexor and hip extensor (semitendinosus) contracts briefly at the beginning of stance and again at the beginning of swing (Engberg and Lundberg, 1969). While the locomotor patterns generated in deafferented or immobilized spinal preparations tend to be simpler than normal stepping patterns shortly after spinalization (Pearson and Rossignol, 1991), more complex patterns can be generated with the application of additional drugs (such as clonidine) or after a period of training (Barbeau and Rossignol, 1987, Belanger *et al*, 1988, Pearson and Rossignol, 1991, Rossignol *et al*, 1996). Moreover, by changing the level of tonic sensory input, a variety of patterns can be generated in immobilized decerebrate preparations (Perret and Cabelguen, 1980). From these observations, it is apparent that complex and diverse motor patterns can be generated by spinal networks. Thus it is evident that the half-centre model is insufficient to explain the muscle activity generated during locomotion.

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As a result of these findings, alternate configurations of the locomotor CPG have been proposed. One very influential model put forth by Grillner (1981, 1985) proposed that the CPG can be subdivided into smaller functional units, termed "unit burst generators", which control the muscles around each joint or body segment. This organizational model has been corroborated by findings in various species, including the tadpole (Roberts *et al*, 1997), the lamprey (Matsushima and Grillner, 1992), and the mudpuppy (Cheng *et al*, 1998b). In the lamprey, for example, each spinal segment contains a neural network capable of generating the rhythmic alternating activity in motoneurons on the two sides to produce swimming. By changing the local excitability of different segments of the spinal cord, the phase coupling between segments can be changed, thus resulting in different directions of swimming (Matsushima and Grillner, 1992). Moreover, support for this scheme has come from the analysis of forward and backward walking in humans and crustaceans, in which distinct phase shifts in the timing of activity in different muscles have been observed (Ayers and Davis, 1977, Clarac, 1984, Thorstensson, 1986). However, this model does not provide an explanation for the muscle activity observed in both swing and stance in bifunctional muscles like sartorius and rectus femoris (Engberg and Lundberg, 1969). Therefore, while the unit burst generator model proposed by Grillner (1981) succeeds in explaining some of the diversity in locomotor patterns generated by CPGs, the pattern generating networks for different behaviours can be more complex than simply altering the coupling between discrete functional units in the network.

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An alternative theory suggests that the half-centre model is maintained, but with the incorporation of a more complicated interneuronal network operating between the half-centres and motoneurons (Burke, 2001, Perret and Cabelguen, 1980, Perret, 1983). This idea has led to the proposal of a two-level CPG, which may be able to account for more of the complexity in rhythmic movements. This model postulates that CPGs are composed of separate networks for rhythm generation (i.e. the half-centre or "clock" specifying the basic timing of flexion and extension) and for pattern formation (i.e. the distribution of excitation and inhibition to specific motoneuron pools) (Burke, 2001, Lafreniere-Roula and McCrea, 2005, Rybak *et al,* 2006a, Rybak *et al*, 2006b – see Figure 1.1). The output of the CPG feeds down to motoneurons either directly or indirectly via last-order interneurons (Burke, 2001). All levels of this model (i.e. rhythm generating network, pattern formation network, last-order interneurons and motoneurons) may be affected by supraspinal influences or peripheral feedback. One advantage of this model is that the pattern formation networks are thought to distribute excitation and inhibition to specific muscle synergies (Ivanenko *et al,* 2006). These synergies can consist of muscles operating across several different joints and thus motor behaviours coordinated by the CPG are not limited to those that are simply variations in the phase-coupling between different limb/body segments (as in Grillner's model). Therefore, the two layer model offers the attractive possibility that many similar motor tasks may share components of a common pattern generating network (also see Zehr, 2005).

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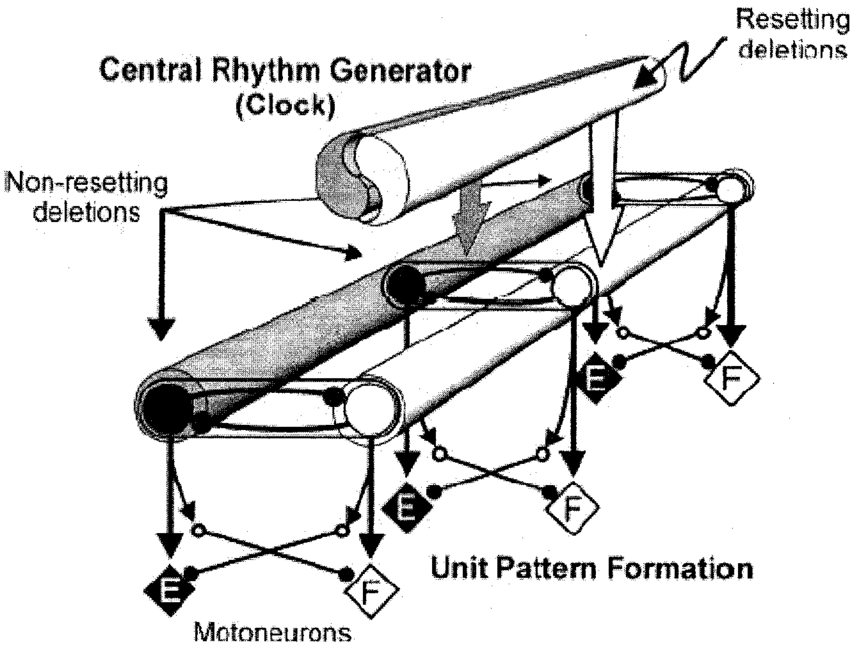

Figure 1.1: **Schematic representation of the proposed structure of the "two layer" CPG model.** Networks for rhythm generation (Central Rhythm Generator) set the overall timing of the pattern whereas pattern formation networks (Unit Pattern Formation) distribute excitation and inhibition to specific motoneuronal pools. Spontaneous deletions that affect the locomotor rhythm (i.e. resetting deletions) may result from perturbations to the Central Rhythm Generator, whereas deletions in which the locomotor rhythm is maintained (i.e. non-resetting deletions) may result from perturbations to the Unit Pattern Formation networks. From Lafreniere-Roula and McCrea (2005).

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Initial evidence supporting the two-layer model has come from studies of spontaneous failures (i.e. "deletions") in the normally robust flexor and extensor activity during fictive locomotion and scratch in the cat (Lafreniere-Roula and McCrea, 2005, Rybak *et al*, 2006a). These studies have demonstrated that the rhythmic pattern that re-emerges after a deletion is often not phase-shifted, which suggests that some neuronal mechanism (i.e. the rhythm generator) maintains the locomotor period when motoneuron activity fails. In this situation, the deletion is likely the result of a perturbation of the pattern formation networks which does not affect the rhythm generator. At times, there was a resetting of the locomotor rhythm after a deletion, which may be a result of a perturbation to the rhythm generator itself (Lafreniere-Roula and McCrea, 2005, Rybak *et al*, 2006a – see Figure 1.1). Further evidence for this two-level model can be taken from studies of the effects of afferent stimulation during fictive locomotion. For example, during the extensor phase of fictive locomotion, stimulation of extensor muscle group I afferents prolongs the extensor phase, which may occur with or without a resetting of the locomotor cycle, depending on the degree to which the sensory input affects rhythm generating and pattern formation circuits, respectively (Rybak *et al,* 2006b, also see Schomburg 1999). It is particularly interesting that even when perturbations cause changes in phase durations, the system (i.e. rhythm generator) can remember and maintain the original motor pattern (also see McCrea, 2001, Perreault *et al*, 1995, Stecina *et al*, 2005).

To verify the validity of the multilayer CPG model, interneurons that comprise the rhythm generating and pattern formation networks should ultimately be identified.

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Recent advances in combined genetic and electrophysiological techniques have shown a great deal of promise in unraveling these networks (reviewed in Gordon and Whelan, 2006). For example, a class of interneurons that express the transcription factor Hb9 has been identified as possible rhythm generating neurons (Hinckley *et al,* 2005, Hinckley and Ziskind-Conhaim, 2006, Wilson *et al,* 2005). These Hb9-positive interneurons are located in the medial areas of lamina VII, which is where rhythmically active neurons have been found in the neonatal rat preparation (Kiehn and Butt, 2003). These cells exhibit many properties consistent with a role in rhythmogenesis. They are glutamatergic and are rhythmically active during locomotion. Activity in these cells is linked to the recorded activity in ventral roots and the rhythmicity appears to be intrinsically generated since it persists even when voltage-gated sodium channels are blocked by tetradotoxin (Hinckley *et al*, 2005, Hochman *et al,* 1994, Wilson *et al*, 2005).

It should be noted that there is some debate regarding the necessity of neurons with bursting properties, such as Hb9-positive interneurons, in rhythm generation (see Johnson, 2007 for review). For example, several studies examining CPGs for respiration have suggested that inspiratory bursts of action potentials are an emergent property of non-pacemaker neurons that are interconnected by synaptic interactions (i.e. neural networks) and pacemaker neurons are not necessarily required for rhythmogenesis (Rekling and Feldman, 1998, Feldman and Del Negro, 2006, Pace *et al,* 2007). While the precise role for pacemaker neurons in rhythmogenesis is subject to debate, they are excellent candidates for constituting at least a part of the pattern generating networks.

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1.3 Spinal Contributions to Locomotion in Humans

The evidence for locomotor pattern generators in bipedal humans is more indirect than that in other animals. By definition, a spinal pattern generator must be capable of producing recognizable and reproducible patterns of rhythmic output in the absence of phasic drive from other parts of the central nervous system (CNS) or from peripheral feedback (Grillner, 1981). While this can be tested in animals by using spinalized and deafferented preparations, this line of evidence for the existence of a locomotor CPG in humans could only be obtained from an individual with a complete spinal cord injury and complete deafferentation of the lower limbs who could be made to produce stepping movements. Needless to say, such an individual has not been identified to date. A few investigators have attempted to elicit fictive locomotion in primates, with varying degrees of success. Eidelberg and colleagues (1981) were unable to elicit fictive locomotion in spinalized macaque monkeys even when the monkeys were treated with pharmacological agents that were effective in eliciting the locomotor rhythm in other species (e.g. L-DOPA, noradrenalin, serotonin). However, Fedirchuk and others (1998) could induce components of the fictive locomotor pattern in marmoset monkeys after administration of excitatory amino acids or clonidine. Nonetheless, the increased difficulty in eliciting fictive locomotor patterns in primates suggests that the primate locomotor CPG may depend more on supraspinal input than in other vertebrates.

There have been three main lines of evidence that provide indirect support for locomotor CPGs in humans. The first comes from reports of rhythmic movements and muscle contractions in spinal cord injured individuals. The second comes from human infants, in

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whom coordinated stepping occurs in the relative absence of descending volitional input. The third comes from studies of reflex modulation patterns in neurologically-intact individuals during locomotion. These three lines of evidence will be discussed below.

1.3.1 Evidence from spinal cord injured subjects

Many human studies have provided evidence of oscillatory neural networks that interact with afferent feedback with limited or no detectable supraspinal input. Over the past century, there have been several reports of spontaneous locomotor-like movements in individuals with complete or incomplete spinal cord injury (Calancie *et al*, 1994, Holmes, 1915, Kuhn, 1950). Stepping movements can also be elicited in complete spinal cord injured patients by applying tonic epidural electrical stimulation to the spinal cord via implanted electrodes (Dimitrijevic *et al,* 1998, Gerasimenko *et al*, 2002). Furthermore, locomotor-like EMG patterns can be induced after complete spinal cord injury when leg movements are externally assisted to provide stepping-related sensory cues to the spinal cord (Dietz *et al*, 1994, Dietz *et al*, 1995, Dobkin *et al*, 1995, Harkema *et al*, 1997). These patterns could not be solely attributed to recurrent muscle stretch reflexes, which suggests that central mechanisms (like CPGs) were also involved (Beres-Jones *et al*, 2003, Beres-Jones and Harkema, 2004, Harkema *et al*, 1997).

Other evidence for locomotor CPGs in humans comes from studies of reflex responses after spinal cord injury. In patients with clinically complete spinal cord injury, electrical stimulation of the FRA evokes late flexor discharges on one side while inhibiting contralateral late flexor discharges, which resembles FRA reflex responses observed in

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the cat (Roby-Brami and Bussel, 1987, 1990, 1992). Since the FRA reflex network in cats is believed to be intrinsically linked with the locomotor pattern generator (Jankowska *et al*, 1967a, 1967b), it is likely that similar reflex networks are present in humans and these networks may also be intertwined with pattern generation. Taken together, the evidence from spinal cord injured humans supporting the existence of locomotor CPGs is compelling.

1.3.2 Evidence from human infants

Prior to the onset of independent walking, human infants are capable of stepping when supported over ground or on a moving treadmill (Forssberg, 1985, Thelen, 1986, Yang *et al*, 1998a, Zelazo, 1983). This stepping response appears very early on in life – the human fetus can show well-coordinated stepping-like movements in utero at 10 gestational weeks (De Vries *et al*, 1984). Anencephalic infants are also capable of stepping, which indicates that the coordination of this behaviour takes place within the spinal cord and the brainstem (Peiper, 1963). Further evidence to support the claim that infant stepping is relatively free from volitional control comes from histological, radiological, electrophysiological, and behavioural observations in human infants (as discussed below). All of these suggest that while the peripheral nervous system (PNS), spinal cord, and brainstem are all quite mature by birth, the motor cortex and cerebellum follow a more protracted course of maturation.

The PNS is capable of mediating reflexive movements very early in prenatal life. The earliest closure of a reflex arc occurs around 7.5 gestational weeks (Altman and Bayer,

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2001). Orofacial reflexes can be elicited by cutaneous stimulation at approximately 7.5 gestational weeks and reflexive responses in the hands and feet can be elicited by cutaneous stimulation around 10-10.5 weeks and 10.5-11 weeks, respectively (Humphrey, 1964). However, the conduction velocities of various peripheral nerves in the upper and lower extremities at birth are about half of those found in neurologically intact adults, due to the small fiber diameter and lack of myelination. Adult conduction velocities are not reached until approximately 5 years of age (Eyre *et al*, 1991, Muller *et al*, 1991). Despite the slower conduction velocity, transmission in reflex pathways in infants at birth often resembles that of adults with corticospinal tract lesions. For example, the Babinski response is extensor in infants as it is in adults with corticospinal lesions (Connolly and Forssberg, 1997). Therefore, while peripheral nerve conduction is slower in infants than in adults, the responses in infants still resemble those in adults when there is a lack of corticospinal drive to the spinal cord, suggesting that the reflex pathways are quite mature in infants.

Evidence suggests that the spinal cord is also quite mature at birth. Eyre and others (2000) have studied growth-associated protein (GAP-43) expression during development as a marker of neurite outgrowth. While GAP-43 immunoreactivity is prominent throughout the spinal cord at 24-25 gestational weeks, at 30-33 weeks GAP-43 expression is reduced in all areas except the corticospinal tract. Therefore, with the exception of the corticospinal tract, the other major tracts in the spinal cord have likely completed most of their growth by the 33rd week. In particular, many of the descending pathways originating from the brainstem are well myelinated and likely functioning at

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birth in humans (Sarnat, 1989), although it is difficult to test the functionality of these pathways in infants. However, since the brainstem undergoes its greatest maturation during fetal life (Dambska and Kuchna, 1996), it must be assumed that descending brainstem pathways could be functioning to activate locomotor circuits in the spinal cord at birth.

Neural structures associated with the production and refinement of voluntary movement (such as the corticospinal tract, motor cortex, and to some extent the cerebellum) follow a much more protracted course of maturation (Brody *et al,* 1987, Rorke and Riggs, 1969, Yakovlev and Lecours, 1967). The cerebellum is one of the first brain structures to begin to differentiate, but it is one of the last to mature (Wang and Zoghbi, 2001). Development of the cerebellum is progressing at birth, starting with phylogenetically older areas (Chugani and Phelps, 1986, Yakovlev and Lecours, 1967). The myelination of inferior and superior cerebellar peduncles begins prenatally and begins to resemble adult myelination by 3 to 8 post-natal months (Yakolev and Lecours, 1967). On the other hand, myelination of the lateral cerebellar hemispheres is almost absent at birth and progresses slowly after birth, with 50% of infants having mature myelin by 47 post-natal weeks (Kinney *et* al, 1988). The development of coordinated movements associated with cerebellar function is also delayed (Connolly and Forssberg, 1997). For example, infants are not able to make smooth and accurate arm movements towards a target until around 9 months of age and interlimb coordination during walking, running, or skipping continues to be refined throughout childhood (Connolly and Forssberg, 1997). Therefore, while the

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cerebellum may not be fully mature in infants, the role of the cerebellum in coordinating infant stepping cannot be negated.

The motor cortex and corticospinal tract are quite immature at birth. The motor cortex is largely unmyelinated at birth, and rapidly grows and develops over the first 2 years of life (Richardson, 1982). ^{18}FDG positron emission tomography shows that glucose utilization increases in the sensorimotor cortex until approximately 1 year of age, when a pattern resembling that of an adult is seen (Chugani and Phelps, 1986). While synaptogenesis is progressing in the neocortex by the 3rd trimester of gestation, adult values of synaptic numbers and density are not attained until adolescence (Huttenlocher, 1979, Huttenlocher and Dabholkar, 1997). The corticospinal tract is also immature in infancy: histological studies have shown that while the corticospinal tract reaches the lumbosacral spinal cord by birth, the adult appearance of myelin is not attained until 2 years of age (Altman and Bayer, 2001, Yakovlev and Lecours, 1967). Additionally, corticospinal tract axons are approximately 10 times smaller in newborns than in adults, and the conduction velocity in the corticospinal tract is roughly 10 times slower (Eyre *et al*, 2000). As a result of the immaturity of the corticospinal tract and motor cortex, the threshold for eliciting activity in upper and lower limb muscles in response to transcranial magnetic stimulation over the motor cortex is very high in children under the age of 1 (Eyre *et al*, 1991, Koh and Eyre, 1988, Muller *et al*, 1991). Also, some reflex responses of infants resemble those of adults with corticospinal tract lesions. For example, abnormal Babinski reflexes (as mentioned above) persist until ~18 months of age (Connolly and Forssberg, 1997). Infants also demonstrate 'reflex irradiation' in which short-latency excitatory responses

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from neighbouring muscles (both agonists and antagonists) result from tendon taps (Myklebust and Gottlieb, 1993). Reflex irradiation is also seen in children with cerebral palsy and in adults with upper motoneuron disorders, which suggests that this may be due to immature descending inhibitory control from the cortex. Therefore, observations from a variety of experimental approaches support the assumption that stepping in infants younger than 1 year of age is under very little volitional control.

Is the same neuronal circuitry used for infant stepping and adult walking? The kinematic pattern of stepping is certainly different between infants and adults. Unlike adult walking, infant stepping is characterized by a digitigrade mode of progression and synchronous motion of the hip, knee, and ankle (whereas adults show a more segmented pattern) (Forssberg, 1985). However, the immature kinematics of infant stepping patterns persist as the child becomes independent in walking and gradually mature over time (Cheron *et al*, 2001a, Cheron *et al*, 2001b, Dominici *et al*, 2007, Forssberg, 1985, Ivanenko *et al*, 2004, Ivanenko *et al*, 2005, Ivanenko *et al*, 2007). Since there is a gradual progression from infant stepping to adult walking, it has been suggested that stepping movements in infants and independent locomotion may be controlled by the same neuronal circuitry (Forssberg, 1985). Moreover, the sensory control of walking is quite similar in infants and adults. For example, when the foot is perturbed early in swing phase, an exaggerated flexion of the leg and prolongation of swing phase were observed in infants (Lam *et al,* 2003) and adults (Eng *et al,* 1994; Schillings *et al,* 2000). Unexpected loading of the limb during stance phase resulted in a prolongation of stance phase in both infants (Pang and Yang, 2000) and adults (Stephens and Yang, 1999),

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although the magnitude of the response was greater in infants. The similarity in the ways adults and infants respond to sensory perturbations suggests that similar neural circuitry is involved.

The major limitations of the infant model are that (1) the amount of input coming from other areas of the CNS (particularly the brainstem) is not known with certainty and (2) details of the relative state of nervous system maturation in different infants is unknown. However, one substantial advantage of the infant model is that the human pattern generator is in its intact state; this is in contrast to the state of the pattern generator after spinal cord injury when adaptive changes may have taken place by the time of study (Dimitrijevic *et al*, 1998). Therefore, based on the above evidence, infants provide a particularly useful model for the study of human pattern generators for walking (reviewed in Yang *et al*, 2004).

1.3.3 Evidence from reflex studies

It has been known for some time that reflexes are modulated in amplitude and sometimes even reverse in sign during locomotion in cats (Duysens and Pearson, 1976, Forssberg *et al*, 1975) and in humans (Duysens *et al*, 1990, Van Wezel *et al*, 1997, Yang and Stein, 1990, Zehr *et al*, 1997). Intracellular recordings from motoneurons during fictive locomotion in cats have provided clear evidence that the locomotor CPG exerts powerful control of transmission through reflex pathways as assessed by phasic modulation of synaptic potentials (Andersson *et al*, 1978, LaBella *et al*, 1992, Schomburg and Behrends, 1978). Since reflex transmission in cats is subject to CPG control, these

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reflexes can provide clues as to the organization of pattern generator circuits (Burke, 1999).

In neurologically intact humans, the locus of mechanisms controlling reflex modulation has been subject to some debate. The H-reflex has been shown to be phase- and task-dependent during rhythmic movement, thus H-reflexes may be gated by CPG networks (Capaday and Stein, 1986, Crenna and Frigo, 1987, Dietz *et al,* 1990, Stein and Capaday, 1988. However, Brooke and others (1997) have shown that H-reflexes are modulated during passive movement in a similar fashion to how they are during active movement, suggesting that afferent feedback is a contributor to the modulation pattern. On the other hand, the modulation of cutaneous reflexes during locomotion was not related to afferent feedback (Brooke *et al,* 1999). Rather, Brooke and others (1999) suggested that the cutaneous afferent discharge may be primarily modulated by the complex interneuronal circuits of the CPG. Also supporting this hypothesis is the finding that cutaneous reflexes are task-dependent: reflexes evoked during active movement (e.g. walking) are different from those evoked while quiescent or during static contraction (e.g. standing) (Duysens *et al,* 1993, Kanda and Sato, 1983, Komiyama *et al,* 2000). This task-dependency is likely of central origin since background EMG amplitude and afferent feedback regarding the position or loading of the limbs were similar (also see Zehr *et al,* 2001). Furthermore, Duysens *et al* (1996) found clear differences in cutaneous reflex modulation patterns during forward and backward walking that could not be explained by differences in afferent feedback, background muscle activity, or supraspinal drive to muscles. Therefore, since these other factors were ruled out, reflexes were proposed to

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be affected by pre-motoneuronal gating by pattern generating networks. All of these studies have thus added credence to the notion that cutaneous reflexes are modulated by locomotor CPGs and that cutaneous reflexes may be used as a "neural probe" to investigate the operation and organization of CPGs.

One limitation of using reflexes to investigate locomotor CPGs is that the degree to which supraspinal areas affect transmission in these pathways is not known with certainty. Tibialis anterior (TA), in particular, is thought to be close linked to the corticospinal tract during walking (Capaday *et al*, 1999, Pijnappels *et al*, 1998, Schubert *et al*, 1997), and there is some evidence that the gating of suppressive cutaneous reflex pathways onto TA motoneurons may be related to cortical contributions during gait. Suppressive reflexes in TA during gait are often seen at the end of swing (Duysens *et al*, 1990, Van Wezel *et al*, 1997, Yang and Stein, 1990, Zehr *et al*, 1997), when cortical control of TA is probably higher than at other points in the step cycle (Schubert *et al*, 1997). Duysens et al. (2004) found that suppressive TA reflexes could be evoked during stance during an unstable walking task (i.e. split-belt treadmill walking), possibly as a result of increased cortical drive to TA during stance. Furthermore, in a study of mildly affected spinal cord injured individuals, an absence of end-swing suppressive responses in TA was noted (Jones and Yang, 1994). In contrast, the end-stance facilitatory reflexes in TA were mostly spared. However, Zehr et al. (1998) have found that the reflex responses to superficial peroneal nerve stimulation were mostly preserved after stroke. Nonetheless, the general consensus is that end-stance reflex facilitations may be primarily

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under the influence of the spinal CPG while end-swing suppressions may be more under the control of the cortex.

1.4 Coordination of Bipedal Gait

As outlined above, the available evidence suggests that humans, like other animals, possess pattern generating networks within the spinal cord that are capable of coordinating the basic walking pattern. However, supraspinal areas appear to have a more substantial role in generating human gait compared to other animals (reviewed in Capaday, 2002, Nielsen, 2003, Yang and Gorassini, 2006). This has been poignantly demonstrated by studies showing that body weight support treadmill training is far more effective in retraining gait in quadrupeds (Lovely *et al*, 1986, Rossignol *et al*, 1986), as compared to humans (Dietz *et al*, 1994, 1995, Dobkin *et al*, 1995, Harkema *et al*, 1997) or primates (Eidelberg *et al,* 1981; Fedirchuk *et al,* 1998). Often improvements in the walking of individuals after stroke or incomplete spinal cord injury can be partially correlated with increased corticospinal drive to muscles and/or increased activity in cortical areas (Dobkin *et al*, 2004, Thomas and Gorassini, 2005, Winchester *et al*, 2005). The degree to which the motor cortex contributes to walking is subject to debate; however, it cannot be denied that it is of some importance.

In addition to the enhanced role of cortical areas, another distinguishing feature of human locomotion is that we are bipedal. The development of bipedal gait may have coincided with evolutionary differences to how pattern generators controlling the movement of each

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limb are coupled with one another during gait (i.e. interlimb coordination). This will be explored in the remainder of this introduction.

1.4.1 Bilateral coordination between the legs

The regulation of walking requires a close coordination of muscle activation between the two sides. Indeed, the coordination between the legs during gait may be more critical for humans than for other animals given the added challenge of stability in bipedal walking. With only two legs to stand on, normally one leg must be in stance phase when the contralateral leg is in swing to maintain balance during locomotion.

In animals, there is some degree of interdependence between pattern generators controlling the limbs on each side. Grillner and Rossignol (1978) found that the initiation of swing phase was dependent on the position of the contralateral leg: swing could only be initiated in spinalized cats when the other leg was either in mid-swing or mid-stance. They suggested that the former response represented interlimb coupling during alternating forms of gait, such as walking or trotting, whereas the latter response represented interlimb coupling during synchronous forms of gait, such as galloping (Grillner and Rossignol, 1978). Disturbances that prolong swing phase in cats have also been shown to cause compensatory reactions in the contralateral limb in order to preserve ground contact and stability (Forssberg *et al*, 1977, Matsukawa *et al*, 1982). Furthermore, the response to stepping into a hole was different in cats depending on the state of the contralateral leg. If the contralateral leg was in stance, this stance phase was extended until the ipsilateral side regained ground contact; if the contralateral leg was in

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swing, the flexion response on the ipsilateral side was delayed until the contralateral side regained ground contact (Gorassini *et al*, 1994, Hiebert *et al*, 1994).

Animals as diverse as cats and stick insects (Kulagin & Shik, 1970; Halbertsma, 1983; Forssberg et al., 1980; Foth and Bassler, 1985) maintain coordination while stepping on a split-belt treadmill, with two belts running at different speeds. However, their limbs can also operate somewhat independently, as demonstrated by the limb on the faster treadmill belt sometimes taking more steps than the other leg(s) on the slower belt(s) (i.e., asymmetrical stepping). This type of flexible coordination has been reported for many animal preparations, including stick insects (Foth & Bassler, 1985), spinal (Forssberg et al., 1980), decerebrate (Kulagin & Shik, 1970) and intact cats (Halbertsma, 1983), and spinal turtles (Stein & McCullough, 1998). Even during this asymmetrical stepping, the legs interact to prevent the co-occurrence of swing on both sides. These results indicate that the pattern generators for each leg have some degree of autonomy while interacting with each other for coordination.

Interlimb coordination between the legs in humans bears some resemblance to that in other animals. For example, when swing phase is initiated early in one limb (via a rapid extension of the hip), swing on the contralateral side is truncated so that this limb can regain ground contact (Berger *et al*, 1984, Dietz *et al*, 1984, Pang and Yang, 2001). Coordinated responses in both legs are generated in response to a stumble or perturbation during swing in both adults and infants (Dietz *et al*, 1986, Eng *et al*, 1994, Pang and Yang, 2001, Schillings *et al*, 2000, Yang *et al*, 1998b). Also, there is a similar interaction

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between the two legs to maintain 1:1 alternating stepping on a split-belt treadmill (Dietz *et al*, 1994, Prokop *et al*, 1995, Thelen *et al*, 1987). In contrast to observations in other animals, there have been no reports of adult humans adopting an asymmetrical stepping rhythm on the split-belt treadmill (Dietz *et al*, 1994, Jensen *et al*, 1998, Prokop *et al*, 1995), which may reflect a lack of autonomy between the two sides. However, adaptations to large speed differentials have not been studied in humans, so it may be that this behaviour has not yet had the opportunity to emerge.

In summary, bilateral coordination of the legs in humans is very similar to that in other animals. The main difference is that the coupling between the two sides may be stronger in humans than in animals, possibly as a result of the increased importance of maintaining ground contact with at least one leg during bipedal walking. Whether the stronger coupling between the legs occurs on a subcortical level or is due to volitional control remains to be seen.

1.4.2 Bilateral coordination between the arms

Substantial evidence has been accumulating supporting the hypothesis that rhythmic arm movements in humans are coordinated in part by CPGs that are similar to those controlling rhythmic leg movements (reviewed in Zehr *et al*, 2004). For example, cutaneous reflexes evoked in arm muscles during arm swing of walking and rhythmic arm cycling showed phase modulation across the movement cycle that was generally unrelated to background muscle activity and, thus, indicative of pre-motoneuronal gating by central control mechanisms, such as CPGs (Zehr and Kido, 2001, Zehr and Haridas,

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2003). Furthermore, task dependence and nerve specificity of cutaneous reflexes have been demonstrated during arm cycling, which were akin to that observed for leg cutaneous reflexes during locomotion (Zehr and Kido, 2001). Recently, Carroll et al. (2006) found that H-reflexes conditioned by subthreshold transcranial magnetic stimulation (TMS) were smaller during arm cycling than they were during static contractions of arm muscles, indicating that the corticospinal drive to these muscles was reduced during rhythmic activity. This suggests that some of the role of coordinating rhythmic arm movement was delegated to lower centres of the nervous system, such as the brainstem and spinal CPGs.

While there are a number of similarities between the neural control of rhythmic arm and leg movements, the degree to which the two sides are coupled is different between the arms and legs: the coupling between the legs is stronger than that between the arms. For instance, while passive movement of the contralateral leg caused H-reflexes to be suppressed in a stationary leg (Cheng *et al*, 1998a, Collins *et al*, 1993, Misiaszek *et al*, 1998), there was little effect of contralateral arm movement on H-reflex amplitude in a stationary arm (Zehr *et al*, 2003). Similarly, cutaneous reflex modulation patterns during arm cycling were found to be highly conserved regardless of differences in activity on the contralateral side (Carroll *et al*, 2005, Hundza and Zehr, 2006). Therefore, cutaneous and H-reflexes in the arms were primarily dependent on the state of the limb in which the muscle resides, whereas reflexes in the legs were also affected by the state of the contralateral limb.

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Functionally, it is perhaps not surprising that there is stronger coupling between the legs than there is between the arms. During bipedal walking, the legs perform symmetric, coordinated, out-of-phase locomotor movements while the arms have the option of moving independently of one another. In other words, the roles of the arms and legs in human walking are different and the strength of coupling between pattern generators controlling each limb may have developed to reflect this.

1.4.3 Interlimb coordination between the arms and legs

The presence of propriospinal interlimb connections between the hind- and forelimbs has been clearly demonstrated in the cat (Gernandt and Megirian, 1961, Gernandt and Shimamura, 1961, Miller *et al*, 1973, Skinner *et al*, 1980) and in the neonatal rat (Juvin *et al*, 2005). Both ascending (Akay *et al,* 2006, Gernandt and Megirian, 1961, Gernandt and Shimamura, 1961, Juvin *et al*, 2005) and descending (Akay *et al,* 2006, Skinner *et al*, 1980) pathways may assist in the coordination between the limbs. It has been proposed that similar interlimb connections exist in humans to link the activity of the arms and legs during locomotion (reviewed in Dietz, 2002, Zehr and Duysens, 2004). Behaviourally, the arms and legs can be observed to be coordinated during locomotor activities like walking, creeping, and swimming. Wannier et al. (2001) have shown that during all of these activities, an integer frequency relationship was maintained between the limbs, which was suggestive of a coupling between neuronal circuits controlling arm and leg movements that corresponds to that observed in systems consisting of two coupled oscillators (von Holst, 1973). Also indicative of interlimb linkages in humans is the finding that interlimb reflexes can be evoked in arm muscles after electrically or

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mechanically perturbing the foot (Dietz *et al*, 2001, Haridas and Zehr, 2003) and can also be evoked in leg muscles after electrically perturbing the hand (Zehr and Haridas, 2003). Finally, in anatomical studies of the human nervous system, Nathan and colleagues (Nathan and Smith, 1955, Nathan *et al*, 1996) have identified long projecting propriospinal neurons that couple the cervical and lumbar enlargements in humans, similar to those found in animals.

While substantial evidence exists to support the existence of neural connections between the arms and legs in humans, the function of these pathways during gait is less clear. Segmental cutaneous reflexes in the legs are precisely gated and likely have a functional role in maintaining and restoring stability during walking (Haridas *et al*, 2005, reviewed in Zehr and Stein, 1999); however, interlimb reflexes in the arms are more variable and have not been specifically linked to a role in stumble correction (Haridas and Zehr, 2003, Misiaszek, 2003). This may be due to the large number of degrees of freedom in arm movement strategies for restoring balance. That is, during locomotion arm movement is relatively unconstrained and since the arms are not directly interacting with the ground, they are not in a mechanical position to immediately and directly modify stability. Recently, Haridas and others (2006) reported a general facilitation of interlimb reflexes in the arms when the arms were crossed in front of the body during an unstable walking task. This facilitation appeared in several muscles and a general role for interlimb reflexes in corrective responses during locomotion was postulated.

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Therefore, neuronal connections between the cervical and lumbar pattern generators are retained in bipedal humans, despite the fact that the arms do not directly generate propulsion during walking. It is likely that these reflexes are functional in coordinating whole-body responses to perturbations during gait; however, the specific role of these reflexes has yet to be determined.

1.5 Objectives of Thesis:

While it is becoming clear that CPGs have a role in generating locomotion, how these networks are organized to coordinate different types of rhythmic movement is not as apparent. Have the demands of bipedal walking been met by evolutionary changes to human CPGs for locomotion? Also, compared to what is known about CPGs controlling leg movements, relatively little is known about the neural control of arm movements. Therefore, the purpose of this thesis was two-fold: (1) to determine the extent to which human CPGs for leg movement resemble those of other animals and (2) to characterize the neural control of human arm movements, particularly with respect to how the arms interact with each other and with the legs during coordinated locomotion.

The first part of the thesis examined the flexibility of human CPGs for leg movement, in order to investigate the degree to which human CPGs resemble those of other animals. Chapter 2 investigated the task-dependency of cutaneous reflexes during different locomotor tasks to determine whether different forms of locomotion have a common central neural control just as in other animals. The data suggested that underlying neural mechanisms involved in coordinating level walking could be modified to also coordinate

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incline walking and stair climbing, thus indicating a common pattern generator for a number of different locomotor tasks. Chapter 3 examined whether pattern generators controlling each leg retain some degree of autonomy, as in other animals. It was found that CPGs for human infant stepping behaved in a remarkably similar way to other animals, and the two legs were autonomous from one another except when they needed to interact for coordination (i.e. at the beginning of swing phase).

The objective of the second part of this thesis was to characterize the neural control of the arms in humans. In chapter 4, an asymmetrical arm cycling task was used to examine whether neural coupling between arm CPGs is revealed when the two arms perform very different activities that could potentially interfere with each other. Evidence for coupling between the arms was found in the control of ongoing muscle activity but not in the regulation of cutaneous reflexes, suggesting a separation in the neural control of these two outputs from arm CPGs. In general, the coupling between the arms was weaker than that between the legs, which is probably reflective of the increased independence that humans have in arm and hand movements. Finally, chapter 5 investigated whether interlimb reflexes in arm muscles after electrically stimulating a nerve in the foot have a functional role during locomotion. It was found that reflexes were enhanced in certain arm muscles when subjects held a hand rail. Specifically, reflex facilitation occurred selectively in arm muscles that could be important in using the rail for support. Therefore, reflexes in the arms may be gated to have an enhanced role in stumble correction when the arms have a larger mechanical role in restoring stability.

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Overall, this thesis is devoted to studying the operation of CPGs during human rhythmic movement. This thesis highlights the similarities and differences between CPGs for locomotion in humans and animals and contributes to the characterization of upper limb CPGs in humans. Moreover, the results shed light on possible functional roles of reflex pathways and interlimb connections in the coordination of human gait.

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CHAPTER 2: TASK-SPECIFIC MODULATION OF CUTANEOUS REFLEXES EXPRESSED AT FUNCTIONALLY RELEVANT GAIT CYCLE PHASES DURING LEVEL AND INCLINE WALKING AND STAIR CLIMBING[*]

2.1 Introduction

Reflexes are exquisitely sensitive to the task that is being performed at the time they are evoked. This phenomenon of reflexes changing in amplitude or sign between motor tasks has been termed "task-dependency" (for reviews see Brooke et al., 1997; Zehr and Stein, 1999). Cutaneous reflexes evoked during active movement (e.g. walking) are different from those evoked while quiescent or during static contraction (e.g. standing) (Kanda and Sato, 1983; Duysens et al., 1993; Komiyama et al., 2000). For example, reflexes evoked by sural nerve stimulation during standing are predominantly suppressive and graded with background EMG, whereas facilitatory reflexes evoked during movement do not correlate with background EMG (Duysens et al, 1993; Komiyama et al, 2000).

While it is clear that tasks with obvious differences in motor output and peripheral feedback (e.g. sitting and walking) have different reflex modulation patterns, it is less clear to what degree motor tasks need to be different in order to observe a shift in the neural control, as evidenced by changes in the modulation of reflexes. Duysens et al. (1996) found that tasks that were similar in muscle activity and joint kinematics, such as forward and backward walking, exhibited similar patterns of cutaneous reflex modulation, despite the reversal in the direction of limb movement. Recently, it was

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shown that alterations to a rhythmic arm cycling task that affected mainly EMG amplitude (i.e. changing direction of movement and changing crank length) had little effect on reflex control (Zehr and Hundza, 2005; Hundza and Zehr, 2006). Therefore, these modifications to rhythmic tasks did not induce a global change in the underlying neural control.

We were interested in whether the neural control would be conserved across three locomotor tasks that were different in terms of EMG amplitude, joint kinematics, and stability demands. Our working hypothesis was that the three tasks of level and incline walking and stair climbing would share common neural components; thus, reflex modulation patterns would be generally retained across tasks. Reflex modulation patterns were defined as being different, and indicative of differences in neural control, if there were significant differences in the phasing of reflexes. An example of such a difference is a reflex reversal between different tasks, where there is a reversal in reflex sign at a given latency, akin to what is observed between standing and walking (e.g. Komiyama et al, 2000).

2.2 Materials and methods

2.2.1 Subjects and tasks

Nine neurologically intact subjects (7 males, aged 21-44 years) participated in the experiment with informed, written consent under approved protocols for human subjects at the University of Alberta and according to the Declaration of Helsinki.

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Subjects performed three ~7 minute locomotor tasks: (1) treadmill walking while level (LEVEL) and (2) while inclined to 15° (INCLINE) (Spirit Manufacturing Inc, Jonesboro, AR); and (3) stair climbing on a stepping mill (StepMill 7000PT, StairMaster, Kirkland, WA, USA) (STAIRS). Treadmill speed was set to 1.1 m/s (~1 step/second) and the stepping rate for stair climbing was also ~1 step per second. The inclination for the stepping mill was 40° (rise/run for each step = 20.3/20.3cm). Subjects moved their arms naturally during all of these tasks.

2.2.2 Electrical Stimulation

The right sural nerve at the ankle was stimulated via bipolar Ag-AgCl surface electrodes (Vermont Medical, Inc., VT, USA) using a Grass S88 stimulator connected to an SIU5 isolation unit and a CCU1 constant current unit (Astro-Med, Inc., West Warwick, RI USA). Stimulation was applied in trains of 5 x 1.0 ms pulses at 300 Hz and at a non-noxious intensity of ~2x the radiating threshold (RT - see Zehr et al., 1998; Haridas and Zehr, 2003 for details). Stimulations were pseudorandomly delivered at a rate such that no more than one stimulation occurred within a step cycle.

2.2.3 Electromyography (EMG) and step-cycle detection:

Once the skin was cleaned with alcohol, bipolar Ag-AgCl surface electrodes (Vermont Medical, Inc, VT, USA) were placed over the target muscles. Posterior deltoid (PD) was recorded bilaterally and all other muscles were recorded ipsilaterally. EMG was obtained from all 9 subjects for ipsilateral PD (iPD), erector spinae (ES), rectus femoris (RF), vastus lateralis (VL), biceps femoris (BF), tibialis anterior (TA), and medial

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gastrocnemius (MG). EMG was recorded from anterior deltoid (AD) in 4 and from contralateral PD (cPD) in 5 subjects. Ground electrodes were placed over electrically neutral tissue (on the lateral malleolus at the ankle and the patella for lower limb muscles, on the acromion procress for upper limb muscles, and over a vertebral spinous process for the trunk muscle). EMG signals were amplified 5000x and filtered from 100-300 Hz (Grass P511, Astro-Med, Inc., West Warwick, RI, USA). Heel contact and toe-off were recorded using custom-made force sensors taped to the insoles of both shoes.

2.2.4 Data acquisition and EMG analysis

Data were sampled simultaneously at 1 kHz with a 12 bit A/D converter connected to a computer running custom-written LabView virtual instruments (National Instruments, Austin, TX, USA). Off-line analysis was performed to separate the step cycle into 8 equal phases, beginning with initiation of stance on the right side, using an interactive custom-written MatLab program (the Mathworks, Natick, MA). During the off-line analysis, EMG data were full-wave rectified and low-pass filtered at 40 Hz with a dual-pass Butterworth filter.

Sweeps of rectified EMG were collected around each stimulation, from 100 ms pre-stimulus to 200 ms post-stimulus. Stimulated sweeps that occurred within the same movement phase (based on the time of stimulation) were averaged together (10-20 sweeps in each phase). Averaged EMG from non-stimulated step cycles was subtracted from the averaged stimulated sweeps to obtain subtracted reflex traces. This subtraction resulted in negative values when the stimulated EMG was less than the non-stimulated

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EMG. We concentrated on middle latency reflexes (~80-120 ms after stimulation) which tend to be larger and occur most frequently (Baken et al., 2005). Reflexes were quantified as the peak amplitude (from a 10 ms window centred on the peak latency) within the middle latency. Averaged reflexes for each subject were considered significant if the peak exceeded a 2-standard deviation band around the mean pre-stimulus EMG level. Within each subject and muscle, if the stimulus intensity was sufficient to evoke a significant reflex in at least one of the conditions tested, we selected corresponding windows of muscle activity for all conditions.

2.2.5 Statistics

Level walking was used as the reference or "control" and all data (background and reflex EMG) were normalized to the peak background EMG amplitude during this condition. Linear regression analysis was used to determine significant correlations between reflex amplitudes and background EMG levels. Repeated-measures ANOVAs were used and post-hoc analysis of significant main effects and interactions was conducted using Student-Newman-Keul's test. Statistical significance was set to $P < 0.05$.

2.3 Results

Figure 2.1 shows mean background EMG (top, line plots) and middle latency reflex amplitude (bottom, bars) during the three tasks. Results from statistical analyses are shown on the graphs and are explained in the figure caption. The phasing of background EMG was largely maintained regardless of task in the arms (AD, iPD, cPD) and trunk (ES); however, note that there were some differences in amplitude for iPD and ES. In the

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leg muscles (RF, VL, BF, TA, MG), there were differences in the phasing of EMG and some differences in amplitude. For example, the activity in quadriceps (RF and VL) was greater at the stance-to-swing transition (phase 5) during LEVEL when compared to INCLINE or STAIR. Also, activity was larger near the swing-to-stance transition (phases 8 & 1) during STAIR. Activity phasing in the BF was different in STAIR. Also, activity in TA was greater in STAIR during early swing (phases 5 & 6). EMG was also enhanced in MG for INCLINE and STAIR at mid-stance (phase 3). There were significant task main effects (indicating global differences in amplitude) in iPD, ES, RF, VL, and TA and task x phase interactions (indicating differences in phasing) for all of the leg muscles.

Middle latency reflex modulation patterns were also generally conserved in most arm and trunk muscles, but there was a facilitation of the reflex in contralateral PD and in ES during STAIR, compared to LEVEL and INCLINE. Many of the leg muscles showed differences in middle latency reflex amplitude: amplitudes increased in RF, BF, and MG during STAIR. However, for these muscles and VL, there were fewer differences in the phasing of reflexes across the step cycle compared to the number of differences in the phasing of background EMG.

An interesting reflex emerged at the stance-to-swing transition (phase 5) in TA. The reflex at this phase was facilitatory during LEVEL and INCLINE, but was suppressive during STAIR. This reversal in reflex sign within the middle latency was also apparent in

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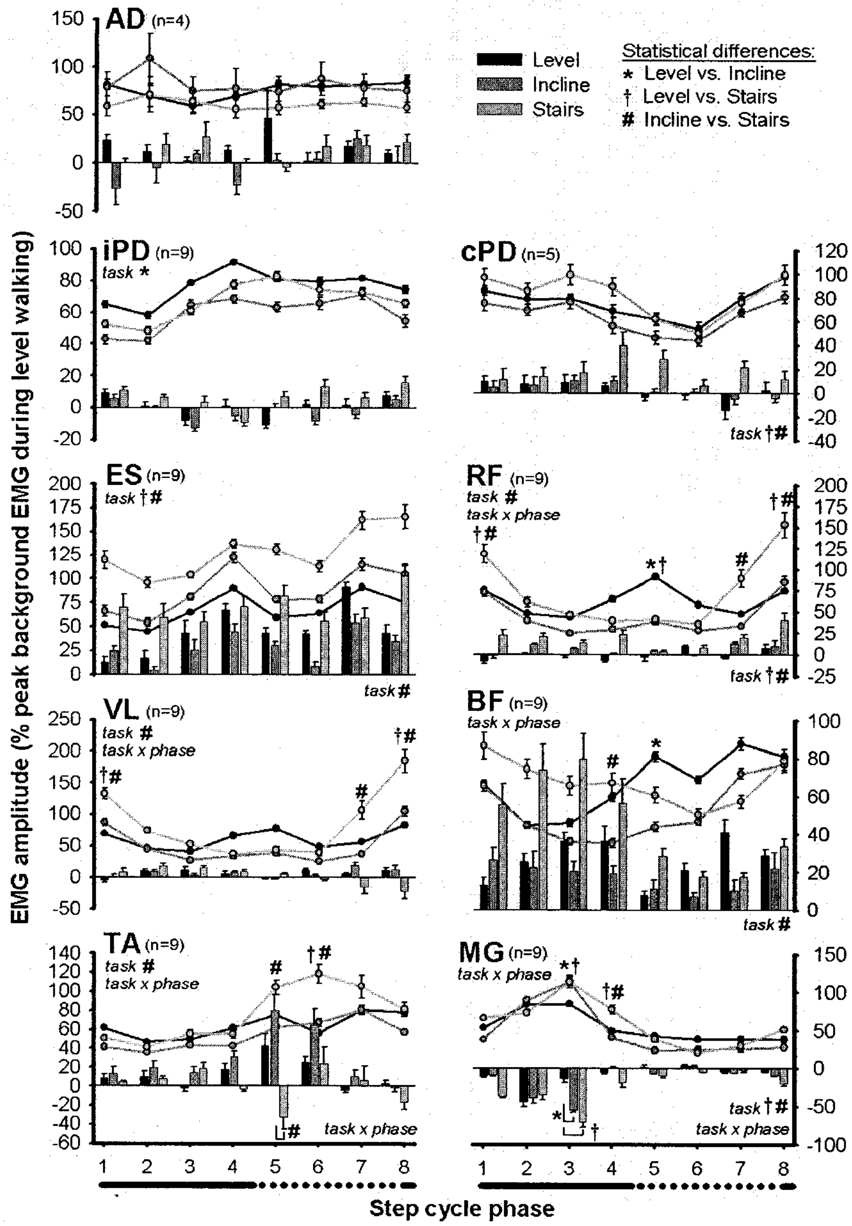

Figure 2.1: Normalized background EMG and middle latency reflex amplitudes during level walking, incline walking and stair climbing.

Background EMG is shown in line plots and reflex amplitude is shown in bar plots. Values were averaged across all subjects for each task and context (± standard error) and normalized to the peak undisturbed EMG during level walking. Abbreviations are as follows: AD, anterior deltoid; iPD, ipsilateral posterior deltoid; cPD, contralateral posterior deltoid; ES, erector spinae; RF, rectus femoris; VL, vastus lateralis; BF, biceps femoris; TA, tibialis anterior; MG, medial gastrocnemius. The number of subjects (n) for each muscle is indicated beside the muscle name. The horizontal bars at the bottom of the graphs denote stance (solid line) and swing phase (dotted line). The stance-to-swing transition occurred at a similar phase of the step cycle for all tasks (averages: level=phase 4.54; incline=phase 4.55; stairs=phase 4.69). For background EMG, significant task main effects (*task*) and task x phase interactions (*task x phase)* are indicated in text below the name of each muscle (top left corner of graph). For reflex amplitude, these are indicated at the bottom right. Symbols representing results from post-hoc analyses are either placed beside the text (for task main effects) or on the plots (for task x phase interactions - above the line plots for background EMG and below the bar plots for reflex amplitude). The α-level was set at 0.05.

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the single subject data (Figure 2.2). There was a significant task x phase interaction for TA (at phase 5), indicating that a change in phasing occurred in this muscle.

There was also a significant task x phase interaction in MG due to a shift in phasing (peak at phase 2 in LEVEL and at phase 3 in INCLINE and STAIR). However, upon testing correlations between reflex amplitude and background EMG, we found two instances of significant correlation for MG (Table 2.1). Therefore, differences in reflex phasing could be explained by differences in background EMG (note the peak background EMG is also shifted to phase 3 during INCLINE and STAIR). The amplitude of the remaining reflexes was not directly related to background muscle activity, thus reflex modulation was not largely due to fluctuations in motoneuronal excitability (e.g. automatic gain compensation - Matthews, 1986; Toft et al., 1991)

2.4 Discussion

In this study, we examined the effects of variations in locomotor tasks (level walking, incline walking, and stair climbing) on cutaneous reflex modulation. Generally, we found that patterns of cutaneous reflex modulation across the entire body were maintained across tasks, suggesting that transmission in segmental and interlimb reflex pathways is gated in a similar manner during different locomotor tasks. An exception was found in one muscle, TA, for which the reflex modulation pattern during stair climbing was different from that observed during level or incline walking. We suggest that similar pattern generating elements are shared across all three tasks and specific reflex differences may emerge during different locomotor tasks due to changes in

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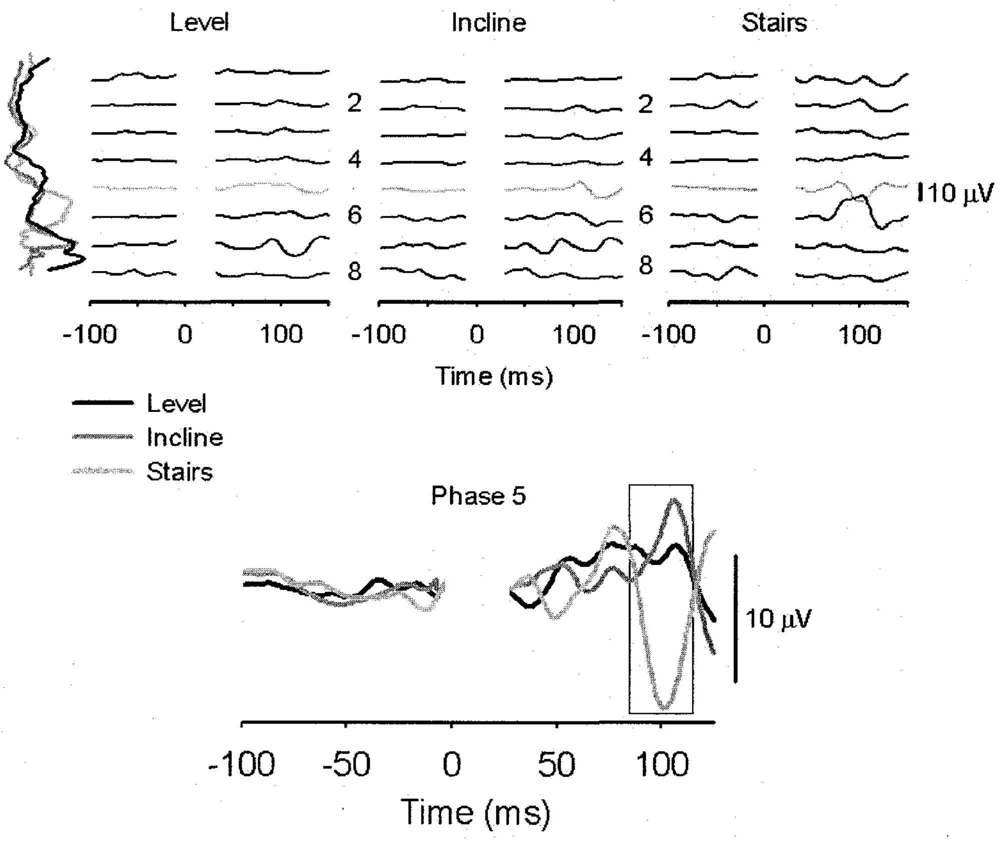

Figure 2.2: Subtracted reflex EMG traces in TA from a single subject during level walking, incline walking and stair climbing.

Sweeps of data are shown from 100 ms before stimulation to 150 ms after stimulation. For the upper graphs, the step cycle phase is represented between the plots (even numbers only), with phase 1 corresponding to heel contact and the stance-to-swing transition occurring between phases 5 and 6. Stimulation occurred at time 0 and the stimulation artefact (between 0 – 30 ms) was removed. The vertical traces plotted on the left of the graphs indicate the averaged background EMG during each task across the step cycle. In the lower graph, reflex traces from the same subject in phase 5 are shown during level walking, incline walking and stair climbing. The rectangle highlights reflexes observed during the middle latency window (80-120 ms after stimulation).

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Table 2.1: Correlation coefficients between middle latency reflex amplitude and background EMG during 3 locomotor tasks.

Values shown in shaded cells indicate significant Pearson r values at the level of $p < 0.05$
Critical r (n = 9) = 0.667
Abbreviations are defined in Figure 2.1.

MUSCLE	TASK		
	Level	**Incline**	**Stairs**
AD	0.06	-0.32	0.34
iPD	-0.29	-0.42	-0.04
cPD	-0.32	0.09	-0.04
ES	0.47	0.45	0.49
RF	-0.15	-0.01	0.30
VL	0.08	0.07	-0.48
BF	0.11	0.17	0.44
TA	0.06	0.40	-0.29
MG	-0.48	-0.67	-0.73

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descending drive or afferent feedback, which may be important in specifically shaping the motor output to the demands of the ongoing task.

2.4.1 Task-dependent modulation of cutaneous reflexes

The tasks examined in this study were different in terms of background EMG amplitude (represented by task main effects) and phasing (represented by task x phase interactions). Generally, most differences occurred between STAIR and the two walking tasks: note the greater number of differences in LEVEL vs. STAIR († in Figure 2.1) and INCLINE vs. STAIR (#) compared to LEVEL vs. INCLINE (*). Additionally, previous work has demonstrated changes in hip, knee and ankle angles during INCLINE and STAIR compared to LEVEL (Laubenthal et al., 1972; Andriacchi et al., 1980; McFadyen and Winter, 1988; Livingston et al., 1991; Leroux et al., 1999; Riener et al., 2002). Therefore, these tasks are different both in limb movement and in muscle activation patterns.

Although these tasks differed significantly in movement patterns, attempts were made to control for other perceptual elements. For example, the use of the stepping mill allowed subjects to step "in place", similar to stepping in place on a moving treadmill. Therefore, there was no forward movement during locomotion and visual and vestibular feedback was likely similar for all three tasks. To our knowledge, this is the first study that uses a stepping mill for reflex studies and offers a good comparison with treadmill walking.

Despite the differences in background EMG, cutaneous reflexes were generally similar in phasing across the three tasks; however, some differences in amplitude were observed.

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Similar to background EMG, reflexes during STAIR differed the most from the other tasks (Figure 2.1). Indeed, reflexes were generally amplified during STAIR in many muscles (cPD, ES, RF, BF, and MG). This may be a reflection of the less stable nature of stair climbing compared to walking. Since responses to electrical stimulation of cutaneous nerves may reflect functional strategies to overcome an obstacle or recover stability (Schillings et al., 1996; Zehr and Stein, 1999), these reflexes may be enhanced during less stable tasks because of the increased probability of losing one's balance. Amplification of reflexes has also been shown during unstable forms of standing (Burke et al., 1991) and walking (Haridas et al., 2005; Misiaszek and Krauss, 2005). Therefore, our result showing reflex amplification during STAIR is generally consistent with these previous findings.

The phasing of cutaneous reflex modulation was in fact different in only two muscles: TA and MG. As stated above (see results), the shift in reflex phasing in MG could be explained by the shift in peak muscle activity observed across these tasks (Figure 2.1). However, the difference in TA phasing during stair climbing could not be explained by differences in muscle activity and may reflect a functional role of the reflex in this task. We suggest that the reflex near the stance-to-swing transition (phase 5) becomes suppressive during stair climbing to prevent contact-evoked dorsiflexion. Dorsiflexion at this point of the step cycle could result in tripping on the upcoming stair (if swing has been initiated) or collapse of the supporting ankle (if at end stages of stance). In this way it may be an enhanced expression of a task-specific stumbling correction (e.g. see Van Wezel et al., 1997; Zehr et al., 1997; Haridas and Zehr, 2003).

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2.4.2 Possible neural mechanisms and functional relevance

There are several lines of evidence supporting the idea that level and incline walking are coordinated by similar neuronal mechanisms in humans. First, gradual changes in joint angles and muscle activity with progressively increasing slope during walking have been shown previously (Tokuhiro et al., 1985; Leroux et al, 1999). That is, no abrupt transitions occurred that would indicate a shift in neural control (such as between standing and walking). Furthermore, we found only one difference in cutaneous reflexes between LEVEL and INCLINE (MG phase 3, Figure 2.1) and Simonsen et al. (1995) found that patterns of H-reflex modulation changed very little between these two tasks. Therefore, it is likely that these two tasks share common pattern generating elements (i.e. they are coordinated by a common core pattern generator - for review see Zehr, 2005).

It has been suggested that the transition from walking to stair climbing does not progress as gradually as the transition from level to incline walking (e.g. Riener et al, 2002). We also found more differences in reflex modulation between stair climbing and the two walking tasks than between level and incline walking. However, even though stair climbing was more different from the other tasks, reflex phasing was still generally conserved across all tasks despite substantial differences in muscle activity. Thus, components of the pattern generating networks may be shared across all three locomotor tasks; however, stair climbing involves specific task constraints (e.g. much greater chance of accidental tripping due to foot contact and increased stability demands) which further modify the expressed reflex patterns observed during this task. Specifically, two

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differences in reflex patterns were noted during stair climbing as compared to level or incline walking. First, reflex amplitudes were enhanced in many muscles during stair climbing, possibly reflecting a functional adaptation to decreased stability during this task (as noted above). Since this was an adjustment of reflex gain, rather than modulation patterns, this enhancement is probably not indicative of a switch in coordinating circuits (or pattern generating networks).

A more substantial difference in reflex modulation, suggestive of a difference in neural control, was seen in TA with the reversal of the middle latency response at the stance-to-swing transition during stair climbing (as compared to level and incline walking). Two neural mechanisms have been proposed to account for reflex reversal within a muscle (see Yang and Stein, 1990, De Serres et al., 1995). The first postulates that parallel excitatory and inhibitory pathways from cutaneous afferents converge on the α-motoneuron pool to facilitate or suppress muscle activity. This mechanism has been shown to account for reflex reversals in TA during human gait (De Serres et al., 1995). The second mechanism proposes that reflex reversals occur due to the recruitment and activation of different functional "units" of motoneurons that respond differently to cutaneous stimulation (Kanda et al., 1977; Burke, 1981; Nielsen and Kagamihara, 1993). For example, Nielsen and Kagamihara (1993) found that suppressive reflexes were more easily evoked in small (type I) motor units and facilitatory reflexes were more easily evoked in large (type II) motor units of TA, thus allowing for reflex reversals when different populations of motor units are recruited However, we found that suppressive reflexes occurred in TA when EMG was relatively high (phase 5 STAIR, Figure 2.1), in

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contrast to Nielsen and Kagamihara's (1993) findings. Moreover, TA is predominantly composed of type I muscle fibres (~76% type I – Jakobsson et al., 1988) and it is unlikely that the contraction levels experienced during stair climbing were high enough to recruit significant numbers of type II fibres. Therefore, a change in the relative excitability of parallel facilitatory and suppressive interneuronal pathways from cutaneous afferents onto the motoneuron pool is a more likely mechanism to account for the observed reflex reversal.

There is some evidence that that gating of suppressive reflex pathways onto TA motoneurons may be related to cortical contributions during gait. During level walking, suppressive reflexes in TA are often seen at the end of swing (Duysens et al., 1990; Yang and Stein, 1990; Van Wezel et al., 1997; Zehr et al., 1997), when cortical control of TA is probably higher than at other points in the step cycle (Schubert et al., 1997). During stair climbing, an additional phase where cortical input to TA may be increased is early swing phase since control over dorsiflexion is critical to ensure clearance of the upcoming step. In these phases where descending control may be enhanced, reflexes in TA are suppressive. Furthermore, Duysens et al. (2004) found that suppressive TA reflexes could be evoked during stance during an unstable walking task (i.e. split-belt treadmill walking), possibly as a result of increased cortical drive to TA during stance. Therefore, supraspinal input may be related to suppressive TA reflexes; however, afferent feedback and pattern generator activity may also play a role in gating reflex pathways.

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2.4.3 Conclusion

We propose that level walking, incline walking, and stair climbing share some common pattern generating elements based on the observation that reflex modulation patterns are similar across tasks. Our findings are complementary to the "common core hypothesis" proposed previously (Zehr, 2005), which describes an oscillatory core that sets up a basic rhythm and a network of spinal interneurons which can be dynamically facilitated or suppressed by cortical or sensory influences to produce different patterns of rhythmic human limb movement.

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CHAPTER 3: SPLIT-BELT TREADMILL STEPPING IN INFANTS SUGGESTS AUTONOMOUS PATTERN GENERATORS FOR THE LEFT AND RIGHT LEG IN HUMANS*

3.1 Introduction

Pattern generators controlling rhythmic locomotor movements in vertebrates are thought to have a distributed (reviewed in Kiehn & Butt, 2003) and modular organization (Grillner & Zangger, 1979; Grillner, 1981; Jordan, 1991; Stein 2005). While the constituents of a module are unclear, we know that groups of neurons, such as neurons from each side of the spinal cord (Kudo & Yamada 1987; Soffe 1989), or neurons controlling a set of muscles (Cheng et al., 1998; Stein & Daniels-McQueen, 2002, 2004) have considerable rhythmogenic and autonomous ability. Behaviourally, the independence of each hemispinal cord can be reflected in the coordination of leg movements on a split-belt treadmill, in which the belts are run at different speeds. Animals as diverse as cats and stick insects (Forssberg et al., 1980; Foth & Bassler, 1985) maintain coordination while stepping on a split-belt treadmill, but in addition, their limbs can also operate somewhat independently, as demonstrated by the limb on the faster treadmill belt sometimes taking more steps than the other leg(s) on the slower belt(s) (i.e., asymmetrical stepping). These results indicate the pattern generators for each leg have some degree of autonomy, while interacting with each other for coordination.

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There is increasing evidence that the human spinal cord also contains a pattern generator for walking (Calancie et al., 1994; Dimitrijevic et al., 1998). While it is likely that the human pattern generator for walking has retained some or many of the characteristics found in other vertebrates, it is not a foregone conclusion (Capaday, 2002; Nielsen, 2003). The evolution of bipedal walking has imposed greater demands on the lower limbs, demands that may have been met by evolutionary changes to the pattern generator in the spinal cord, and/or the role of other centres in the nervous system (e.g., Capaday et al. 1999; Petersen et al., 2001).

All previous reports of human adults stepping on split-belt treadmills have reported alternate stepping, with left and right steps alternating in a 1:1 fashion (Dietz et al., 1994; Prokop et al., 1995; Jensen et al., 1998). No asymmetrical stepping rhythms were reported. Volitional control may have overridden the behaviour of the pattern generator, however, in these adult subjects. In the single study with infants (Thelen et al., 1987), 2:1 steps were mentioned, but they seemed infrequent and no details were provided. Since the speed differential between the belts was small (2 fold), the full potential of the pattern generators may not have been revealed.

In this study, we examined split-belt treadmill stepping in infants, because their stepping is less likely to be under strong cortical control compared to adults (Forssberg, 1985; Yang et al. 1998). We tested stepping with a large variation in belt speeds. In addition, we tested the condition in which the belts were run in opposite directions, since other animals show such behaviour when making tight turns in walking (Graham 1985), or

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swimming (Field & Stein, 1997a&b). Preliminary data have been published (Lamont et al., 2003) and included in a review paper (Yang et al., 2004).

3.2 Materials and Methods

3.2.1 Subjects

A total of 45 infants, ranging in age from 5.0 to 11.8 months (mean±SD=9.4±1.3 months), were studied. None of these infants were able to walk independently at the time of the study. Infants were recruited through local health clinics. Parents were instructed to practice stepping with their infants as described in Yang et al. (1998). Once the infant was able to perform 10 consecutive steps (as reported by a parent), an experiment was scheduled. Ethical approval for this study was obtained through the Health Research Ethics Board, University of Alberta and Capital Health, Edmonton. Informed and written consent was obtained from a parent prior to the experiment. All experiments were conducted in accordance with the Declaration of Helsinki for experiments on humans.

3.2.2 Instrumentation

Walking was studied on a custom-built split-belt treadmill (model INFSBT-FP, designed and built by R Gramlich and S Graziano, University of Alberta). The two treadmill belts are driven by separate motors, which could be electrically coupled to run at the same speed (tied-belt condition) or uncoupled to run at different speeds (forward split-belt condition). The belts are also capable of running in the opposite direction (opposite split-belt condition), by reversing the motor drive. The speed of each treadmill belt was estimated by the rotation of the main drive shaft for each belt, and confirmed with the

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video data. A Plexiglas partition (15 cm in height) was placed between the 2 belts to ensure that the infant's legs remained on separate belts. Force plates located under each belt recorded vertical ground reaction forces.

Stepping movements were recorded with a video camera (Canon Elura 50) in the saggital plane (right side) at 30 frames per second. Infants were dressed in black stockings to enhance the contrast of the white markers (2 cm in diameter). Markers were placed over the superior border of the iliac spine, the greater trochanter, the knee joint line, the lateral malleolus and the head of the fifth metatarsal of the right leg, and the medial malleolus and first metatarsal of the left leg. An electrogoniometer (Penny & Giles, Biometrics, Blackwood, Gwent, UK) was placed over each hip joint. The arms of the goniometer were aligned with the mid-axillary line of the trunk and the longitudinal axis of the femur. The video and analog signals were synchronized by a custom-made timer, which generated pulses at 1 Hz.

In some experiments (17 infants), surface electromyograms (EMGs) were also recorded from the legs bilaterally. Pairs of infant-sized, silver-silver chloride EMG electrodes (Kendall LTP, Chicopee, MA, USA) were applied over the tibialis anterior (TA) and gastrocnemius-soleus (shortened as SOL) muscles after the skin was cleaned with alcohol. The signals were conditioned with an AMT-8 amplifier (Bortec Biomedical Ltd., Calgary, Canada), bandpassed at 10 to 10k Hz. The EMG signals were full-wave rectified and low-pass filtered at 30 Hz before digitization.

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All signals were digitized on-line using a commercially available A/D program (AXOSCOPE, Axon Instruments Inc., Foster City, CA, USA), at a rate of 340 Hz. The force plate and electrogoniometer signals were also low-pass filtered at 30 Hz. All unprocessed analog signals were also recorded on VHS tape with a pulse code modulated encoder (Vetter, Rebersburg, PA, USA) for backup.

3.2.3 Experimental procedures

The experiments were approximately 1 hour long. The infant was held under the arms from behind by one of the experimenters or a parent, over the treadmill belts. Forearm supports were provided for the person holding the infant to limit the possibility of imposing movements on the infant. The infant was allowed to support as much of his/her weight as possible. The infant's body weight was measured at the end of the experiment.

Each experiment began with a trial in which both belts ran at the same moderate speed (0.3 – 0.6 m/s) to familiarize the infant with treadmill stepping. The order of subsequent trials (see below) was arbitrarily chosen, and typically consisted of one or two of the following.

(1) Forward split-belt stepping

All infants were tested in this condition, with 39 generating useful data. The ratio of the two belt speeds ranged from 1.8 to 22.5, with the speed of the slow belt ranging between 0.04 – 0.31 m/s and the speed of the fast belt ranging between 0.23 – 1.69 m/s. Not all

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infants were tested at all speeds. In the first 10 infants tested, we focused on how they adjusted to the belt speeds when they were first put on the treadmill, to determine if there was a period of learning before a stable pattern was achieved. Only infants who showed at least 15 alternate steps in the first split-belt trial were included.

(2) Matched split-belt and tied-belt speeds in forward stepping

Twelve infants were tested under tied-belt conditions in which the belt speed was matched to the fast and slow belt speeds during the split-belt condition in turn (less than 20% difference between split-belt and tied-belt conditions). In this way, these infants could serve as their own control for comparisons of tied- and split-belt stepping. In 4 other infants, we occasionally stopped one leg on the split-belt treadmill by placing a piece of cardboard under the foot and holding it stationary (see Figure 2 in Pang & Yang, 2000). We anticipated the stepping rate in the contralateral leg to change back to what would be expected under tied-belt conditions when the influence of the opposite leg was removed.

(3) Treadmill belts running in opposite directions

Ten infants were studied with the right treadmill belt running backwards, while the left was running forwards. The speeds of the two belts were the same under this condition, and ranged between 0.25 – 0.4 m/s.

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3.2.4 Data analysis

Successful sequences of forward stepping (at least 3 consecutive steps) were identified off-line from video. Opposite direction stepping was deemed successful only if the backwards stepping foot was placed behind the hip joint marker to initiate stance phase (Lamb & Yang, 2000), and a minimum of 3 steps were seen on each side in the sequence.

The onset of stance was defined as the time when the foot made contact with the treadmill. The onset of swing was defined as the time when the toe marker changed from moving backward (stance phase) to moving forward (swing phase) for forward stepping, or visa versa for backward stepping. Force plate data provided confirmation of the onset of stance and swing phases. If there were discrepancies between the video and force plate data, the video data was used. Individual steps were selected from the digitized data and analyzed using custom-written programs in MatLab (Math Works, Natick, MA, USA). Signals from the selected steps were averaged, stance and swing durations for each step were calculated, as were the average level of force from the force plates during the stance phase, and the average level of muscle activity during the stance and swing phases.

The number of steps using either alternate or asymmetrical coordination was determined and expressed as a percentage of the total steps. Step counts always referred to steps made by the leg on the slow belt. In addition, a phase lag was calculated by expressing the onset of the step cycle in one leg as a function of the step cycle of the contralateral leg, and expressed as a percentage. When there were more steps on the fast belt, those

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steps were expressed as a function of the leg on the slower belt. While phase lag is technically an inappropriate term for asymmetrical steps because the frequency of stepping is different on each side, we will still use this term to refer to the coupling for simplicity.

Asymmetrically coupled steps are particularly suited for studying the relationship between the flexor and extensor generating centers for each leg. Only subjects with a sequence of asymmetrical steps (>2 in a row) and EMG signals free of crosstalk were used for this analysis. Crosstalk was defined as a Pearson's Product-Moment correlation >0.2 between the flexor and extensor muscles on a leg in the sequence of interest. EMG onset and offset times were estimated visually based on crossing a threshold that defined noise level. The duration of coactivity between homologous muscles from the 2 legs was expressed as a percentage of the total sequence time.

3.2.5 Statistics

To determine if there were time-dependent effects (i.e., learning) when subjects were first exposed to split-belt stepping, average step cycle durations were calculated for groups of 3 steps each, in chronological order. The first 3 groups of steps and the last 2 groups were compared with a Repeated-Measures Analysis of Variance (ANOVA). Paired t-tests were used for post-hoc analysis. Paired t-tests were also used for the comparison of step cycle, stance and swing phase durations under different experimental conditions in which the subject served as his own control. Comparison of the cycle structure in the fast leg for asymmetrically coupled steps were made with a paired t-test, and a repeated-

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measures ANOVA for the 2:1 and 3:1 coupled steps, respectively. An independent t-test was used to compare coactivation levels between homologous muscles on the 2 sides during asymmetrically coupled steps. The level of significance was 0.05 for all comparisons, except for the post-hoc tests, which were adjusted for the number of comparisons.

3.3 Results

3.3.1 Success Rate of Split-belt Walking

The majority of infants (39 out of 45 infants) who could step on the treadmill with both belts running at the same speed could also step when the belts were running at different speeds. The 6 infants who were unsuccessful still showed some stepping, but the sequences of stepping were not long enough to meet our definition of a successful trial. When the two belts were running in opposite directions, 6 out of 10 of the infants who could step in the tied-belt condition were also successful in this task. The 4 infants who were not successful also showed some stepping with the belts running in opposite directions, but in 2 infants the sequences were too short to be included in the analysis, and the other 2 failed to meet our definition of backward stepping. In summary, the ability to step under split-belt conditions was very high.

3.3.2 Time-dependent adaptation

Infants adjusted rapidly to the split-belt condition, which ranged from a speed differential of 2 to 13 (average 5.2). There was a trend for the first 3 step cycles to be slightly shorter in duration than the others. The one-way repeated measures ANOVA on cycle duration

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was significant for the slow leg only. Among the many post-hoc comparisons, only the average of the first 3 steps on the slower treadmill belt was significantly different from the other groups of steps. Thus, aside from the first 3 steps, all steps were representative of the steady state split-belt stepping.

3.3.3 Forward split-belt walking

Infants could adapt to large differentials between the 2 belt speeds. The maximum speed ratio between the 2 belts accommodated was 22.5. When the speed differential of the belts was low, infants tended to maintain alternate stepping by changing their cycle structure and duration to accommodate the differences (see below). As the differential increased, the infants sometimes used asymmetrical steps (i.e., 2:1, 3:1 or other). The coupling pattern varied step-to-step, with alternate steps randomly mixed with asymmetrical steps. A typical example is shown in Figure 3.1. The force plate data show when the legs were in stance and swing phase. The activity of a flexor and extensor muscle is shown for each leg. The coupling pattern is shown at the bottom of the graph. In general, the occurrence of asymmetrically coupled steps was more common with larger speed differentials between the 2 belts (Figure 3.2). Steps with 2:1 and 3:1 patterns were common, while 4:1 and 5:1 steps were occasionally observed. The ratio of fast to slow steps was always an integer.

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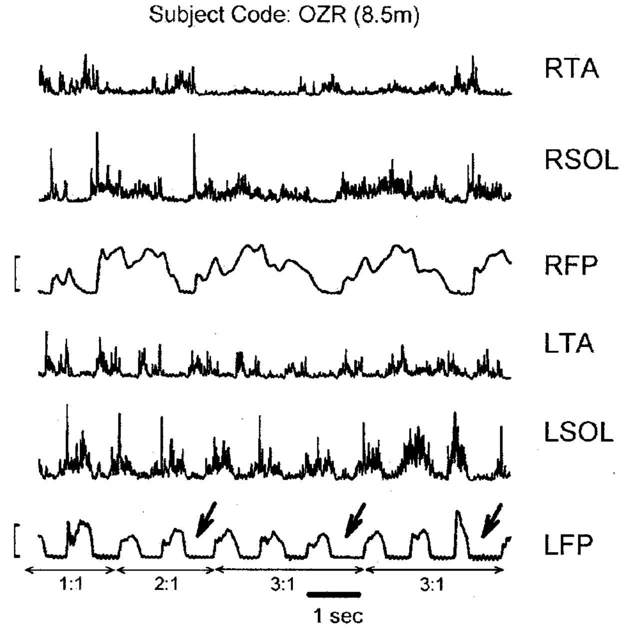

Figure 3.1: Typical data from a single subject (8.5 months old) during split-belt treadmill walking. The belt speeds were 0.06 m/s and 0.49 m/s for the right (R) and left (L) sides, respectively. Gastroc-soleus (SOL) and tibialis anterior (TA) EMGs are shown together with force platform (FP) data. In this sequence of walking, the subject showed a mixture of 1:1, 2:1 and 3:1 coordination between the legs. The EMGs are in arbitrary units, while the vertical brackets to the left of the force plate traces indicate 50% body weight. Diagonal arrows point to the second and third steps of the leg on the fast belt during 2:1 and 3:1 steps. These steps were systematically longer than the others.

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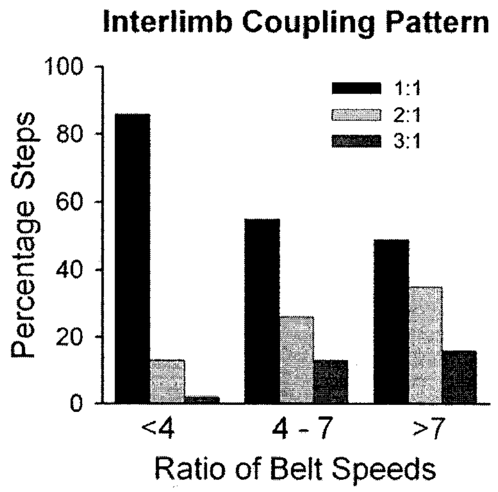

Figure 3.2: Ratio of treadmill belt speed influences the type of interlimb coordination. The percentage of steps showing 2:1 or 3:1 coordination increases as the ratio of belt speed increases. All infants included in this figure provided data with belt speed ratio in the range of <4, and at least one trial with >4 (n=24 infants).

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One-to-one coupling

During alternate stepping on the split-belt treadmill, the step cycle duration was intermediate between that during tied-belt stepping at each of the belt speeds. Figure 3.3A illustrates the step cycle duration as a function of the treadmill belt speed for all the trials recorded. Tied-belt stepping is shown by the black-filled circles. The data from the tied-belt condition was well fit by a power curve ($y=ax^{-b}$), as shown previously (Yang et al., 1998). Split-belt stepping is shown in the lighter symbols, with the step cycles from the slow belt in gray, and those from the fast belt as open symbols. Each symbol represents an average from a specific stepping condition in an infant. Most infants provided a number of data points, because stepping was obtained under a few conditions. It is clear the adaptation to split-belt walking was made by both legs, with the leg on the fast belt taking a slower step than it would normally do at that speed under tied-belt conditions (Figure 3.3A, many open circles above black circles), while the leg on the slow belt did the opposite (many gray circles below the black circles). The leg on the fast belt slows down by lengthening its swing phase (Figure 3.3B, many open circles above black circles), while the leg on the slow belt speeds up by shortening the stance phase (Figure 3.3C, many gray circles below black circles). Generally, the changes were more dramatic in the leg on the slower treadmill belt, such that the average step cycle duration was closer to what would be expected on the fast belt. This is seen by plotting the average step cycle duration measured during split-belt stepping, against the predicted cycle duration (Figure 3.3D). The predicted cycle duration is the mathematical average of the cycle duration during tied-belt stepping at each of the treadmill speeds, estimated from the equation $y=0.71x^{-0.59}$, in Figure 3.3A. If the prediction is correct, the points

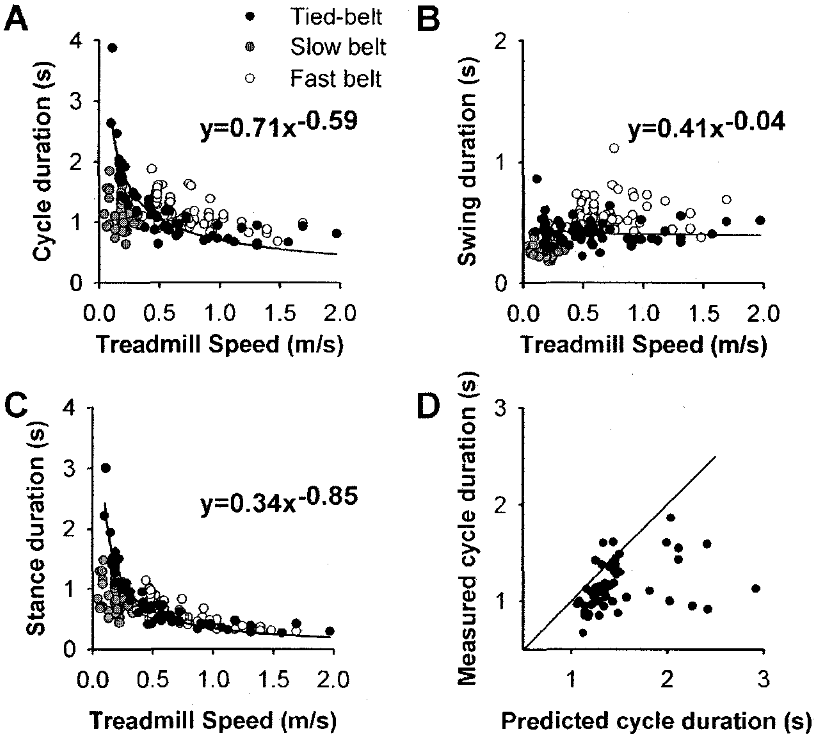

Figure 3.3: Step cycles during tied and split-belt stepping.
The step cycle (**A**), swing (**B**) and stance (**C**) phase durations are shown as a function of treadmill speed. Tied-belt measures (black filled circles) were fitted with the power function $y=ax^{-b}$, shown as the solid line on the graph, with parameters for the best-fitting line displayed. The measures taken from split-belt stepping are shown in gray filled circles for the slow leg and open circles for the fast leg (n=30 infants). Each point represents an average of at least 8 steps in a trial. Comparison of the measured and predicted cycle duration is shown in **D**. The predicted cycle duration is the mathematical average of that estimated from measures obtained during tied-belt stepping using the equation in **A**. The unit line indicating perfect prediction is shown. Most measured durations were shorter than the predicted.

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should fall on or near the unity line. The vast majority of points fell below the line, indicating the measured cycle durations were shorter than the predicted. Comparison of the cycle, stance and swing phase durations under the tied- and split-belt conditions with matched speeds are shown in Figure 3.4. Measures obtained from the leg on the slow belt and the fast belt are shown. Note that tied-belt measures were obtained from 2 separate trials, whereas split-belt measures were obtained from the same trial. It is clear that the step cycle duration during split-belt stepping is much closer to that during tied-belt stepping at the fast speed (Figure 3.4A), indicating a greater adjustment made by the leg on the slow belt. This adjustment included shortening of both the stance phase (Figure 3.4B left) and swing phase (Figure 3.4C left) during split-belt stepping. The leg on the fast belt lengthened its cycle duration (Figure 3.4A right) mostly by lengthening the swing phase duration (Figure 3.4C right). To verify the influence of the pattern generator of one leg affecting that of the other on a moment-by-moment basis, we occasionally stopped the stepping of one leg during forward split-belt stepping. Under this situation, the step cycle on the contralateral side reverted immediately back to what was observed during tied-belt stepping at the same speed (not shown).

Asymmetric coupling

The infants performed asymmetric stepping by increasing the step cycle duration on the slow side, while the fast side continued stepping at much the same rate as during 1:1 coupling. This was easily seen from the raw data (Figure 3.1). The cycle duration is lengthened on the slow side primarily by lengthening the stance phase, such that the stance phase occupied a disproportionately long period of the step cycle. Paired t-tests

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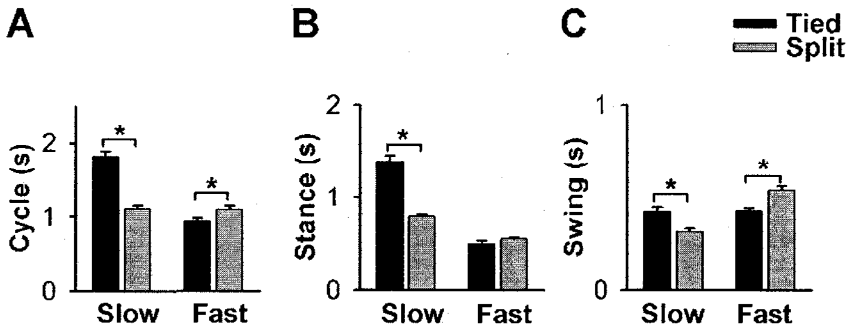

Figure 3.4: Cycle structure in tied and split-belt stepping with matched treadmill speeds.
The average step cycle, stance and swing phase durations for infants (n=12) with exactly matched speeds in tied-belt and split-belt conditions are shown for steps exhibiting 1:1 coordination (means±SEM). Black filled bars measured from 2 separate trials during tied-belt stepping (i.e., fast and slow speeds). Gray bars measured from split-belt stepping with one belt at the fast and one at the slow speed. **A**. The step cycle duration during tied-belt stepping is very different for the fast compared to the slow speed (i.e., height of black bars). When stepping under split-belt conditions, the step cycle on the 2 sides become similar (i.e., height of gray bars). The cycle duration changes more for the leg on the slow belt compared to that on the fast belt during split-belt stepping (difference greater between black and gray bars on left compared to right). During split-belt stepping, shortening of the step cycle on the slow belt (**A** left) comes from shortening of the stance phase (**B** left), and the swing phase (**C** left). The slight lengthening of the step cycle in the leg on the fast belt (**A** right) comes mostly from lengthening of the swing phase (**C** right). The * denotes statistical significance (paired t-tests).

showed significant lengthening of the stance phase across subjects, and no change in the swing phase (not shown). The multiple step cycles on the fast side were not equal in length (Figure 3.5A), with the second fast step in 2:1 coupling, and the third fast step in 3:1 coupling being longer. The duration of the stance phase in that step was very slightly lengthened, and it corresponded in time to the contralateral swing phase. The subsequent swing phase was also longer (see arrows in single subject data of Figure 3.1). To ensure that this difference (particularly the length of the swing phase) was not a result of the stepping condition in the following step (which could be a 1:1 coupled step), we reanalyzed a subset of the 2:1 steps using only those steps which were followed by another 2:1 or 3:1 coupled step. The 3:1 steps were not analyzed this way because of insufficient numbers. With this analysis, the differences remained (Figure 3.5B). We determined if the difference in phase durations was accompanied by differences in the intensity of muscle activation or the amount of weight-bearing. The average muscle activity for the tibialis anterior (swing phase) and gastroc-soleus (stance phase), and the average force plate (FP) signal (stance phase) were calculated for each of the fast steps during 2:1 coupling. There were no significant differences in the TA activation between the 2 fast steps in the swing phase, but there were significant differences in both the amount of weight bearing (force plate signal) and the gastroc-soleus EMG during the stance phase (Figure 3.5C).

The extent to which homologous muscles from the legs were coactive was estimated. Example from an individual sequences can be seen in Figure 3.1. There was significantly

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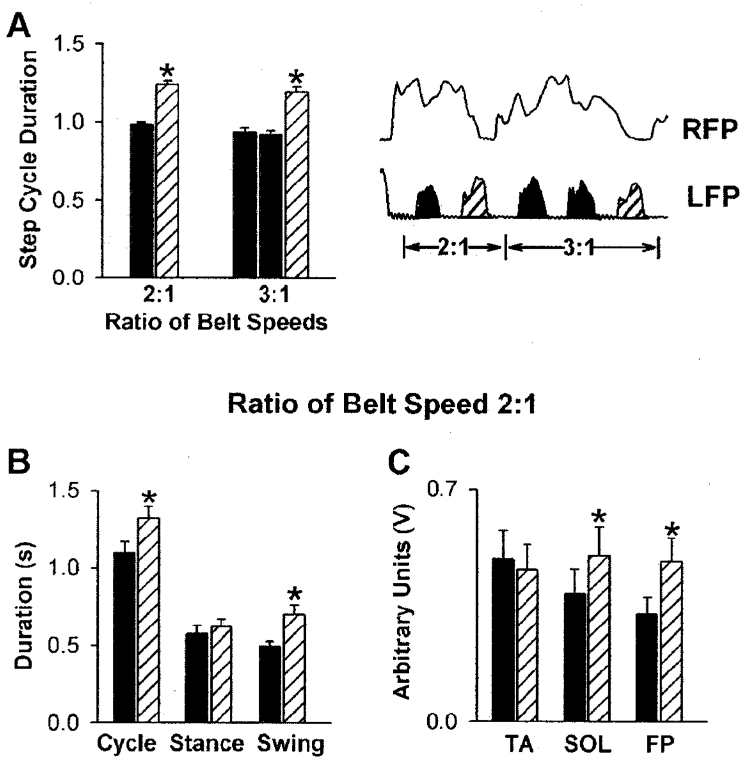

Figure 3.5: Cycle structure and muscle activation in asymmetrically coupled steps.
A. In asymmetrically coupled steps, the step cycles on the fast belt were not identical in duration. An example of raw data from the left (L) and right (R) force plates (FP) are shown on the right, to illustrate the shading of the different steps. The step that corresponded to swing phase on the contralateral side (hatched) was significantly longer than the other steps (black filled). Bar graph shows mean±SEM with statistically significant differences (*). (n=250 steps from 33 infants for 2:1 steps, and n=90 steps from 27 infants for 3:1 steps). **B**. A subset of the data from **A** which consisted of 2:1 steps that were followed by a 2:1 or 3:1 step are shown. The difference in cycle duration for the first and second step in the fast leg remained. This difference was largely a result of a longer swing phase. **C**. The average gastroc-soleus (SOL) EMG amplitude and the average weight-bearing was higher in the stance phase of the second step, while the average tibialis anterior (TA) EMG was not different between the 2 steps.

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more coactivation of extensors (mean±SD=31%±2%) compared with flexors (13%±4%) (Student's t-test). Regardless of the type of coupling between the legs, the stepping was very coordinated. For the most part, one leg went into swing phase only when the contralateral leg was in the stance phase. Figure 3.6A shows that when the infants were stepping with an alternating pattern (1:1 coupling), regardless of the speed differential, the phase lag was close to 50% (mean=53%). The phase lag was further analyzed for different split-belt speed ratios to determine if the phase interval was a function of the speed differential. No relationship was found. During asymmetrically coupled steps, the phase lags for each fast step is shown in different shading (Figure 3.6 B&C). There was considerable range in the phase lag for each of the steps, with means 30% and 74% for 2:1 coupling, and 22%, 52%, and 82% for 3:1 coupling. There were relatively few steps with a phase lag near 0% and 100%, which is the time the contralateral leg on the slow belt entered the swing phase.

3.3.4 Stepping in opposite directions

Stepping in opposite directions is depicted in Figure 3.7 for a single subject. The force plate and goniometer signals are shown at the top left. To illustrate the coordination more clearly, 5 specific time points (A through E) are identified with dotted lines, and the corresponding position and motion of the legs are shown in the sketches, made from the corresponding video frames. The position of the heel marker is also illustrated for the frames from A to E in the top right of Figure 3.7, with arrows indicating the direction of motion. While stepping in this manner, infants maintained an alternate relationship between the limbs. The phase lag is shown for all subjects in Figure 3.6D. The forward-

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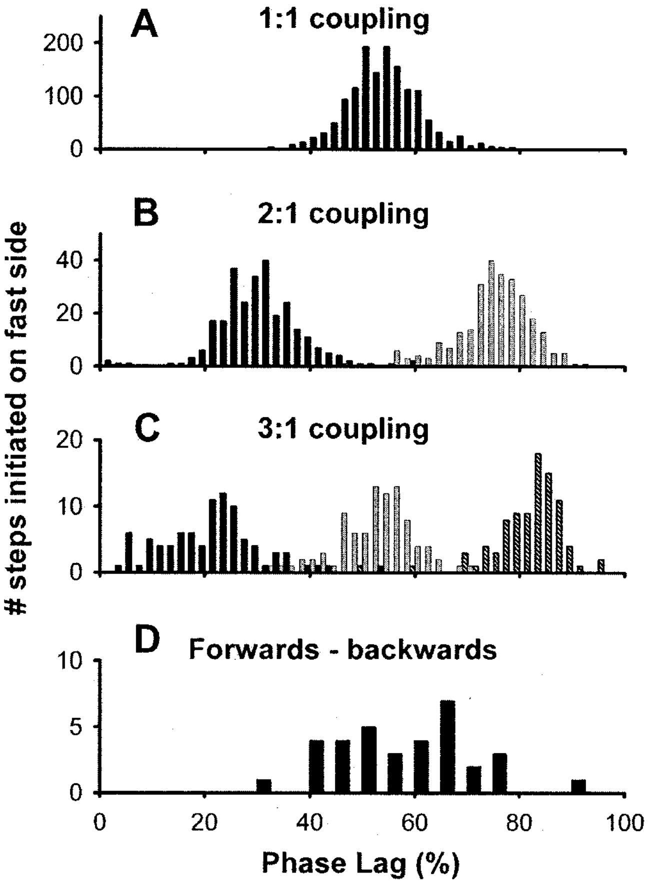

Figure 3.6: Phase relationship between the two legs for different types of stepping.
Initiation of steps on the fast leg as a function of the step cycle on the slow leg is defined as the phase lag. A phase lag of 0% means the two steps were initiated simultaneously, while a phase lag of 50% means the steps were exactly alternate. Steps that were coupled 1:1 show a phase lag centered around 50% (**A**). Phase lags for steps coupled 2:1 and 3:1 are shown in **B** and **C**, respectively, with each step on the fast side represented by different shading. Data obtained from 1416 steps with 1:1 coupling from 35 infants, 269 steps with 2:1 coupling from 32 infants, and 90 steps with 3:1 coupling from 27 infants. **D** shows coupling when the 2 belts were running in opposite directions. Phase lag here refers to the initiation of the forward stepping leg as a function of the backward stepping leg. Data is from 31 steps in 6 infants.

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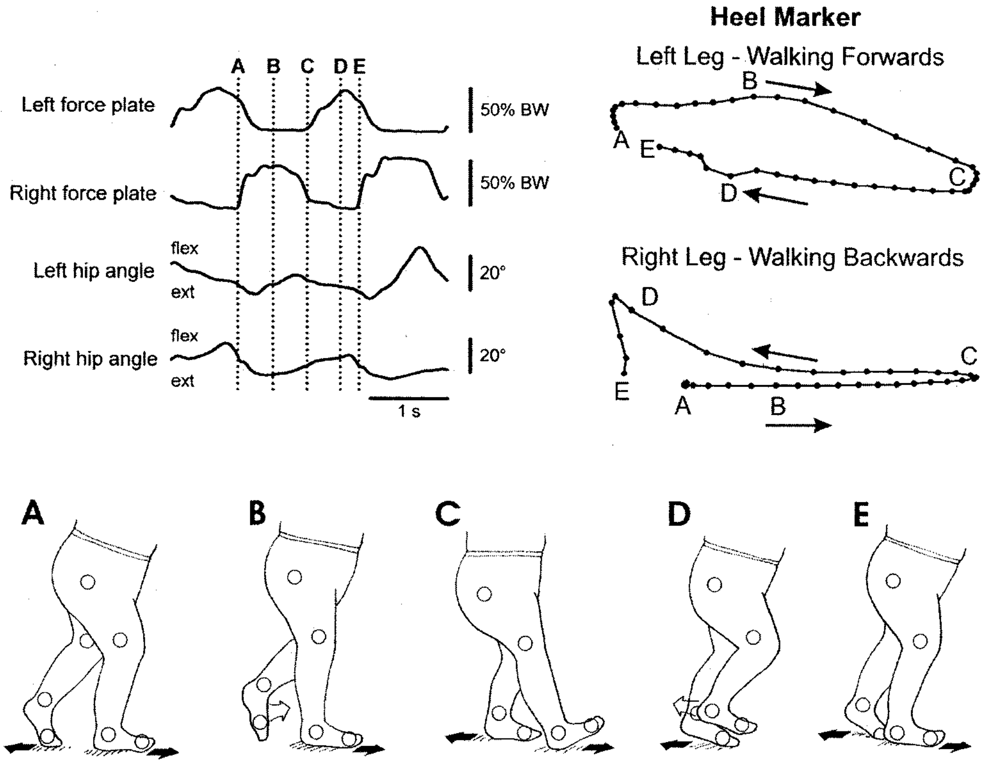

Figure 3.7: Data from a single subject during opposite direction split-belt stepping.
The left leg was stepping forward while the right leg was stepping backward. Sketches from the video frames corresponding to 5 specific time points (A, B, C, D, E) during the stepping are illustrated at the bottom of the figure. The arrows in the sketches illustrate the direction of motion of the leg (solid arrows show motion of foot during stance phase, open arrows show motion of foot during swing phase). The corresponding 5 points in time are shown by vertical lines on the force plate and goniometer data at the top left. The trajectory of the heel marker in the sagittal plane is shown in the top right for the time interval from times A to E, with arrows indicating the direction of movement. BW is body weight.

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stepping side initiated stance phase approximately mid-way through the step cycle on the backward-stepping side (mean phase interval 54%). Thus, the reciprocal relationship between the legs seen during other forms of stepping was also seen when the legs stepped in opposite directions.

3.4 Discussion

We have shown for the first time that infants can step in a coordinated fashion on a split-belt treadmill with the belts running at remarkably different speeds (up to 22 fold difference), and when the belts are running in opposite directions. The two legs could adopt different patterns of stepping, but they remained coordinated so that only one leg entered the swing phase at a time.

3.4.1 Inter-dependence of the pattern generators for each limb

The stepping in each limb showed some dependence on its counterpart on the other side. When the 2 belt speeds were different, a step cycle duration was adopted by each leg that was intermediate between that seen during tied-belt stepping at each of the belt speeds, just as has been shown for a variety of other animals (Kulagin & Shik, 1970; Halbertsma, 1983; Forssberg et al., 1980; Foth & Bassler, 1985). Moreover, if one limb was temporarily held stationary in the stance phase during split-belt stepping, the other limb immediately returned to its preferred cycle duration seen during tied-belt stepping. Thus, the influence of one limb on the other is clear, reminiscent of von Holst's (1973) demonstration of "coupled oscillators" controlling fish fins.

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Another example of the interdependence of the two limbs is seen during asymmetrically coupled steps. During 2:1 and 3:1 steps, the step cycles of the fast leg were not identical. The stance phase on the fast side coinciding with swing phase on the contralateral side was slightly longer than the others. Moreover, the subsequent swing phase on the fast leg, which corresponded to the beginning of the stance phase on the slow leg, was also substantially longer than the other swing phases. A similar asymmetry has been reported for split-belt stepping in intact cats (Halbertsma, 1976), and can be seen in the raw data reported for chronic spinal cats (Figures 7&8 in Forssberg et al., 1980). Such interaction between rhythm generators on each side of the body has also been reported for fictive locomotion in acute spinal cats (Grillner & Zangger, 1979) and fictive scratching in spinal turtles (Stein & McCullough, 1998). Lengthening of that step could be explained by either central or peripheral factors. Peripheral factors that might lengthen the stance phase include the higher load borne in that step because the contralateral limb is in swing phase, compared to other steps when the contralateral limb is in the stance phase. Peripheral factors that might lengthen the subsequent swing phase are less clear. Lengthening of the swing phase is a very strong effect, and is not accompanied by stronger activation of the TA muscle.. It is interesting that a similar lengthening of the flexor burst duration is seen in fictive preparations (Stein & McCullough, 1998), suggesting central factors may also play a role.

The relationship between the pattern generators on each side is also reflected in the degree to which homologous muscles on each leg could be coactive during stepping. This is most clearly seen during asymmetrically coupled steps, when the pattern

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generators are not operating in a strictly reciprocal way. Based on the timing of muscle activity, we found a much greater degree of coactivity in the extensors on the two sides compared with the flexors. In many sets of raw data, however, there was some depression in the extensor EMG when the contralateral extensor is active (some evidence in Figure 3.1). This phenomenon could be explained by changes in sensory input, because the limb load is also varying in exactly the same way (see force plate signal). Overall, there seems to be a stronger reciprocal inhibition between the flexor generating centers on each side compared with the extensor generating centers, just as has been shown in cats (Forssberg et al., 1980; Hiebert et al., 1996).

3.4.2 Independence of the pattern generator for each limb

The pattern generators for each limb were also able to operate independently, since at more extreme differences in belt speed, the fast limb could take more steps than the slow limb. This type of flexible coordination has been reported for many animal preparations, including stick insects (Foth & Bassler, 1985), spinal (Forssberg et al., 1980), decerebrate (Kulagin & Shik, 1970) and intact cats (Halbertsma, 1983), and spinal turtles (Stein & McCullough, 1998). Split-belt studies in human adults showed some independence of the pattern generators for each leg in relation to learning (Prokop et al. 1995), but asymmetrical stepping has not been reported. We confirm here that the putative human infant pattern generator for each limb shows some autonomy much like that in other animals. We attribute the scarcity of asymmetrical stepping in previous split-belt studies in humans to the lower speed differentials used (Dietz et al., 1994; Thelen et al., 1987), and to intervention from higher centres in the nervous system in the case of adults,

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perhaps to maintain equilibrium. It remains to be seen whether the adult pattern generator retains such independence as seen here with infants.

We also showed that the two limbs can step in opposite directions simultaneously. The stepping is not passive, because we defined successful steps to be those in which placement of the stepping limb at the time of foot-ground contact to be in front of (for forward stepping) or behind (for backward stepping) the hip joint, which requires an active motion. Further evidence this is an active process is the fact that swing phase does not coexist on the two sides during this stepping, just like the other forms of stepping. This type of locomotion in which the legs on each side of the body operate in opposite directions has been reported for insects pivoting or making very tight turns (reviewed in Graham 1985), and for spinal turtles making turns during swimming (Field & Stein, 1997a&b). Adult humans rarely show this type of coordination, but the capability is present. Perhaps this is a reflection of our evolutionary history.

The ability to step forwards in one limb while backwards in the other implies that the relative weighting of sensory input to the pattern generator is highly flexible. For example, one of the important sensory signals to initiate swing phase is hip extension for the forward stepping leg, while it is hip flexion for the backward stepping leg (Pang & Yang, 2002). Hence, swing phase is initiated by opposite sensory signals from the hip for each leg. This is similar to walking sideways, when the motion of the two limbs are opposite, with swing phase triggered by hip adduction in the leading limb, and hip abduction in the trailing limb (Pang & Yang, 2002). Together, these data suggest that the

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pattern generator might be better thought of as composing a swing phase controller and a stance phase controller, rather than the more traditional flexor and extensor half-centre. The swing phase controller can be functionally coupled to whatever muscles are needed to generate the required movement, such as hip flexors, extensors, abductors or adductors. Moreover, each of the controllers can be inhibited or excited by the appropriate sensory input for the particular walking direction. We speculate the pattern generators have considerable flexibility in which muscles they control, how they relate to each other, and how sensory input affects them.

3.4.3 Adjustments in step cycle to accommodate split-belt stepping

During alternate 1:1 stepping on the split-belt treadmill, the intermediate value of the step duration was almost always closer to that in tied-belt stepping at the faster speed. This suggests the faster leg exerted a greater influence on the overall pattern of walking, just as in adults (Dietz et al., 1994). Perhaps the sensory input from the fast leg is stronger and therefore more dominant.

In infants, adjustments in the step cycle occurred in both stance and swing phases in split-belt stepping. During 1:1 stepping, for example, the stance phase duration was longer in the slow leg, while the swing phase duration was longer in the fast leg (Figure 3.4). Hence, the proportion of the step cycle spent in the stance and swing phases are changeable, just as has been reported by Forssberg et al. (1980). Our data do not support the idea that the step cycle structure is immutable (Kulagin & Shik, 1967). The stance phase, however, appears to be more flexible in duration than the swing phase.

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3.4.5 Conclusions

The pattern generators for human infant stepping behave in a remarkably similar way to that in other terrestrial animals. The pattern generator for each leg has some autonomy, since different types of coupling (such as 2:1, 3:1), and opposite directions of stepping are possible simultaneously in both legs. At the same time, the pattern generator for each leg communicates with the other to ensure swing phase occurs in one limb at a time. The flexor generating centres in each leg likely inhibit each other, since co-existence of flexor activity was low. In contrast, coexistence of extensor activity on both sides was relatively common. Since different types of coupling are possible between the legs and even opposite directions of walking, it suggests the coupling relationship among the pattern generators is extremely flexible.

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CHAPTER 4: ASYNCHRONOUS ARM CYCLING REVEALS BILATERAL LOCOMOTOR COUPLING TO INCREASE MOVEMENT STABILITY[*]

4.1 Introduction

The evolution of upright stance and gait has allowed for a great deal of flexibility in how the upper limbs interact with each other. The arms can be coordinated in alternating patterns (e.g. when walking), in synchronous patterns (e.g. when rowing), and in patterns that require differential contributions from each arm (e.g. when playing a musical instrument). Evidence of synchronization during fine motor tasks has led to the proposal that synchronization is the default mode of coordination between the arms (Kelso, 1995;Bressler and Kelso, 2001;Varela et al., 2001;Spencer et al., 2006;Spencer and Ivry, 2007). For example, during finger-tapping and continuous circle drawing, movements in which homologous muscles are active together (i.e. in-phase movements) are most stable (Tuller and Kelso, 1989;Varela et al., 2001;Swinnen, 2002). This interaction between the arms is most apparent when trying to perform different movements simultaneously or during very fast movements.

In contrast to the arms, the legs are biased towards out-of phase coordination (Ting et al., 2000;Pang and Yang, 2001;Kautz et al., 2002;Kawashima et al., 2005), which is the pattern used for the majority of locomotor movements. Interestingly, during locomotor-like rhythmic arm movement, little evidence for bilateral interaction has been found (Zehr et al., 2003;Carroll et al., 2005;Hundza and Zehr, 2006). Rhythmic arm cycling

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has significant relation to the form of arm and leg activity occurring during walking and is thought to be coordinated in part by central pattern generators (CPGs – Zehr et al., 2004); however, crossed-effects due to changing contralateral activity were not observed in various unilateral and bilateral arm cycling tasks (Zehr et al., 2003;Carroll et al., 2005;Hundza and Zehr, 2006). Since previous investigations have examined arm cycling tasks that utilized simple temporal and spatial coordination, it is possible that coupling between the arms may be revealed during a more complex task. Alternatively, since synchronization may be the default mode of coordination between the arms, inter-arm coupling may be suppressed during locomotor-like arm movement to allow for alternating activity.

The purpose of this study was to determine whether inter-arm coupling would be revealed during an asynchronous arm cycling task, in which one arm cycled at twice the frequency of the other. We hypothesized that this temporally complex task would reveal coupling between CPGs for each arm, as evidenced by alterations in amplitudes and modulation of muscle activity and cutaneous reflexes across the movement cycle when the temporal coordination between the arms was altered. Preliminary data have been published in abstract form (Lamont et al., 2005;Lamont and Zehr, 2006).

4.2 Materials and Methods

4.2.1 Subjects

Twelve neurologically intact subjects (three males and nine females, aged 20-44 years) participated in the experiment with informed, written consent under approved protocols

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for human subjects at the University of Victoria conducted according to the Declaration of Helsinki.

4.2.2 Protocol

Participants performed rhythmic arm cycling using a custom-built, hydraulic arm ergometer that has been described previously (Zehr and Kido, 2001;Zehr et al., 2003). The positions of the ergometer cranks were defined relative to the clock face, with the top position specified as 12 o'clock, and the other positions following the clock when the ergometer was viewed from the subject's right (ipsilateral) arm. The two arms of the ergometer could be mechanically coupled together or allowed to rotate independently. Subjects performed seven different arm cycling tasks, each for ~5 minutes. The tasks included two unilateral (UNI), three bilateral (BILAT), and two asynchronous (ASYNC) arm cycling tasks (see Figure 4.1A for schematic). The two unilateral cycling tasks were (1) 1 Hz ipsilateral cycling while the contralateral arm was stationary at the 6 o'clock position (UNI 1Hz) and (2) 2 Hz ipsilateral cycling while the contralateral arm was stationary at the 6 o'clock position (UNI 2Hz). The three bilateral tasks were (1) 1 Hz out-of-phase cycling with the cranks coupled (or constrained) 180° apart (BILAT CON), (2) 1 Hz out-of-phase cycling with the cranks uncoupled (BILAT 1Hz), and (3) 2 Hz cycling with the cranks uncoupled (BILAT 2Hz). The two asynchronous tasks were (1) 1 Hz ipsilateral cycling and 2 Hz contralateral cycling (ASYNC 1:2) and (2) 2 Hz ipsilateral cycling and 1 Hz contralateral cycling (ASYNC 2:1) (also see Figure 4.1B and C for explanation). Visual and verbal feedback about cycling frequency was provided to the subjects. Asynchronous cycling was demonstrated and practiced for five to ten

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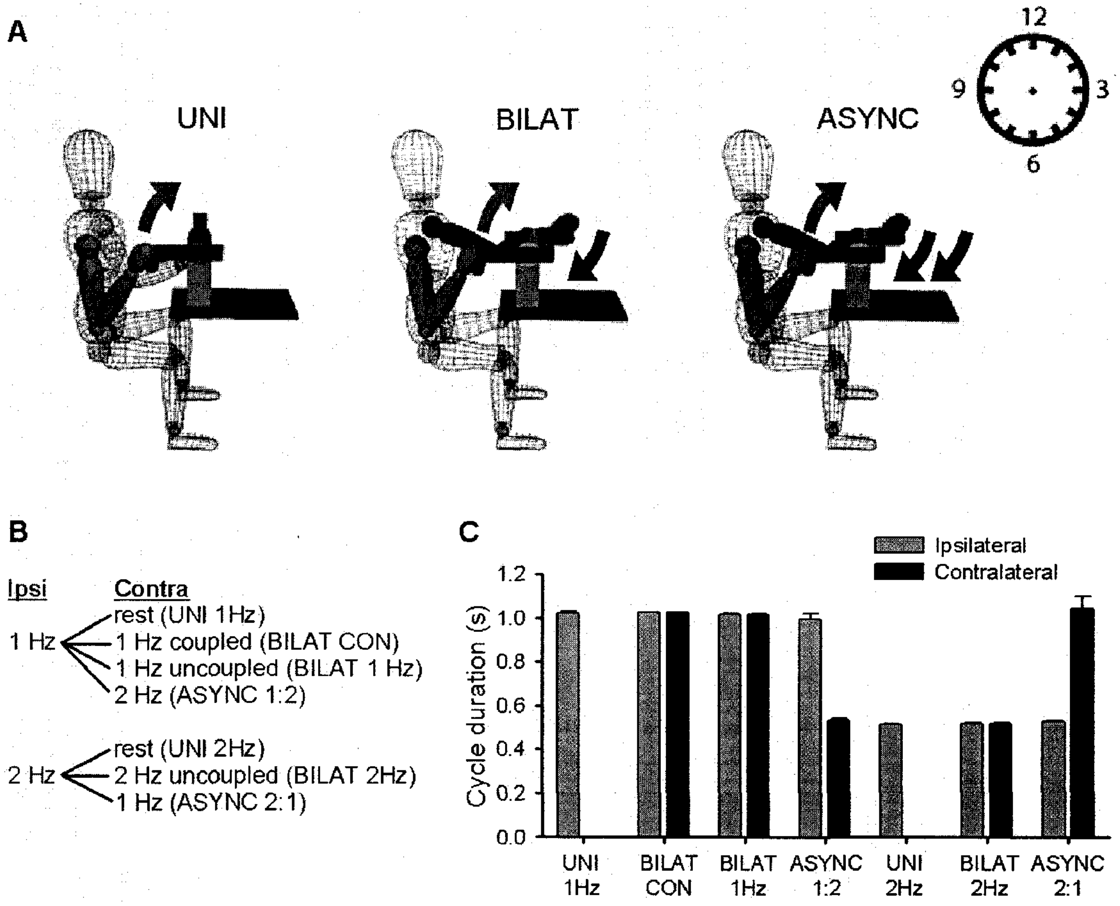

Figure 4.1: Experimental protocol and cycling tasks.
(A) Schematic illustration of arm cycling tasks (UNI only ipsilateral arm cycled, BILAT both arms cycled out-of-phase, ASYNC one arm cycled at twice the rate of the other). A stationary arm is represented by a mesh outline; ipsilateral (grey) arm movement is represented by a grey arrow showing the cycling direction; contralateral (black) arm movement is represented by a black arrow showing the cycling direction. Two arrows show when one arm was cycling twice as fast as the other. The positions of the arm cranks were defined using the clock face (with 12 o'clock at the top) when viewing the ergometer from the subject's right arm. (B) The cycle frequencies of the ipsilateral (Ipsi – grey text) and contralateral (Contra – black text) arms are shown for each of the seven tasks. In the first four tasks shown (UNI 1Hz, BILAT CON, BILAT 1HZ, and ASYNC 1:2), the ipsilateral arm always cycled at 1 Hz while the cycling rate varied on the contralateral arm. In the remaining tasks (UNI 2Hz, BILAT 2Hz, ASYNC 2:1), the ipsilateral arm always cycled at 2 Hz while the contralateral cycling rate varied. (C) Mean cycle durations (± standard error) across all subjects are shown for the seven tasks. Grey bars show the cycle duration on the ipsilateral arm, while black bars correspond to the contralateral arm.

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minutes prior to testing. The order of the trials was randomized and subjects were allowed to take breaks if they felt fatigued.

4.2.3 Electrical Stimulation

The right superficial radial nerve was stimulated at the wrist via bipolar Ag-AgCl surface electrodes (Thought Technology, Montreal, QC) using a Grass S88 stimulator connected to an SIU5 isolation unit and a CCU1 constant current unit (Astro-Med, Inc., West Warwick, RI). Stimulation was applied in trains of 5 x 1.0 ms pulses at 300 Hz and at a non-noxious intensity of ~2x the radiating threshold (RT - see Zehr and Chua, 2000; Zehr and Kido, 2001 for details). Stimulations were pseudorandomly delivered at a rate such that no more than one stimulation occurred within a cycle. Approximately 100 stimulations were obtained from each trial.

4.2.4 Electromyography (EMG)

Once the skin was cleaned with alcohol, bipolar Ag-AgCl surface electrodes (Thought Technology, Montreal, QC) were placed over the target muscles. EMG recordings were obtained bilaterally from flexor carpi radialis (FCR), biceps brachii (BB), triceps brachii (TB), anterior deltoid (AD), and posterior deltoid (PD). Muscles were defined as being either ipsilateral (e.g. iBB) or contralateral (e.g. cBB) to the site of stimulation. Ground electrodes were placed over electrically neutral tissue (on the olecranon process of the elbow and the clavicle). EMG signals were amplified 5000x and filtered from 100-300 Hz (Grass P511, Astro-Med, Inc., West Warwick, RI).

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4.2.5 Data acquisition and analysis

Data were sampled simultaneously at 1 kHz with a 12 bit A/D converter connected to a computer running custom-written LabView virtual instruments (National Instruments, Austin, TX). Off-line analysis was performed to separate the arm cycle into 6 equal phases (each 60° or "2 hours" in length), beginning with the 12 o'clock position, using an interactive custom-written MatLab program (the Mathworks, Natick, MA). During the off-line analysis, EMG data were full-wave rectified and low-pass filtered at 40 Hz with a dual-pass Butterworth filter.

Sweeps of rectified EMG were collected around each stimulation, from 100 ms pre-stimulus to 200 ms post-stimulus. Stimulated sweeps that occurred within the same movement phase (based on the time of stimulation) were averaged together (10-20 sweeps in each phase). Averaged EMG from non-stimulated cycles was subtracted from the averaged stimulated sweeps to obtain subtracted reflex traces. This subtraction resulted in negative values when the stimulated EMG was less than the non-stimulated EMG. Reflexes that peaked between 50-80 ms after stimulation were classified as early latency and those that peaked between 80-120 ms were classified as middle latency. Reflexes were quantified as the peak amplitude (from a 10 ms window centred on the peak latency) within the middle latency. Averaged reflexes for each subject were considered significant if the peak exceeded a 2-standard deviation band around the mean pre-stimulus EMG level. Within each subject and muscle, if the stimulus intensity was

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sufficient to evoke a significant reflex in at least one of the conditions tested, corresponding windows of muscle activity were selected for all conditions.

Phase relationships between the two arms were quantified as the position of the ipsilateral arm (in degrees) when the contralateral arm was at the 12 o'clock position (shown in Figure 4.2A). In the case of the asynchronous tasks, this was defined as the position of the 2 Hz arm when the 1 Hz arm was at 12 o'clock. Each phase relationship was plotted around a circle of radius 1 and the centre of gravity or mean for the data set was determined using trigonometric functions (see Batschelet, 1981).

4.2.6 Statistics

BILAT CON was used as the reference or "control" condition and all data (background and reflex EMG) were normalized to the peak background EMG amplitude during this condition. Coefficients of variation were calculated for the cycle durations of each arm during ASYNC 1:2 and ASYNC 2:1. T-tests were used to compare these coefficients between the high and low frequency arms. Linear regression analysis was used to determine significant correlations between reflex amplitudes and background EMG levels. To analyze differences in background EMG and reflexes, repeated-measures ANOVAs were used and post-hoc analysis of significant main effects and interactions was conducted using Student-Newman-Keul's test. Since we hypothesized that reflex amplitudes would be different during ASYNC, the phases of movement in which ASYNC differed from at least one other task are shown by asterisks in Figures 3, 5, and 6. A repeated-measures ANOVA and Student-Newman-Keul's post-hoc test were also

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used to analyze differences in perceived mental exertion (Table 4.1). To analyze differences in phase relationships between different tasks (e.g. BILAT CON, BILAT 1Hz, BILAT 2Hz – see Figure 4.2 C), the Watson-Williams test for circular observations was performed (Batschelet, 1981). Statistical significance was set to $P < 0.05$.

4.3 Results

Figure 4.1C shows mean cycle durations (± standard error) for ipsilateral and contralateral arms during each of the tasks. Even during the asynchronous tasks, subjects maintained the cycling rate of each arm such that the targeted frequencies were maintained (i.e. one arm at 1 Hz and the other at 2 Hz). The coefficient of variation for cycle duration of the slow arm was significantly greater than that of the fast arm during for both ASYNC 1:2 and ASYNC 2:1, indicating higher variability in the cycling of the slow arm (data not shown).

Figure 4.2A shows single subject data during ASYNC 1:2. Notice that the coordination pattern clearly shows two rotations with the contralateral arm for every rotation with the ipsilateral arm. An example of how phase relationship polar plots were constructed is shown in Figure 4.2B. For each cycle, the individual phase relationships were plotted around the circumference of the graph. The x and y coordinates of each data point were extrapolated using trigonometric functions and these were averaged to give the coordinates of the mean phase relationship. Figure 4.2C and D show mean phase relationships between the two arms for the bilateral (BILAT CON, BILAT 1Hz, and BILAT 2Hz) and asynchronous (ASYNC 1:2) cycling tasks. Since there were no

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differences in the phase relationships during ASYNC 1:2 and ASYNC 2:1, only ASYNC 1:2 is shown. The mean phase relationship for BILAT CON was 180° (Figure 4.2C) since the arms were physically coupled to maintain this relationship. When the arms were uncoupled during bilateral cycling, the arms were not precisely 180° apart from one another; rather, the mean phase relationship during BILAT 1Hz was ~150° and during BILAT 2Hz was ~135° (Figure 4.2C). Phase relationships during these three tasks were found to be significantly different ($p<0.05$). During ASYNC, the phase relationships were ~80° and ~290°, depending on whether it was the 1st or 2nd pass of the 2 Hz arm, In other words, the 2 Hz arm was at 12 o'clock when the 1 Hz arm was either between 2-3 o'clock or 9-10 o'clock (Figure 4.2D).

Nine of the twelve subjects were asked to quantify the attentional demands of each task by rating "mental exertion" on a scale from 1 to 10. A score of 1 meant that the task was not at all attentionally demanding and subjects felt that they could easily carry on a conversation while performing the task (although no actual speaking was performed during any of the tasks). A score of 10 meant that the task was extremely demanding, and any attempts to speak or do anything other than the task at hand would interfere significantly with the performance of the task. Results are shown in Table 4.1. Subjects reported that ASYNC (both 1:2 and 2:1) and BILAT 2Hz were significantly more attentionally demanding than BILAT CON ($p<0.05$).

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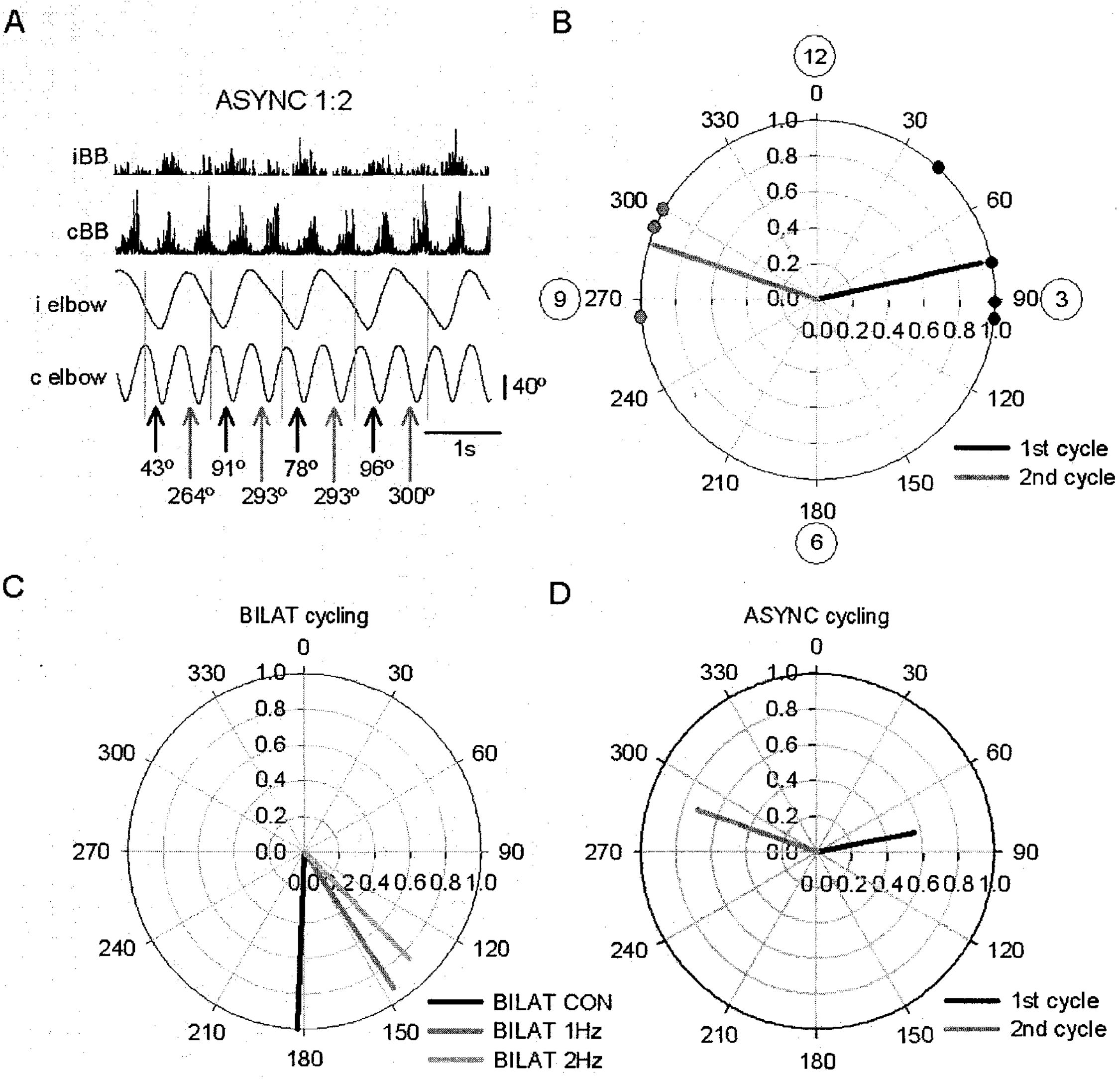

Figure 4.2: Interlimb coordination during bilateral and asynchronous cycling.
(A) Single subject EMG and elbow angle traces are shown during asynchronous cycling (ASYNC 1:2). Abbreviations are as follows: i, ipsilateral; c, contralateral; BB, biceps brachii. The 12 o'clock position of the ipsilateral arm (cycling at 1 Hz) is marked by the vertical grey lines. The 12 o'clock position of the contralateral arm (cycling at 2 Hz) is marked by arrows: black arrows mark the first time the contralateral arm passes 12 o'clock (1st cycle) and grey arrows mark the second time this occurs (2nd cycle). The numbers below the arrows give the phase relationship between the two arms when the contralateral arm is at 12 o'clock. (B) An example showing the extrapolation of polar plots using single subject data shown in (A). The individual phase relationships are plotted as dots around the circumference of the graph (1st cycle in black, 2nd cycle in grey). The x and y coordinates of the phase relationships were extrapolated via the sine and cosine of each angle and these coordinates were averaged to give the coordinates of the mean phase relationship (shown by the black and grey lines for 1st cycle and 2nd cycle, respectively). The length of the line shows the amount of variability in the data set; the closer the length is to 1.0, the less variability is in the data. Clock face positions (in o'clock) corresponding to the phase relationships are shown around the circumference of this plot (C) Mean phase relationships during the bilateral cycling tasks (BILAT CON in black, BILAT 1Hz in dark grey, and BILAT 2Hz in light grey). (D) Mean phase relationships during the 1st (black) and 2nd (grey) contralateral cycles during ASYNC 1:2. ASYNC 2:1 is not shown because the phase relationships are very similar to those in ASYNC 1:2.

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Table 4.1: Ratings of perceived mental exertion self-reported by nine subjects.
A score of 1 indicates that the task was not very attentionally demanding where as 10 indicates that it was extremely demanding. Tasks are explained in Figure 4.1.

Tasks	*Ratings of mental exertion (average ± standard error)*
BILAT CON	1.12 ± 0.16
UNI 1Hz	1.72 ± 0.24
BILAT 1Hz	2.28 ± 0.28
ASYNC 1:2	5.72 ± 0.76 *
UNI 2Hz	2.56 ± 0.68
BILAT 2Hz	3.89 ± 0.76 *
ASYNC 2:1	4.94 ± 0.87 *

* indicates a significant difference ($p<0.05$) from the control condition (BILAT CON).

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4.3.1 Background EMG

Since we were interested in any changes to ipsilateral EMG and reflex amplitudes that occurred as a result of differences in contralateral arm activity, we focused on the ipsilateral muscles in this analysis. Figure 4.3 shows background EMG during all the tasks when the ipsilateral arm was cycling at 1Hz (left column) and 2Hz (right column). With the exception of FCR, the lowest EMG activity when the ipsilateral arm was cycling at 1 Hz occurred in the BILAT CON task. This is not surprising since the two arm cranks were coupled together during this task, thus allowing the contralateral arm to assist in the cycling on the ipsilateral arm. Interestingly, even though the mechanical demands of the remaining three tasks with ipsilateral 1 Hz cycling (i.e. UNI 1Hz, BILAT 1Hz, ASYNC 1:2) were the same, the background muscle activity was altered. Specifically, background EMG during ASYNC 1:2 was increased above that during the other tasks in all muscles (other than AD). In all of the ipsilateral 1 Hz cycling tasks, EMG modulation patterns were similar in the flexor muscles (FCR, BB, and AD); that is, the peak values occurred within the same phase across tasks. However, there were some differences in the modulation of EMG in extensors (TB and PD). Specifically, during ASYNC 1:2, there was little modulation of EMG in TB compared to the other tasks in which the ipsilateral arm was cycling at 1 Hz. Furthermore, the modulation pattern of PD EMG shifted during ASYNC 1:2: the peak activity occurred at 5 o'clock during ASYNC 1:2 and at 7 o'clock during the other tasks. There were significant differences due to task in all of the muscles during 1 Hz cycling on the ipsilateral arm (as indicated by asterisks on the left column graphs).

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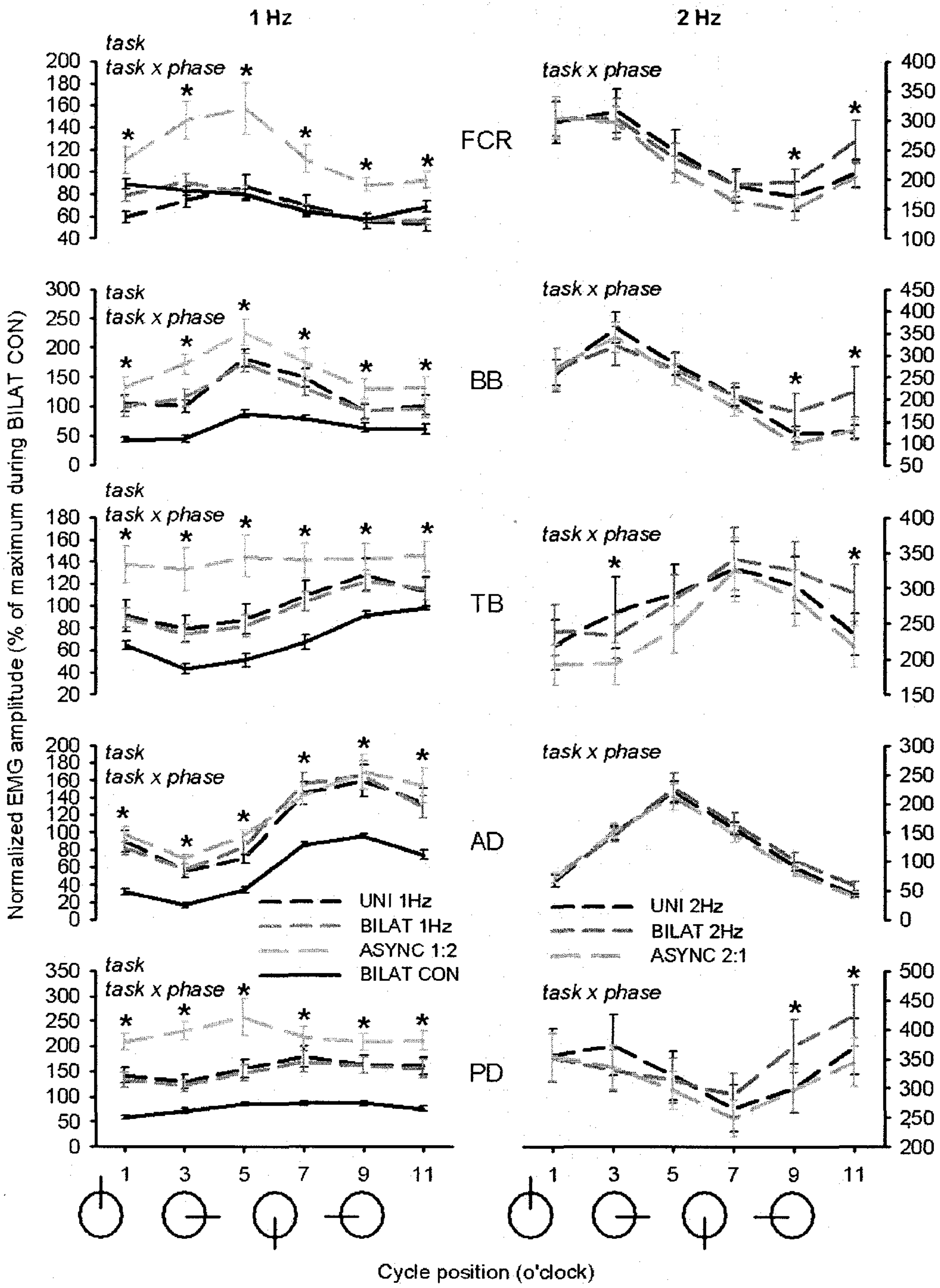

Figure 4.3: Normalized background EMG from ipsilateral muscles during the seven cycling tasks. Values were averaged across all subjects for each task (± standard error) and normalized to the peak EMG during BILAT CON. The left column of graphs shows data when the ipsilateral arm was cycling at 1 Hz (i.e. UNI 1Hz, BILAT 1Hz, ASYNC 1:2, BILAT CON); the right column shows data when the ipsilateral arm was cycling at 2 Hz (i.e. UNI 2Hz, BILAT 2Hz, ASYNC 2:1). The cycle position (according to the clock face) is indicated along the x-axis at the bottom and is also illustrated by the diagrams beneath the x-axis. Abbreviations are as follows: FCR, flexor carpi radialis; BB, biceps brachii; TB, triceps brachii; AD, anterior deltoid; PD, posterior deltoid. Significant main effects for task (task) and task by phase interactions (*task x* phase) are indicated at the top left of each plot. Asterisks denote where there was a significant difference between ASYNC and at least one of the other tasks ($p<0.05$).

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When the ipsilateral arm was cycling at 2 Hz (i.e. during UNI 2Hz, BILAT 2Hz, and ASYNC 2:1 – see Figure 4.3, right column), the EMG amplitude between tasks was quite similar. The modulation patterns across tasks mirrored each other quite closely and the minimum and maximum background EMG tended to occur within the same phases. Near the end of the cycle (9 and 11 o'clock), EMG amplitude was increased during BILAT 2Hz, compared to UNI 2Hz and ASYNC 2:1 in FCR, BB, TB, and PD.

4.3.2 Cutaneous reflexes

An example of subtracted reflex traces from an individual subject for biceps brachii (BB) is shown in Figure 4.4. The tasks shown were the ones in which the ipsilateral arm was cycling at 1 Hz while the contralateral activity varied. Around the 5 o'clock position, there was a suppressive early latency response (50-80 ms after stimulation) and a facilitatory middle latency response (80-120 ms after stimulation) in this subject. This pattern of early and middle latency responses was conserved, regardless of the contralateral activity.

Similar to what was observed in the single subject data, group averages also indicated that early latency reflex amplitudes and modulation patterns were largely conserved for the ipsilateral arm, regardless of the contralateral activity (Figure 4.5). When the ipsilateral arm was cycling at 1 Hz (left column of Figure 4.5), there were few instances where the reflexes were different between ASYNC 1:2 and the other tasks. Main effects for task were noted in BB and AD and task x phase interactions were noted in FCR and

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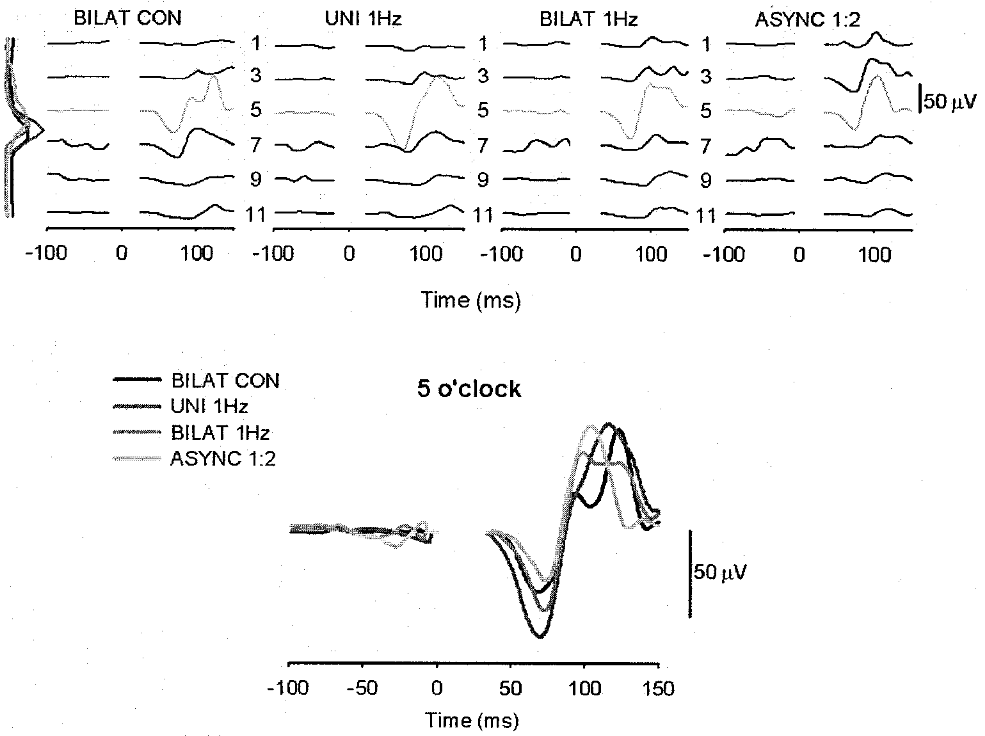

Figure 4.4: Subtracted EMG traces from ipsilateral biceps brachii of a single subject during BILAT CON, UNI 1Hz, BILAT 1Hz, and ASYNC 1:2.

Sweeps of data are shown from 100 ms before stimulation to 150 ms after stimulation. For the upper graphs, the cycle position (expressed as o'clock) is represented by the numbers between the plots. Stimulation occurred at time 0 and the stimulation artefact (between 0 – 30 ms) was removed. The vertical traces plotted on the left of the graphs indicate the averaged background EMG during each task across the step cycle. In the lower graph, reflex traces from the same subject at 5 o'clock are shown during BILAT CON, UNI 1Hz, BILAT 1Hz, ASYNC 1:2.

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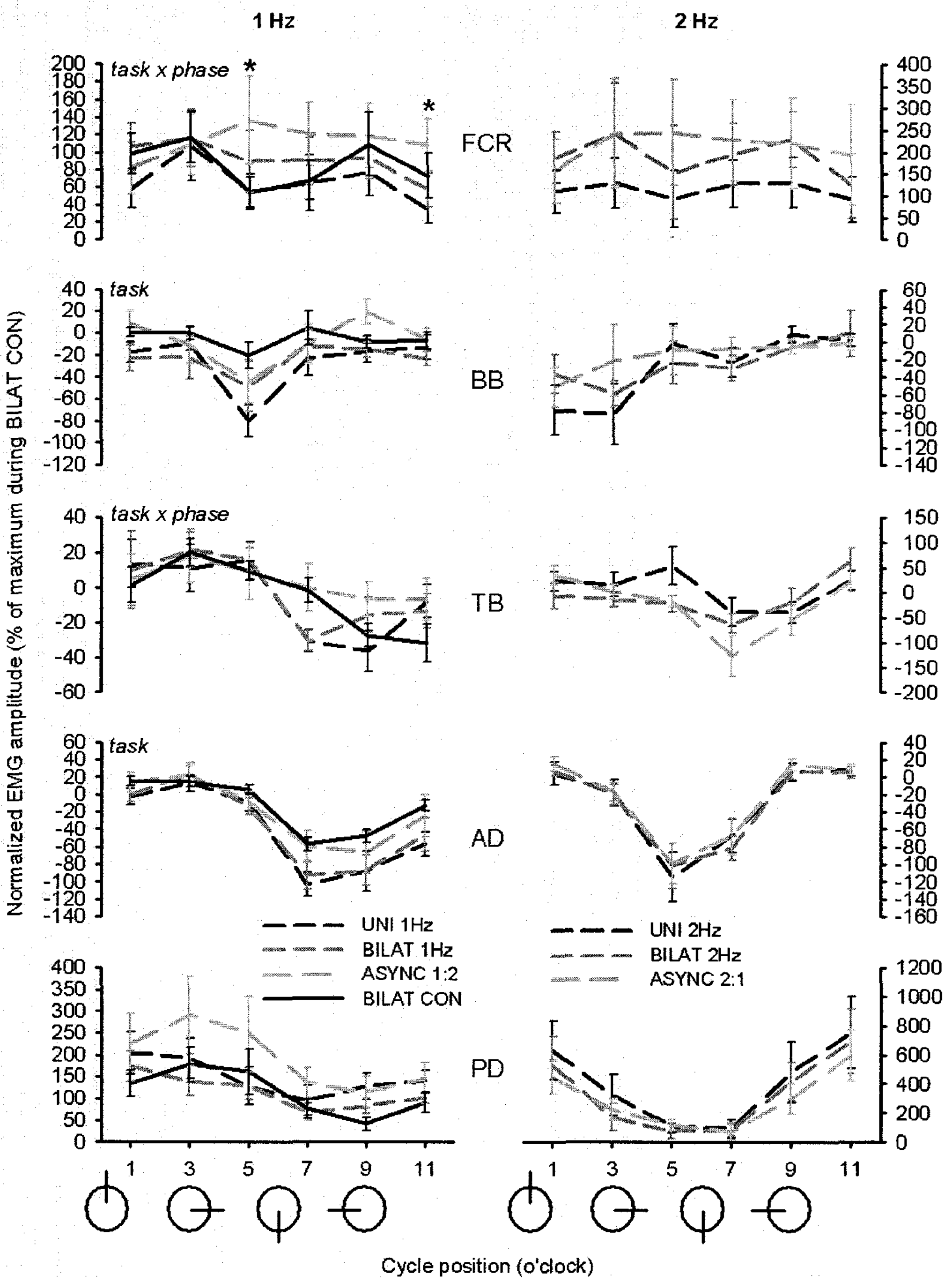

Figure 4.5: Early latency reflex amplitudes from right ipsilateral muscles during the cycling tasks. The format of this figure and abbreviations are the same as in Figure 4.3. The left column of graphs shows data when the ipsilateral arm was cycling at 1 Hz and the right column shows data when the ipsilateral arm was cycling at 2 Hz. The cycle position (according to the clock face) is indicated along the x-axis at the bottom. Significant main effects for task (task) and task by phase interactions (*task x* phase) are indicated at the top left of each plot. Asterisks denote where there was a significant difference between ASYNC and at least one of the other tasks ($p<0.05$).

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TB. The differences in early latency reflex amplitude were not as large or numerous as the differences noted in background EMG (compare to differences in Figure 4.3). In particular, note that the amplitude of early latency reflexes during ASYNC 1:2 was comparable to the other tasks with right arm 1 Hz cycling, despite the large increase in background EMG that occurred during this task (see Figure 4.3). FCR was the only muscle that showed significant differences between ASYNC 1:2 and the other tasks when post-hoc analysis was performed. When the ipsilateral arm was cycling at 2 Hz, there were no differences in early latency reflex amplitudes regardless of the contralateral activity (Figure 4.5, right column).

Middle latency reflexes were also little affected by differences in contralateral activity (Figure 4.6). When the ipsilateral arm was cycling at 1 Hz, there were few differences in middle latency reflex amplitudes (only task main effects in BB and AD and a task x phase interaction in TB). Again, note that reflexes were similar during ASYNC 1:2 and the other tasks during which the ipsilateral arm was cycling at 1 Hz (UNI 1Hz, BILAT 1Hz, BILAT CON). There were no specific differences between ASYNC 1:2 and any of the other tasks noted during post-hoc analysis. When the ipsilateral arm was cycling at 2 Hz, middle latency reflexes were unaffected by contralateral activity (Figure 4.6, right column).

Overall, statistical analysis indicated differences in background EMG due to contralateral activity in all 10 muscles (Figure 4.3). In contrast, differences in early and middle latency reflex amplitudes only occurred in 7 out of 20 muscles (i.e. 4/10 differences in

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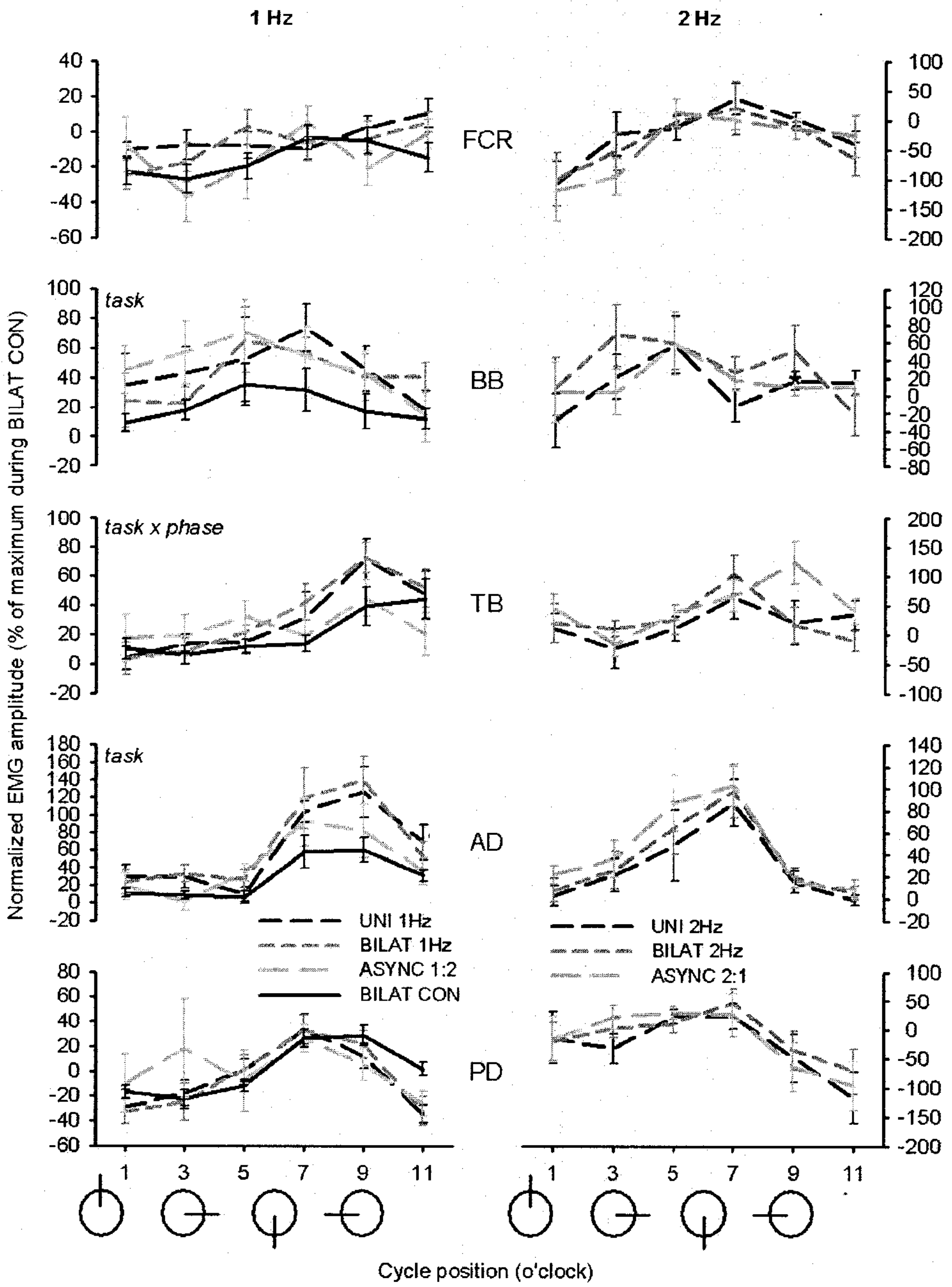

Figure 4.6: Middle latency reflex amplitudes from ipsilateral muscles during the cycling tasks. The format of this figure and abbreviations are the same as in Figure 4.3. The left column of graphs shows data when the ipsilateral arm was cycling at 1 Hz and the right column shows data when the ipsilateral arm was cycling at 2 Hz. The cycle position (according to the clock face) is indicated along the x-axis at the bottom. Significant main effects for task (task) and task by phase interactions (*task x* phase) are indicated at the top left of each plot. Asterisks denote where there was a significant difference between ASYNC and at least one of the other tasks (p<0.05).

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early latency reflexes and 3/10 differences in middle latency reflexes - Figures 5 and 6). This suggests that the differences that occurred in background EMG amplitude did not translate to differences in cutaneous reflexes; thus suggesting that background EMG and reflexes were regulated independently. Further to this point, few significant correlations were found between background EMG and reflex amplitude (Table 4.2). All but one of these significant correlations occurred in AD. With the exception of this muscle, patterns of reflex modulation were largely unrelated to modulations of ongoing EMG during the movement.

4.4 Discussion

In this study, we used an asynchronous arm cycling task to examine inter-arm coupling. We found that changes in contralateral cycling frequency influenced ongoing ipsilateral muscle activity, but had little effect on cutaneous reflex amplitudes. Crossed-effects in background EMG may serve to equalize muscle activity between the arms, thereby increasing stability. The lack of crossed-effects in reflexes suggests that responses to perturbations of the hand are governed through ipsilateral circuits with very little influence from the contralateral arm (Carroll et al., 2005).

4.4.1 Crossed-effects in background EMG equalize activity between the arms

A significant effect of contralateral activity was observed in background EMG amplitude during asynchronous cycling. When the contralateral arm was cycling at a higher frequency, EMG in the ipsilateral arm (cycling at 1 Hz) was significantly increased above

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Table 4.2: Pearson correlation coefficients (*r*) between background EMG and reflex amplitude during the seven cycling tasks.

Statistically significant differences ($p<0.05$) are indicated in bold italic font. Tasks are explained in Figure 4.1. Muscle abbreviations are as follows: FCR, flexor carpi radialis; BB, biceps brachii; TB, triceps brachii; AD, anterior deltoid; PD posterior deltoid.

Muscles		***Tasks***						
		BILAT CON	UNI 1Hz	BILAT 1Hz	ASYNC 1:2	UNI 2Hz	BILAT 2Hz	ASYNC 2:1
Early latency	FCR	0.01	0.04	0.04	0.21	0.11	0.22	0.43
	BB	0.18	0.51	0.51	0.34	0.46	0.14	0.16
	TB	0.41	0.37	0.37	0.11	0.14	0.05	0.45
	AD	***0.76***	***0.82***	***0.82***	***0.74***	***0.74***	***0.79***	***0.75***
	PD	0.32	0.10	0.10	0.49	0.18	0.21	0.04
Middle latency	FCR	0.34	0.16	0.16	0.35	0.19	0.24	***0.61***
	BB	0.37	0.21	0.21	0.40	0.06	0.12	0.04
	TB	0.29	0.01	0.01	0.33	0.22	0.03	0.22
	AD	0.55	***0.76***	***0.76***	0.41	0.34	0.51	0.51
	PD	0.25	0.09	0.09	0.07	0.02	0.04	0.22

Critical r (n=12) = 0.58

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that in the other ipsilateral 1 Hz cycling tasks in most muscles. This suggests that asynchronous cycling promotes inter-arm coupling. This increase in EMG of the lower frequency arm during asynchronous cycling occurred in all muscles except AD. AD was also the muscle in which EMG amplitude was the most similar during 1 and 2 Hz cycling (compare scaling of right and left columns in Figure 4.3). Therefore, muscle activity on the lower frequency arm was only increased when the contralateral homologous muscle activity was much higher and inter-arm coupling may serve to equalize activity in homologous muscles.

During ASYNC, this equalization of EMG occurred primarily in the low frequency arm. This may be because 2 Hz cycling requires greater muscle activity than 1 Hz cycling. It may not be possible for muscle activity to decrease during arm cycling at 2 Hz without a concomitant decrease in cycling frequency. Nevertheless, the rhythm of the high frequency arm was more regular (i.e. less variable) than the rhythm of the low frequency arm (compare rhythmicity in the single subject data in Figure 4.2A). The coefficient of variation for cycle duration was significantly greater for the 1 Hz arm than it was for the 2 Hz arm during both ASYNC 1:2 and ASYNC 2:1, indicating that the higher frequency arm was less variable. This parallels observations during multi-frequency bimanual movement (e.g. finger tapping in 2:1 coordination), in which the higher frequency hand also performed more accurately than the lower frequency hand (Peters, 1985;Jagacinski et al., 1988;Summers et al., 1993;Byblow et al., 1998). It has been proposed that this reflects an asymmetric coupling effect in which the higher frequency limb has a larger influence on the lower frequency limb. There may be a single temporal structure where

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the high frequency arm forms the timeframe into which the low frequency movements are inserted (Peters, 1985;Jagacinski et al., 1988;Summers et al., 1993;Byblow et al., 1998). An asymmetric coupling effect has also been demonstrated when the legs are driven at different rates on a split-belt treadmill. During this asymmetric task, human infants and adults can maintain alternating 1:1 stepping; however, the slow leg makes the greater adjustment to preserve the gait pattern (Dietz et al., 1994;Yang et al., 2005). In other words, the fast leg had a greater influence on the slow leg than vice versa. Therefore, this principle of bimanual coordination may also apply to locomotor coordination.

There is an interesting dichotomy in coordination rules for the arms and legs. While bimanual movements are most stable when synchronized (Tuller and Kelso, 1989;Bressler and Kelso, 2001;Varela et al., 2001;Swinnen, 2002;Spencer et al., 2007;Spencer and Ivry, 2007), the legs are biased towards out-of phase relationships (Ting et al., 2000;Pang and Yang, 2001;Kautz et al., 2002;Kawashima et al., 2005). Interestingly, changing the axis of forearm rotation causes out-of-phase movements to be more stable than in-phase movements (Carson et al., 2007). Perhaps since the axis of rotation is also different between arm cycling (transverse plane) and a bimanual task like circle drawing (horizontal plane), there may be different modes of coupling during these tasks. Indeed, infants as young as 2 post-natal weeks show out-of-phase coordination between the arms during the swimming reflex, which is evoked when infants are held horizontally in the water (McGraw, 1939), suggesting that out-of-phase coordination may be the default mode of arm coordination during locomotor movements. However, in the

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current study, out-of-phase coordination was not particularly stable since the phase relationship shifted away from 180° as the frequency of bilateral cycling increased (Figure 4.2C). Future work could investigate coordination during arm cycling in more detail to determine the relative stability of in-phase and out-of-phase coordination.

4.4.2 Cutaneous reflexes were governed through local circuits

Despite significant differences in background EMG across the tasks, early and middle latency cutaneous reflexes were similar as long as the arm was cycling at the same frequency. That is, when the ipsilateral arm was cycling at 1 Hz, cutaneous reflex amplitudes and modulation patterns were conserved regardless of the activity on the contralateral arm; similarly, when the ipsilateral arm cycled at 2 Hz, cutaneous reflexes were conserved across tasks. In all of the tasks that required ipsilateral 1 Hz cycling, cutaneous reflexes closely resembled the reflex modulation patterns reported previously during bilateral 1 Hz arm cycling when the arm cranks were coupled (Zehr and Kido, 2001), and also resembled those during unconstrained bimanual and unilateral 1 Hz cycling tasks (Carroll et al., 2005). Therefore, the coupling between the two arms that was observed in background EMG during asynchronous cycling was not seen in cutaneous reflex patterns.

Of particular interest is the finding that reflex modulation patterns were conserved during asynchronous arm cycling. Since subjects practiced asynchronous cycling for less than 10 minutes, they were likely in the "cognitive stage" of task performance, during which cortical involvement is high (Puttemans et al., 2005). Self-reports of "mental exertion"

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indicated that subjects found asynchronous cycling to be the most attentionally demanding (Table 4.1), suggesting that this task required significant cognitive activity. Nonetheless, cutaneous reflexes in both the low and high frequency arms were very similar to those during other, less attentionally demanding, arm cycling tasks at equivalent frequencies. Moreover, while cutaneous reflexes during volitional muscle contractions are typically scaled with muscle activity (i.e. automatic gain compensation – Matthews, 1986; Zehr et al., 2007), the majority of reflexes during asynchronous cycling did not follow the level of EMG. This is indicative of pre-motoneuronal gating by spinal networks. Interestingly, Smits-Englesman and colleagues (2006) proposed CPG involvement in cyclic aiming movements, which are also quite volitional. Overall, this suggests that even during cyclic tasks with a significant cortical component, like asynchronous arm cycling, descending signals may be relayed through CPGs to produce the motor pattern.

4.4.3 Implications for neural control

The amplification of ongoing EMG, but not reflex amplitudes, during asynchronous arm cycling indicates a separation in the neural control of each output enabling them to be independent of one another. Since reflexes were largely independent of background muscle activity, this is indicative of CPG involvement in gating reflexes during arm cycling (Zehr et al., 2004). This also supports the hypothesis that CPGs are composed of multiple layers, which can be influenced individually by supraspinal input or somatosensory feedback (Burke et al., 2001;Zehr, 2005;Lafreniere-Roula and McCrea, 2005;Rybak et al., 2006). The general organization of the neural control of rhythmic

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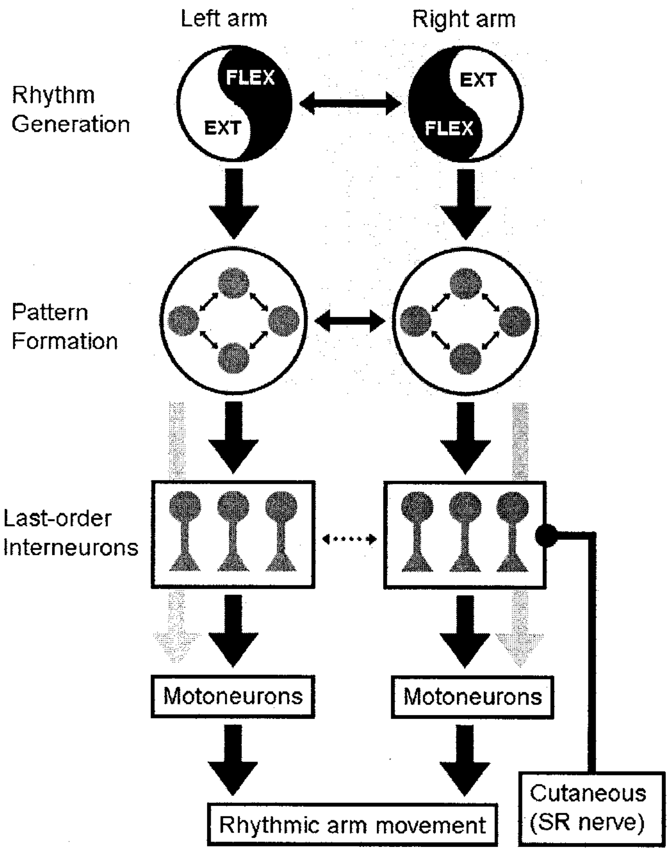

Figure 4.7: Schematic illustration of the possible organization of the neural control of arm movement.

Components of the CPG (i.e. rhythm generating and pattern formation networks) are shown within the grey box. The interaction between the two arms is indicated by reciprocal arrows between these networks. The output of the CPG feeds down to motoneurons either directly (grey descending arrows) or indirectly via last-order interneurons (black descending arrows). Cutaneous information from the superficial radial nerve (SR) is proposed to be relayed through last-order interneurons, without influencing rhythm generating or pattern formation networks. The lack of crossed-effects due to contralateral activity in cutaneous reflexes was proposed to be due to the lack of crossed-connections at the level of the last-order neurons (i.e. dotted reciprocal arrow between last-order interneurons). This figure was adapted from Rybak et al., 2006 (Figure 1) and Zehr, 2005 (Figure 3).

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movement is shown by the schematic in Figure 7. The components of the CPG are shown within the grey box. The rhythm generating networks specify the rhythm or pacing of the movement while the pattern formation networks specify the timing of precise groups of muscles in order to perform a specific task. The output of the CPG feeds down to motoneurons either directly (grey arrows) or indirectly via last-order interneurons (black arrows). All layers of the CPG may be affected by supraspinal or peripheral input (not shown in the schematic). Our finding that cutaneous reflexes were unaffected by contralateral activity may reflect a lack of coupling between last-order interneurons. This follows the findings of Burke and colleagues (2001) that cutaneous afferent information from the superficial peroneal nerve during fictive locomotion in the cat did not directly influence rhythm generating networks. Rather, the afferent information was relayed through last-order interneurons down-stream of the CPG. Muscle activity, on the other hand, is an output of the CPG 'proper', and may have more connections with the contralateral arm (shown by reciprocal arrows between the two arms of rhythm generating and pattern formation networks), possibly via commissural interneurons or coupling in higher centres.

The different effects of contralateral activity on ongoing EMG and cutaneous reflexes may have a functional role during locomotion. The stronger inter-arm coupling observed in EMG equalized the activity between the arms. If extended to walking, this could be a mechanism to prevent asymmetrical muscle activity, which may affect mechanical stability. Conversely, contralateral responses to an external perturbation of the hand would likely be unnecessary in restoring the movement trajectory of the ipsilateral arm.

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Therefore, the coupling between the arms remains weak in interneuronal reflex pathways. Overall, this suggests that inter-arm coupling is selectively expressed when it is important to the stability of the movement.

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CHAPTER 5: EARTH-REFERENCED HAND RAIL CONTACT FACILITATES INTERLIMB CUTANEOUS REFLEXES DURING LOCOMOTION*

5.1 Introduction

Cutaneous reflexes evoked in leg muscles by stimulation of nerves innervating the foot (i.e. segmental cutaneous reflexes) are precisely gated and have a functional role in maintaining and restoring stability during standing (Aniss et al. 1988; Aniss et al. 1990; Burke et al. 1991; Aniss et al. 1992) and walking (reviewed in Zehr and Stein 1999; also see Haridas et al. 2005). For example, stimulation of the sural nerve (innervates the lateral foot margin) evokes facilitatory reflexes in tibialis anterior (TA) during early swing phase of walking, thereby assisting the ankle to dorsiflex and step over an obstacle rather than tripping on it. The same stimulation evokes suppressive reflexes in TA at the end of swing, allowing the foot to make stabilizing ground contact as rapidly as possible (Duysens et al. 1990; Van Wezel et al. 1997; Yang and Stein 1990; Zehr et al. 1998). Therefore, segmental cutaneous reflexes in leg muscles are exquisitely sensitive to the locomotor state of the lower limbs and allow for specific and appropriate responses to maintain stability and forward progression.

It is less clear whether cutaneous reflexes evoked in arm muscles by stimulation of nerves innervating the foot (i.e. interlimb reflexes) have a functional role in maintaining stability

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during locomotion. Behaviorally, the arms can be observed to play a role in common stabilizing reactions including raising the arms or grasping hand rails (McIlroy and Maki 1995; Marigold et al. 2003). The extent to which reflex pathways are involved in recruiting the arms to aid in stability is unknown. Interlimb reflexes can be evoked in arm muscles after electrically or mechanically perturbing the foot (Dietz et al. 2001; Haridas and Zehr 2003), suggesting that these reflexes are involved in assisting the coordination between movements of the arms and legs during locomotion. However, these reflexes could not be specifically linked to a role in stumble correction. Misiaszek (2003) reported rapid responses in the arms following a backwards pull of the trunk during locomotion; however, responses varied greatly between subjects, likely due to the large number of degrees of freedom in arm movement strategies for restoring balance. That is, during locomotion arm movement is relatively unconstrained and since the arms are not directly interacting with the ground, they are not in a mechanical position to immediately and directly modify stability. Haridas and others (2006) reported a general facilitation of interlimb reflexes in the arms when the arms were crossed in front of the body during an unstable walking task. This facilitation appeared in several muscles and a general role for interlimb reflexes in corrective responses during locomotion was postulated.

It is possible that the arms have a limited and variable role in perturbations such as tripping or stumble correction unless a stabilizing object (e.g. a hand rail) is held; that is, unless the locomotor context is appropriate for functional participation by the arms. Holding an earth-referenced hand rail reduces the degrees of freedom in arm movement

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and provides a fixed object for the arm to brace against. As such activity in arm muscles can immediately help to mechanically stabilize the body. Earth-referenced rail contact has been shown to dramatically enhance postural stability while standing, even when the force of contact is subthreshold for directly affecting mechanical stability (Lackner et al. 2000; Lackner et al. 2001). During walking, the perceived stability that a rail provides has also been suggested to affect responses to destabilizing inputs in leg muscles (Rietdyk and Patla 1998; Schneider and Capaday 2003; Haridas et al. 2005).

The purpose of this study was to investigate whether reflex pathways are gated differently when an earth-referenced hand rail is held. We hypothesized that interlimb reflexes in the arms would be amplified when an earth-referenced rail was held during gait, enabling the arm to use the rail for support. Conversely, since the arms could take on more of a mechanically supportive role, we predicted that reflex responses in the legs and trunk would decrease in amplitude with rail contact. Furthermore, we predicted that these effects of holding the rail (context effects) would be greatest during the most unstable part of the step cycle (i.e. swing phase) and during more unstable locomotor tasks (such as incline walking and stair climbing, where the threat of tripping is greater than during level walking). Some portions of the data have been previously described in abstract form (Lamont et al. 2002).

5.2 Materials and methods

5.2.1 Subjects and tasks

A total of nineteen neurologically intact subjects (8 males and 11 females between 21-44 years of age) participated in the experiments with informed, written consent. All subjects

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were healthy and free of documented neurological impairment. All experiments were conducted under approved protocols for human subjects at the Universities of Alberta and Victoria and according to the Declaration of Helsinki.

Experiment 1: Context effects during three locomotor tasks

This experiment tested the effect on reflex amplitudes of holding a rail during three different locomotor tasks. Nine subjects performed a series of three locomotor tasks: (1) walking on a treadmill (Spirit Manufacturing Inc, Jonesboro, AR) with no incline (LEVEL); (2) walking on an inclined treadmill (INCLINE); and (3) stair climbing on a stepping mill (StepMill 7000PT, StairMaster, Kirkland, WA, USA) (STAIRS). These three tasks were selected since motor patterns on the treadmill and stepping mill are similar to those during the every-day activities of walking and stair climbing. All tasks were performed while holding onto a hand rail on the right side. The subjects were instructed to grasp the rail lightly, as they normally would hold a rail during locomotion, and were told not to grip or use the rail for support. The rail was at waist height and was held at a comfortable position for the subjects, slightly in front of the body, and the left arm was allowed to swing normally. The speed of the treadmill was set to 1.1 m/s (~1 step/second) and the level of difficulty on the stepping mill was set to allow subjects to comfortably pace their stepping at 1 step per second to closely approximate the same step cycle timing between tasks. The treadmill was inclined to 15° during incline walking and the inclination for the stepping mill was 45° (rise/run for each step = 20.3/20.3cm). Subjects performed each task for 7 minutes and were allowed to take short breaks if they

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felt tired. Each 7 minute trial resulted in approximately 450 steps on the treadmill and 400 steps on the stepping mill.

To determine the effect of holding the rail, these data were compared to recently published data recorded from the same subjects (on the same experimental day) performing the same three tasks, but without holding a hand rail (see Lamont and Zehr, 2006). In the original experiment, the order of the trials holding and not holding the rail was randomized. The results from the previous study are not revisited, but some of the data are replotted and used for comparison in the present study (Figures 1 and 2).

Experiment 2: Nature of context-dependent effects during incline walking:

This experiment tested whether the observed context effects were dependent on holding an earth-referenced rail. An additional six subjects performed incline walking on a treadmill (DESMO-M, Woodway, Waukesha, WI, USA) inclined to 15° during 5 different rail-holding contexts: (1) not holding a rail with arms moving naturally by sides (NO RAIL); (2) lightly holding a rail (LIGHT HOLD); (3) gripping the rail with the hand (GRIP); (4) leaning on the rail for some body weight support (SUPPORT); and (5) holding onto a hard plastic cylinder that was the same diameter as the rail but not earth-referenced (MOVING). The cylinder weighed 700 g and had a diameter of 7.5 cm and a length of 35 cm. In this last context, the arms were able to move naturally by the sides while holding the freely-moving cylinder. The rail or cylinder was held with the right hand while the left arm was allowed to move naturally. The speed of the treadmill was

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set to 1.1 m/s (pacing = ~ 1 step/second). Each trial lasted between 6-7 minutes (~420 steps were obtained) and the order of the trials was randomized.

To rule out the effect of arm position, a final control experiment was performed on an additional 4 subjects during incline walking. Four contexts were tested: NO RAIL, LIGHT HOLD, RAIL POSITION (subjects held their arm in same position as it was during LIGHT HOLD, without actually holding anything), and BRACE (a brace held the arm in the same position as it was during LIGHT HOLD; subjects did not hold anything). All contexts involved the right arm, while the left arm was allowed to move naturally. Similar to the other experiments, the speed of the treadmill was set to 1.1 m/s and each trial lasted between 6-7 minutes. The order of the trials was randomized. Electrogoniometers (Biometrics Ltd., Gwent, UK) were placed over the elbow and shoulder joints to measure flexion and extension movements.

5.2.2 Electrical Stimulation

The right sural nerve was stimulated just posterior and inferior to the lateral malleolus using a Grass S88 stimulator connected in series with a SIU5 isolation unit and a CCU1 constant current unit (Grass Instruments, Quincey, MA, USA) (Zehr et al., 1998; Lamont & Zehr 2006). Stimulation was applied to the sural nerve using flexible disposable surface EMG electrodes (Experiment 1: Vermont Medical, Inc., VT, USA; Experiment 2: Thought Technology Ltd., Montreal, QC, Canada) with trains of 5 x 1.0 ms at 300 Hz at 2 times the threshold at which clear and full radiating parasthesia into the lateral foot margin was perceived, as described previously (Lamont & Zehr, 2006).

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5.2.3 Electromyography (EMG)

Once the skin was cleaned with alcohol, disposable surface electrodes were placed on the skin over muscles in the arms, trunk, and legs. All muscles were recorded ipsilaterally to the site of stimulation (right side), except posterior deltoid which was recorded bilaterally in Experiment 2. For Experiment 1, EMG recordings were obtained from all nine subjects for posterior deltoid (PD), erector spinae (ES), rectus femoris (RF), vastus lateralis (VL), biceps femoris (BF), tibialis anterior (TA), and medial gastrocnemius (MG). For Experiment 2, EMG recordings were obtained from all six subjects for flexor carpi radialis (FCR), triceps brachii (TB), anterior deltoid (AD), ipsilateral posterior deltoid (iPD), contralateral posterior deltoid (cPD), erector spinae (ES), and tibialis anterior (TA). In four subjects, recordings were also obtained from medial gastrocnemius (MG). In the control experiment testing the effects of arm position, EMG recordings were obtained from four subjects for triceps brachii (TB) and posterior deltoid (PD). Ground electrodes were placed over electrically neutral tissue. EMG signals were amplified at 5000 times and filtered from 100-300 Hz (Grass P511, Astro-Med Grass Inc.).

5.2.4 Data acquisition and EMG analysis

Data were sampled at 1 kHz with a 12 bit A/D converter connected to a computer running custom-written LabView virtual instruments (National Instruments, Austin, TX, USA). Off-line analysis separated the step cycle into 8 equal parts (phases) beginning with the initiation of stance on the right side, which was recorded using custom-made force

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sensors taped to the insole of the shoes and placed beneath the heel and ball of the foot. The stimulations occurred randomly throughout the step cycle and reflexes were separated into phases according to when the stimulations occurred. EMG data were full-wave rectified and low-pass filtered at 40 Hz with a dual-pass Butterworth filter.

EMG from non-stimulated (control) step cycles was subtracted from that in stimulated cycles to obtain subtracted reflex traces. Reflex traces that occurred within the same phase were averaged together (between 10-20 reflexes occurred in each phase for each subject). For more details about the subtraction and averaging of reflexes, refer to Lamont and Zehr (2006) and Haridas and Zehr (2003). Averaged reflexes were considered significant if the peak exceeded a 2-standard deviation band above or below the pre-stimulus mean EMG level. The amplitude of middle latency reflexes were quantified and averaged across subjects for each task and context. We focused our analysis on the middle latency responses (peak latency ~80-120 ms after stimulation), since these responses tend to be larger and occur most frequently (Baken et al. 2005).

5.2.5 Statistics

The mean amplitudes of all data (reflex and background EMG) were normalized to the maximum background (i.e. non-stimulated) EMG level during INCLINE NO RAIL. Normalization was performed for each subject prior to averaging the subject data together. Linear regression analysis was used to determine significant relationships between reflex amplitudes and background EMG levels. Other statistical procedures are described below. For all analyses, statistical significance was set to $P < 0.05$, except

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during post-hoc and planned comparison analyses for which the significance level was adjusted according to the number and type of comparisons made.

Experiment 1:

In this experiment, we were interested in whether holding a rail affected reflexes across the step cycle (i.e. reflex modulation patterns) during different locomotor tasks. To test the effect of holding the rail, we compared the current data set to the previously published data set without holding a rail (Lamont and Zehr, 2006, shown in Figure 5.3) by performing a 3-way (2 contexts X 3 tasks X 8 phases) repeated measures ANOVA. Tukey's HSD test was used for post-hoc analysis of context and task main effects and the context X task X phase interactions. To test the hypothesis that the context effect would change with the difficulty of the task, we performed a 2-way (3 tasks X 8 phases) repeated measures ANOVA on data from the three tasks while holding the rail. We predicted effects of holding a rail would be largest during swing phase and thus planned comparisons were performed on the data from each task for phases 5-8.

Experiment 2:

To compare the different variations of context to one another, a 2-way repeated measures ANOVA was used (5 contexts x 8 phases). In the control experiment for arm position, a 2-way repeated measures ANOVA was also used (4 contexts x 8 phases). Tukey's HSD post-hoc analysis was performed on significant context main effects and context x phase interactions and planned comparisons were performed on the data from each context for phases 5-8.

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5.3 Results

5.3.1 Experiment 1: Context effects during three locomotor tasks

Example background EMG and reflex traces from an individual subject for arm (PD), trunk (ES), and leg (TA) muscles during the different tasks and contexts are shown in Figure 5.1. The middle latency responses are highlighted with rectangles. This subject showed marked facilitatory reflexes in PD during swing phase, which were only present when the rail was held (Figure 5.1A). In ES, some small decreases in reflex amplitude were noted during swing phase when the rail was held (see Figure 5.1B: incline, phase 7 and stairs, phases 7 and 8). Although there were no effects of holding the rail in TA, there was a task effect where the sign of the reflex reversed (became suppressive) during swing phase of stair climbing, as compared to level and incline walking (i.e. phase 5; see Figure 5.1C).

Background EMG

Figure 5.2 shows the mean background EMG amplitude (line plots) during LEVEL, INCLINE, and STAIRS without holding the rail (NO RAIL). This data has been replotted from Lamont and Zehr (2006) to use for comparison with Figure 5.3 showing RAIL data. Note that the data in Lamont and Zehr (2006) was normalized to the maximum background EMG during LEVEL NO RAIL, whereas the replotted data in Figure 5.3 was normalized to that during INCLINE NO RAIL in order to facilitate comparisons between data from experiments 1 and 2. Since the task differences in the

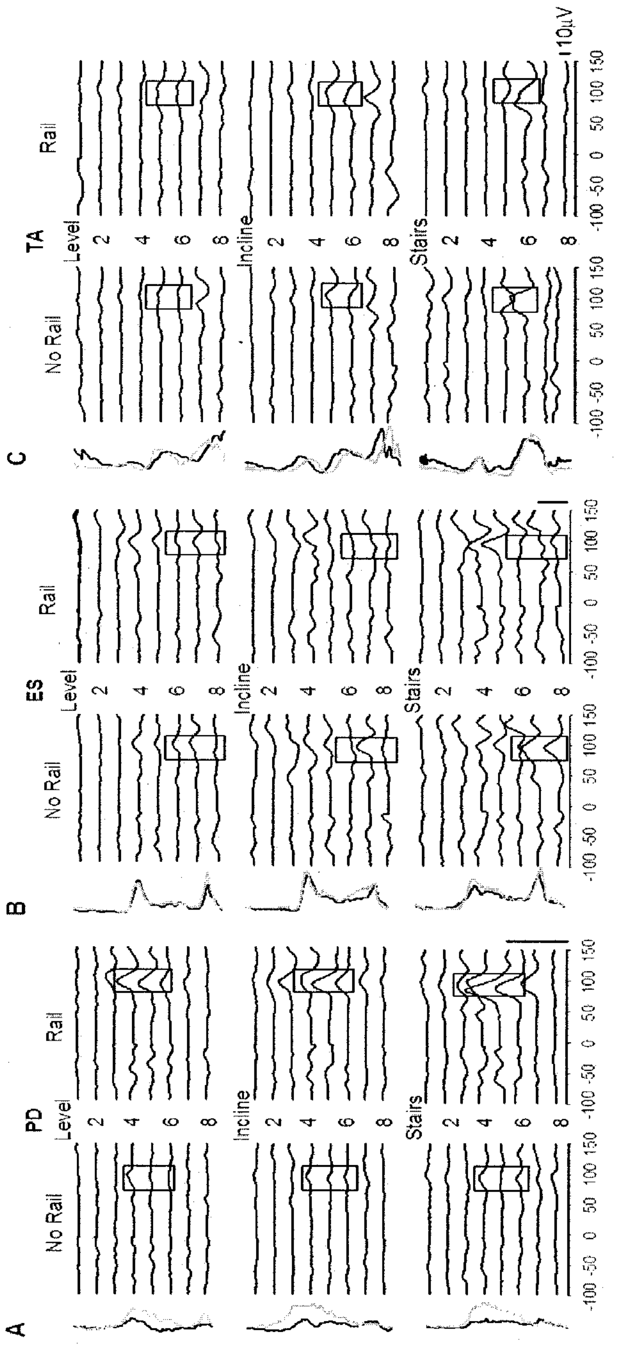

Figure 5.1: Subtracted reflex EMG traces in posterior deltoid (PD – A.), erector spinae (ES – B.) and tibialis anterior (TA – C.) muscles from a single subject during three locomotor tasks while holding and not holding a rail.

The plots on the left side of each graph correspond to when the subject was not holding the rail, and the plots on the right correspond to when the subject was holding the rail. The vertical trace plotted to the left of each figure indicates the averaged background EMG during each task (black = NO RAIL; grey = RAIL) across the movement cycle. The step cycle phase is represented in the centre of the plots (even numbers only), with phase 1 corresponding to stance initiation and the stance-to-swing transition occurring around phase 5. Stimulation occurred at time 0 and the stimulation artifact (between 0 – 40 ms) was removed. The rectangles highlight responses observed during the middle latency window (80-120 ms after stimulation).

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NO RAIL data have been discussed in the previous publication, they will not be revisited here.

Generally, background muscle activity during the rail holding tasks was similar in the arm muscles, but several differences were noted in the trunk and leg muscles (as indicated in Figure 5.3 by the symbols denoting task differences above the line plots). The asterisks above the line plots on Figure 5.4 denote whether background EMG amplitude was different when these three tasks were performed while holding a rail compared to when the same tasks were performed without holding a rail (Figure 5.3). The greatest number of context differences occurred in PD, with fewer seen in other muscles (ES, BF, TA, MG). In PD, holding the rail caused a general decrease in EMG amplitude during swing phase (* above line plots in phases 5-8); the magnitude of this decrease in EMG was between 14-29% of the value during NO RAIL. During stance, holding a rail caused both increases and decreases in PD EMG amplitude, as compared to NO RAIL (* above line plots in phases 1-4; percentage change from NO RAIL was between 13-29%). A few lower limb muscles also showed context effects. Both BF and TA showed context main effects and there were also difference at specific phases due to context in TA and MG (see * above line plots for TA and MG – Figure 5.3).

Middle latency reflex amplitudes

The facilitatory middle latency reflex in PD when holding a rail that was evident in the single subject data (Figure 5.1) can also be seen in the group data (compare PD bar plots in Figure 5.2 to those in Figure 5.3). This large facilitation was only observed during

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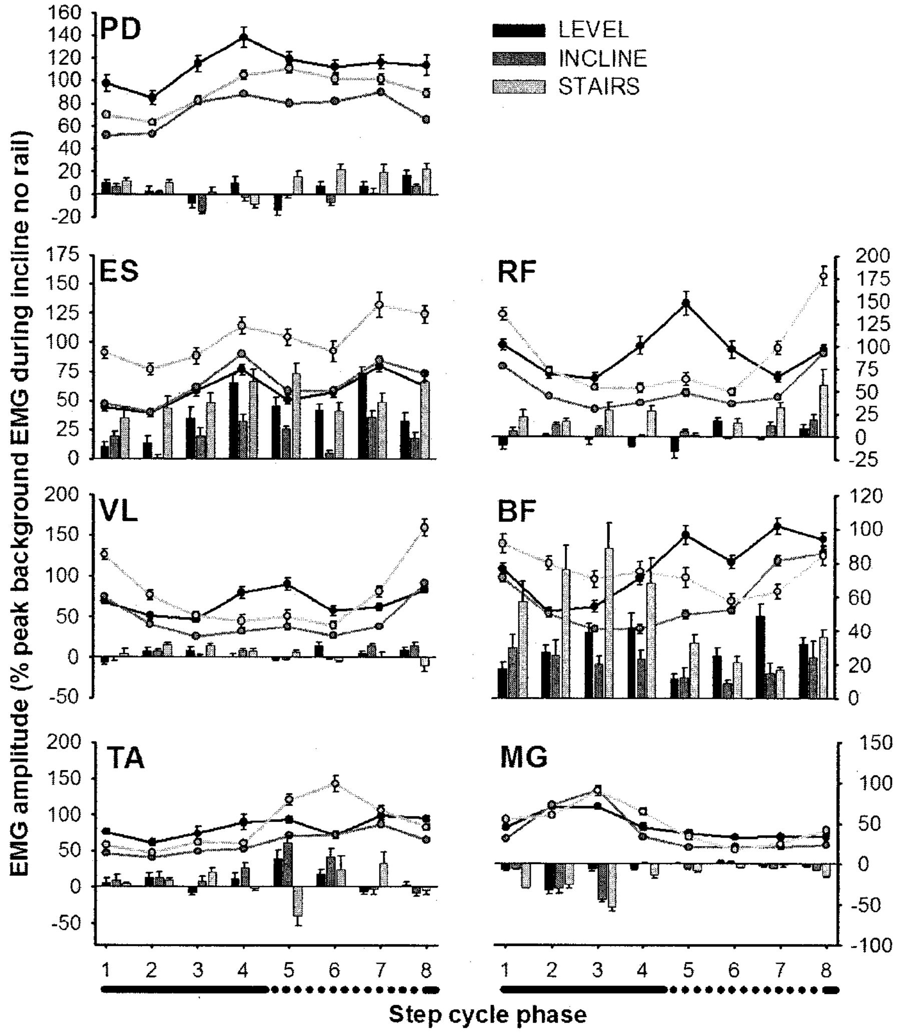

Figure 5.2: Normalized background EMG and middle latency reflex amplitude during LEVEL, INCLINE, and STAIRS NO RAIL.

Values were averaged across all subjects for each task and context (± standard error) and normalized to the maximum undisturbed EMG during INCLINE NO RAIL. Background EMG is shown by line plots and middle latency reflex amplitudes are shown in bar plots. The solid line at the bottom of the graphs marks stance phase and the dotted line marks swing. Abbreviations are as follows: PD, posterior deltoid; ES, erector spinae; RF, rectus femoris; VL, vastus lateralis; BF, biceps femoris; TA, tibialis anterior; MG, medial gastrocnemius.

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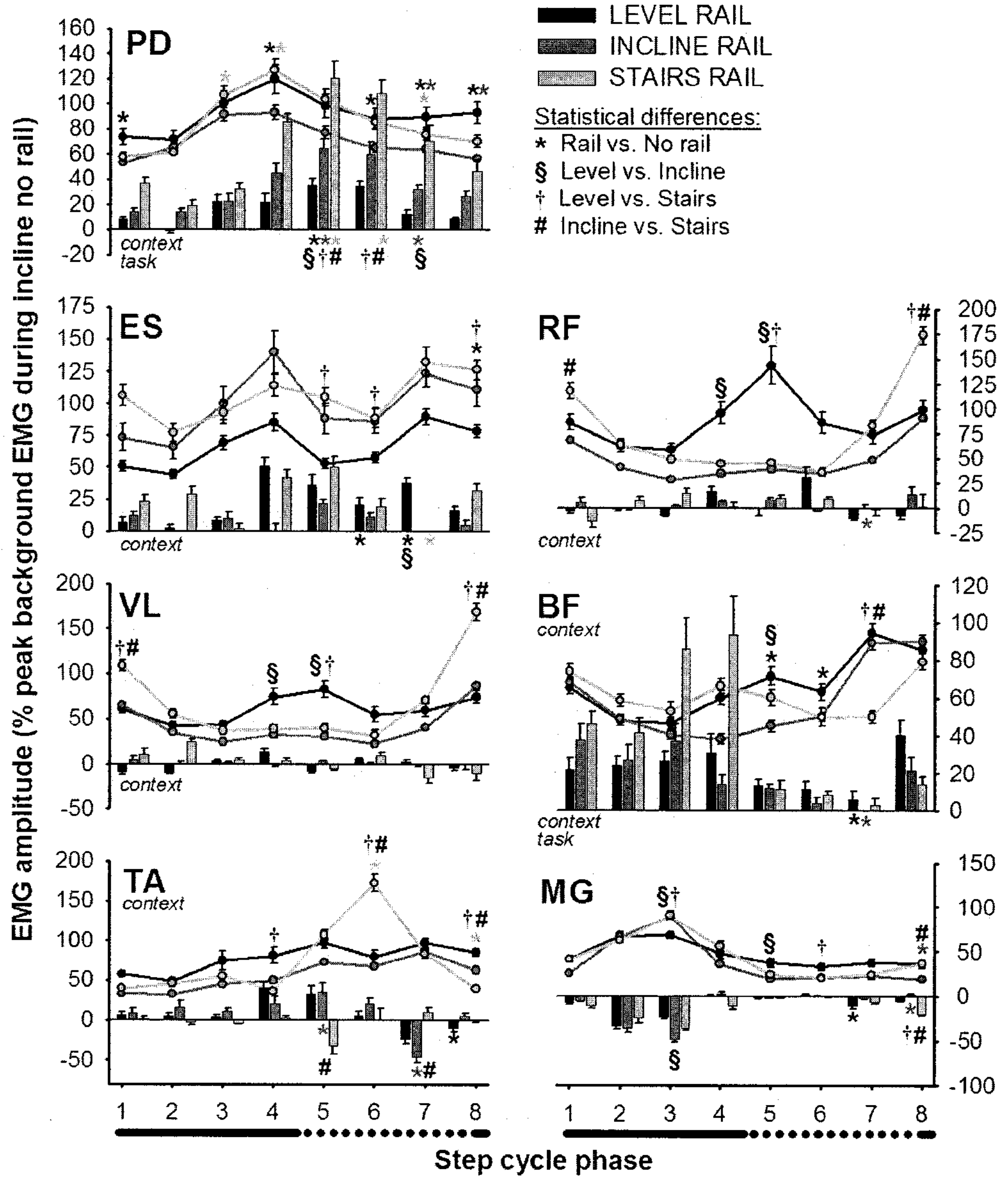

Figure 5.3: Background EMG and middle latency reflex amplitudes during LEVEL, INCLINE, and STAIRS holding a rail (RAIL).

The format of this figure and abbreviations are the same as in Figure 5.2. Background EMG is shown by line plots and middle latency reflex amplitude is shown by bar plots. Significant main effects in background EMG (*context or task*) are indicated at the top right corner of each graph. Asterisks above the line plots denote significant differences in amplitude due to context (i.e. compared to NO RAIL data in Figure 5.2) that were found via post-hoc analysis and planned comparisons. The colour of the asterisk corresponds to the colour of the task during which there was a significant difference due to context. The other symbols above the line plots denote significant differences in background EMG due to task (see legend). Significant main effects in middle latency reflex amplitude (context or task) are indicated at the bottom right of each graph. Asterisks denoting context differences and other symbols denoting task differences in middle latency reflex amplitude are shown below the bar graphs.

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stance-to-swing transition and swing phase (* below bar plots in phases 5-7; percentage change from NO RAIL was between 356-8547%) and was greatest during stair climbing (main effect for context and task). Also, similar to what was observed in the single subject data, there was a general decrease in reflex amplitude for ES when the rail was held (Figure 5.3; percentage change from NO RAIL was between 48-52%). Other differences due to context were observed in RF, VL, BF, TA and MG (as noted on Figure 5.3).

Similar to single subject observations, there was a reflex reversal in TA during early swing (i.e. phase 5) where the facilitatory reflex during level and incline walking switched to being suppressive during stair climbing. This reflex reversal was observed regardless of whether the rail was held (see TA bar plots in Figures 2 and 3). Other significant task differences (Figure 5.3) were observed during RAIL contexts at late swing phase in ES (phase 7), and MG (phases 3 and 8).

5.3.2 Experiment 2: Origin of context-dependent effects

This experiment was conducted to determine which aspects of holding the rail caused the reflex changes. Five rail holding contexts were tested during incline walking: NO RAIL, LIGHT HOLD, GRIP, SUPPORT, and MOVING. Incline walking was chosen to be the locomotor task performed throughout this experiment because we observed significant context-dependent reflex modulation, particularly in PD of the arm holding the rail during this task in the earlier experiment.

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Background EMG

There was at least one significant difference due to context in the background EMG of all of the upper limb muscles (FCR, TB, AD, iPD, cPD) and the trunk muscle (ES) (see * above the line plots in Figure 5.4). The asterisks in Figure 5.4 signify that at least one significant difference between contexts was found upon post-hoc analysis and planned comparisons at that phase. These differences in background EMG are largely consistent with the instructions given to the subjects for each context condition. For example, FCR muscle activity was highest during GRIP and TB muscle activity was highest during SUPPORT. No changes in background EMG were found in the two lower limb muscles (TA, MG); therefore, the muscle activity was consistent between different incline walking trials, regardless of the type of rail contact. The precise statistical differences are listed in Table 5.1.

Middle latency reflex amplitudes

The bar plots in Figure 5.4 shows middle latency reflex amplitudes across the step cycle for the five rail contexts. Reflexes evoked during NO RAIL and MOVING tended to follow a similar pattern of modulation in most of the muscles. For example, there was no difference in reflex amplitude between NO RAIL and MOVING for TB and iPD, even when the other contexts were quite different. Particularly notice the extensive modulation of reflex amplitude in TB during SUPPORT. The middle latency response in this muscle was greatly facilitated during swing phase (* below bar plots in phases 6-7) despite the decreasing muscle activity at this time in the step cycle (compare to background EMG line plots). Statistical analyses are summarized in Table 5.2. There

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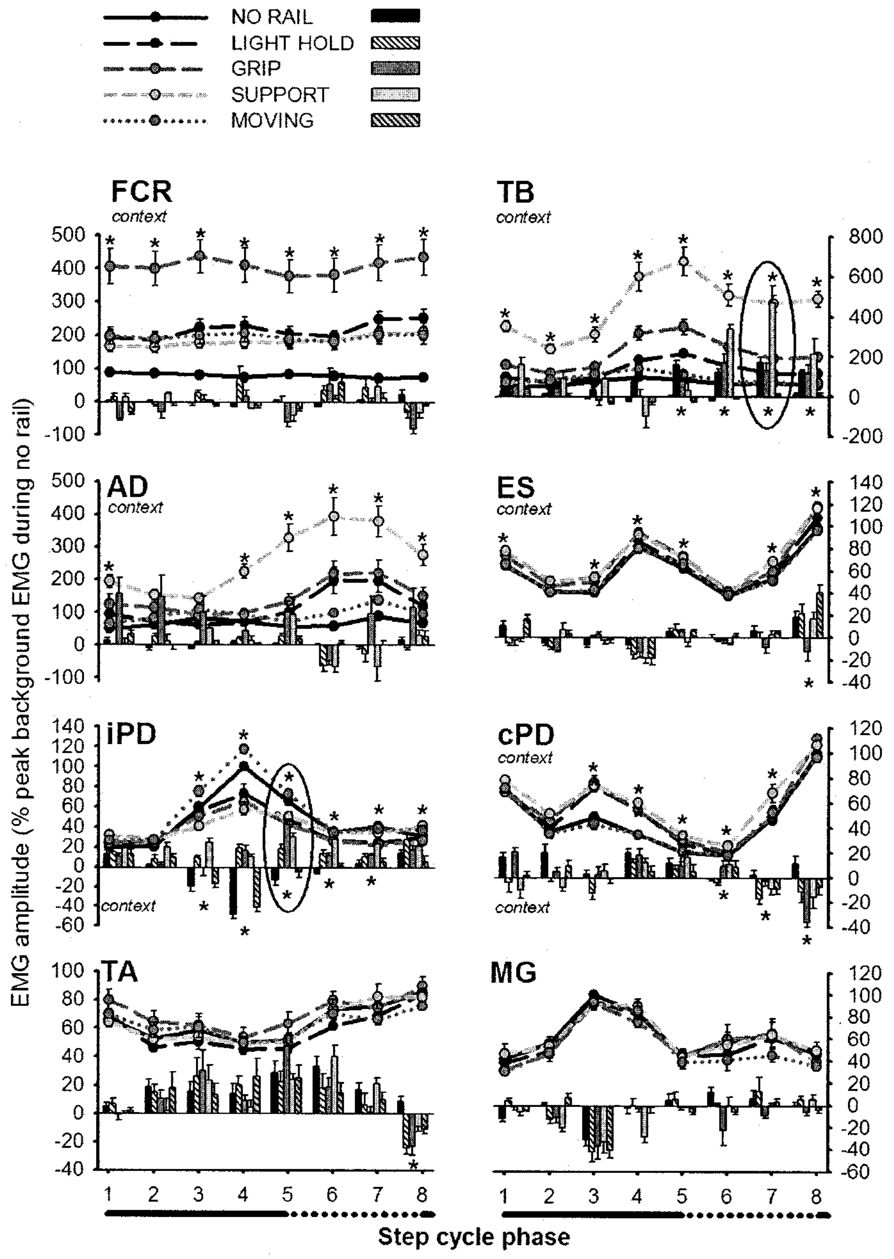

Figure 5.4: Normalized background EMG and middle latency reflex amplitude for incline walking during different rail contexts.

Values were averaged across all subjects for each context (± standard error) and normalized to the peak undisturbed EMG during the no rail context. Background EMG is shown by line plots and middle latency reflex amplitude is shown by bar plots. Significant context main effects (*context*) are indicated by text (top left for background EMG, bottom left for middle latency reflexes). The asterisks above the lines (background EMG) and below the bars (middle latency reflexes) indicate that at least one significant difference occurred between these contexts at this phase (specific differences are listed in Tables 1 and 2). The solid line at the bottom of the graph marks stance phase and the dotted line marks swing. Abbreviations are as follows: FCR, flexor carpi radialis; TB, triceps brachii; AD, anterior deltoid; ES, erector spinae; iPD, ipsilateral posterior deltoid; cPD, contralateral posterior deltoid; TA, tibialis anterior; MG, medial gastrocnemius. The phases highlighted by ovals at the stance-to-swing transition in iPD (phase 5) and at late swing in TB (phase 7) indicate where there are large differences between contexts; these differences are highlighted in Figure 5.5.

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Table 5.1: Summary of results from post-hoc analyses and planned comparisons for background EMG during five different contexts.

Abbreviations for muscle names are listed in Figure 5.4. Abbreviations for contexts are as follows: NO RAIL, no rail; LIGHT HOLD, light rail; GRIP, grip rail; SUPPORT, support rail; MOVING, moving rail. Significant differences are specified by the muscle names (with phase numbers in brackets) listed at the intersections of the context rows and columns. For example, LIGHT HOLD and GRIP are different for FCR (phases 1-8), TB (phases 5 and 6), and ES (phase 8).

	NO RAIL	**LIGHT HOLD**	**GRIP**	**SUPPORT**
LIGHT HOLD	FCR (1-8) TB (5-8) AD (6) iPD (4,5,7) cPD (3,4)			
GRIP	FCR (1-8) TB (4-8) AD (6,7) ES (4,8) iPD (4,5,7) cPD (3-5)	FCR (1-8) TB (5,6) ES (8)		
SUPPORT	FCR (1-8) TB (1-8) AD (1,4-8) ES (1,3,7,8) iPD (4) cPD (3-7)	TB (1-8) AD (4-8) ES (5,7) cPD (7)	FCR (1-8) TB (1,3-8) AD (5-7) iPD (7,8) cPD (7)	
MOVING	FCR (1-8) AD (6-8)	TB (6-8) iPD (4,5,7) cPD (3,4)	FCR (1-8) TB (4-8) ES (4,8) iPD (4,5) cPD (3,4)	TB (1-8) AD (5-8) ES (7,8) iPD (3,4) cPD (3-6)

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Table 5.2: Summary of results from post-hoc analyses and planned comparisons for middle latency reflex amplitude during 5 different contexts.
Statistical differences are noted as in Table 5.1. For example, NO RAIL is different from SUPPORT for TB (phases 6 and 7) and iPD (phases 4-6).

	NO RAIL	LIGHT HOLD	GRIP	SUPPORT
LIGHT HOLD	TB (5-8) iPD (3-5) cPD (7,8) TA (8)	▨	▨	▨
GRIP	iPD (4,5) cPD (8) TA (8)		▨	▨
SUPPORT	TB (6,7) iPD (4-6)	TB (6,7) iPD (7) cPD (6)	TB (6,7) iPD (6,7)	▨
MOVING	cPD (8) TA (8)	TB (5-8) iPD (3-5)	ES (8) iPD (4,5)	TB (6,7) iPD (3-6)

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were no significant differences in reflex amplitude in the two lower limb muscles (TA and MG) across all contexts (Figure 5.4 and Table 5.2). Figure 5.5 highlights the context differences observed in iPD at the stance-to-swing transition (phase 5) and in TB at late swing (phase 7). In iPD, reflexes are small and suppressive during NO RAIL and MOVING. However, this reflex reverses in sign to become facilitatory during LIGHT HOLD, GRIP, and SUPPORT. NO RAIL and MOVING are significantly different from all of the other rail holding contexts (LIGHT HOLD, GRIP, and SUPPORT). In TB, reflexes are smallest during NO RAIL and MOVING and are largest during SUPPORT.

We performed one last control experiment to determine whether the effects of holding an earth-referenced rail could have been due to arm position. Four additional contexts were tested and compared during incline walking: NO RAIL, LIGHT HOLD, RAIL POSITION (i.e. subjects held the arm in the same position as it was during LIGHT HOLD, except without holding anything), and BRACE (i.e. a brace held the arm in the same position as it was during LIGHT HOLD; subjects did not hold onto anything). During LIGHT HOLD, RAIL POSITION, and BRACE, the position of the arm was similar (see elbow and shoulder angle plots, Figure 5.6). However, middle latency reflexes in TB and PD were still facilitated more in LIGHT RAIL than in any of the other contexts (bar plots, Figure 5.6; asterisks below bar plots denote where LIGHT RAIL was different from at least one of the other contexts). Notice that the significant differences in middle latency reflex amplitude due to holding an earth-referenced rail occurred when there were no significant differences in background EMG (compare asterisks above line plots for background EMG to asterisks below bar plots for middle latency reflexes).

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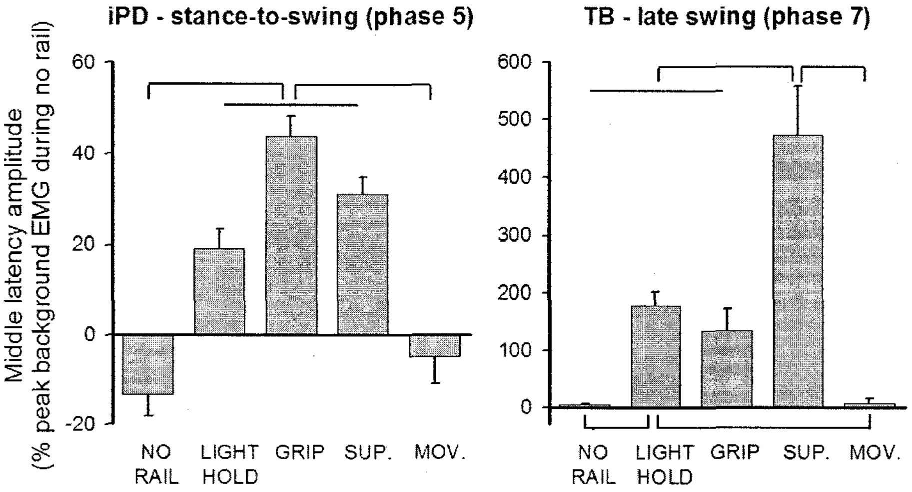

Figure 5.5: Highlighted middle latency reflexes in iPD and TB during different rail contexts. This figure highlights the specific responses in iPD at the stance-to-swing transition (phase 5 – left plot) and in TB at late swing (phase 7 – right plot). Along the x-axis, SUP.=SUPPORT and MOV.=MOVING. Statistical differences are shown by brackets. For example, for iPD at phase 5 (stance-to-swing), NO RAIL and MOVING are different from all of the other contexts (LIGHT HOLD, GRIP, and SUPPORT). For TB at phase 7 (late swing), reflexes during SUPPORT are significantly larger than in any other context. Additionally, reflexes are also larger during LIGHT HOLD than during NO RAIL or MOVING. These statistical differences can also be determined by referring to Table 5.2 (see iPD phase 5 and TB phase 7).

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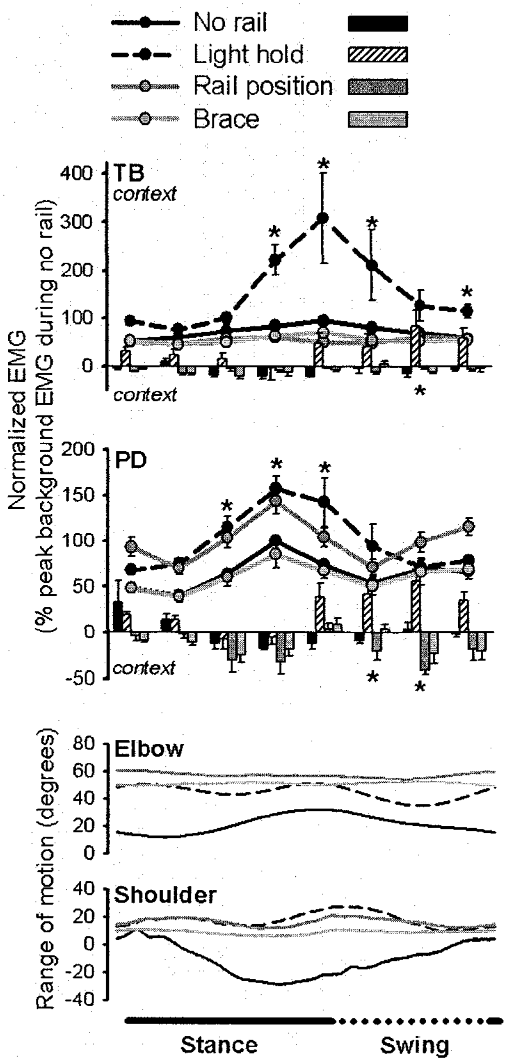

Figure 5.6: Effects of arm position context on background EMG and middle latency reflex amplitude during incline walking.

Values were averaged across all subjects (n=4) for each context (± standard error) and normalized to the peak undisturbed EMG during the no rail context. Background EMG is shown by line plots and middle latency reflex amplitude is shown by bar plots. Significant context main effects (*context*) are indicated by text (top left for background EMG, bottom left for middle latency reflexes). The asterisks above the lines (background EMG) and below the bars (middle latency reflexes) indicate that LIGHT HOLD is different from at least one of the other contexts at that phase in the step cycle. Elbow and shoulder angles across the step cycle for each context are shown in the bottom plots. The solid line at the bottom of the graph marks stance phase and the dotted line marks swing. Abbreviations are as follows: TB, triceps brachii; PD, posterior deltoid.

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As an additional control to ensure that the differences in reflex modulation were not largely explained by fluctuations in background EMG, we tested the correlations between reflex amplitude and background EMG (results shown in Table 3). There were only two instances where the reflex amplitude was negatively correlated with background EMG during the first experiment (RF stairs and MG incline - shown in bolded font in Table 3A); this could be related to the fact that large suppressions can only be observed when there is a lot of background muscle activity. There were no significant correlations found in the second experiment (Table 3B). Therefore, the amplitudes of the majority of reflexes were not directly related to the background muscle activity.

5.4 Discussion

There are two main new findings in this study. Firstly, holding an earth-referenced rail altered cutaneous reflex amplitudes in muscles across the entire body. This effect of the rail was different depending on the walking task being performed. This suggests that transmission in segmental and interlimb reflex pathways is influenced by the stability (i.e. context) of each task, as conveyed by holding a rail, while also being subject to specific gating according to the locomotor task. Secondly, holding the rail amplified reflexes in muscles that were functionally able to make use of the rail to restore balance. Differences due to holding the rail were only observed when the rail was earth-referenced, and were not present when subjects were holding a freely moving cylinder (moving rail). These differences were also not present when the arm was fixed or held in the same position as it was during rail-holding, without actually holding a rail. This suggests that these reflex

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Table 5.3: Correlation coefficients between middle latency reflex amplitude and background EMG during (A) the three locomotor tasks while holding a rail (Experiment 1) and (B) the five rail contexts while incline walking (Experiment 2).

Values shown in bold font indicate significant Pearson *r* values at the level of $p < 0.05$
Critical *r* for A (n = 9) = 0.666
Critical *r* for B (n = 6) = 0.811
Abbreviations of muscle names are defined in Figures 1 (for Table A) and 4 (for Table B).

A

MUSCLE	*LEVEL*	*INCLINE*	*STAIRS*
PD	0.11	0.16	0.60
ES	0.44	-0.06	0.23
RF	-0.40	-0.42	**-0.76**
VL	0.11	-0.08	-0.32
BF	-0.11	-0.04	-0.03
TA	-0.11	0.25	-0.27
MG	-0.39	**-0.73**	-0.61

B

MUSCLE	NO RAIL	LIGHT RAIL	GRIP RAIL	SUPPORT RAIL	MOVING RAIL
FCR	0.15	-0.25	0.35	0.07	-0.17
TB	-0.38	0.54	-0.07	0.10	-0.43
AD	-0.19	-0.76	0.12	0.08	-0.47
iPD	-0.34	-0.18	-0.05	0.32	-0.57
cPD	0.04	-0.53	-0.46	-0.53	-0.34
ES	0.29	0.44	-0.20	0.01	0.26
TA	0.14	-0.06	-0.33	0.05	-0.29
MG	-0.37	-0.45	-0.47	-0.48	-0.51

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changes may contribute to relevant mechanical stabilization of the body during locomotion.

5.4.1 Context-dependent modulation

Holding a rail during the three locomotor tasks altered cutaneous reflex amplitudes (see Figures 1, 2, and 3) most dramatically in the arm holding the rail (PD). The effects were smaller in the trunk (ES) and leg muscles (RF, BF, TA, and MG). Interestingly, the independent expression of reflex control for the arms and legs is similar to the independence of upper and lower body movement following galvanic vestibular stimulation during walking (Bent et al. 2004).

The middle latency reflexes in ES tended to diminish in amplitude while holding the rail, particularly during late swing and the swing-to-stance transition (phases 7 and 8). The trunk muscles typically play a role in restricting excessive trunk movements during locomotion, thus stabilizing the body (Thorstensson et al. 1982). When the rail is held, we suggest that reflex amplitudes decrease because the arm takes on some of the role of stabilizing the body. This could reflect the ability of the nervous system to gate reflexes such that the most effective response to a disturbance results, taking into account the context of the locomotor task (see also Rietdyk and Patla 1998).

Reflexes in the lower limbs exhibited variable context effects; sometimes reflexes were facilitated (i.e. larger in amplitude) when the rail was used, sometimes they were suppressed (i.e. smaller in amplitude), and sometimes reflex reversals were observed (i.e.

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a change from a facilitatory reflex to a suppressive one). Similarly, Rietdyk and Patla (1998) also found that rail contact had a variable effect on reflex responses in the legs. They suggested that the changes in reflex gain produced an optimal recovery strategy, in which unnecessary responses were minimized and more relevant responses were enhanced. In the current study, generally facilitation tended to decrease and suppression to increase when the rail was held (i.e. facilitatory reflexes were smaller and suppressive reflexes were larger). Both of these observations yield the same overall trend in reducing motor activity and could reflect the phenomenon reported by Misiaszek and Krauss (2005) that responses to disturbances in leg muscles were smaller during more stable locomotor tasks. Another interesting point to note with regard to leg reflexes is that the reflex reversal in TA at the stance-to-swing transition (i.e. phase 5) during stair climbing (no rail) that was reported by Lamont and Zehr (2006 – data shown in Figure 5.2) was still present when the rail was held (see Figure 5.3).

The large increase or facilitation of PD reflex amplitude may reflect a “switch” in the potential function of the arm and yield access to relevant interlimb reflex pathways. Without external support from the rail, the arms can be observed to elevate (to shift the centre of mass forwards) or move towards a rail in response to a perturbation (McIlroy and Maki 1995; Marigold et al. 2003). However, when the rail is held, it offers the arm a much larger mechanical role in restoring balance after a disturbance. For example, if tripping were to occur, the arm could be used to support the body against the rail and prevent falling; specifically, PD may abduct the arm to better position it to use to the rail for support. The reflexes in PD may be gated to reflect this functional role, thus enabling

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this muscle to have a role in the corrective response. The response in this muscle is enhanced only when the arm can actually aid in restoring balance and this facilitation is only observed near the stance-to-swing transition and swing phase, when a perturbation is most likely to have a destabilizing effect. Also, further facilitation is observed as subjects perform tasks during which tripping is more likely (e.g. stair climbing as opposed to level walking). This corroborates the findings of Haridas et al. (2006), who also found that interlimb reflexes were scaled to the degree of postural threat and were also affected by the arms being positioned in front of the chest, where they were constrained from participating in corrective responses. That is, having the arms crossed in front of the body limits the ability to use arm motion in corrective responses.

In the second experiment using five variations of rail holding during incline walking, a similar facilitatory response in PD was observed when the rail was held with this arm (regardless of whether it was a light, gripping or supporting hold), but only if the rail was earth-referenced. When subjects held a freely moving cylinder, reflex modulation followed a similar pattern to when they were not holding a rail at all (see iPD – Figure 5.4 and 5.5). Furthermore, when subjects held their arm in the same position as they would when holding the rail, but without holding anything, there was no facilitation of ipsilateral PD reflexes (Figure 5.6). Therefore, the facilitation of the middle latency response in this muscle was not simply due to somatosensory feedback from the hand or the position of the arm. Rather, this may reflect a switch in the "functional set" of reflexes with the added stability that the fixed rail provides. We propose that this functional set takes into account the availability of the rail and adjusts the automatic recovery strategy accordingly. Interestingly this outcome parallels the suggestions of

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Misiaszek (2006) that postural and human locomotor control utilizes fuzzy logic IF/THEN rules for integration and response to perturbations. In this example, IF the hand is in contact with an earth-referenced support THEN interlimb reflex facilitation in PD is present.

Triceps brachii (TB) also exhibited a facilitation of the middle latency response when an earth-referenced rail was held (Figures 4, 5, and 6). Similar to ipsilateral PD, reflex modulation was similar between the no rail and moving rail conditions, and the reflexes were not facilitated when the arm was simply held in the rail position, indicating that facilitation did not occur unless subjects were holding an earth-referenced object. In contrast to PD, the response in TB when the subjects were supporting themselves with the rail was further amplified than in any of the other fixed rail conditions. Note that the amplitude of muscle activity was increased throughout the step cycle when subjects were using the rail for support, but the reflex was only amplified during swing phase (when the muscle activity was decreasing). The reflex may be gated during stance phase because exerting more force on the rail via an increased TB contraction is not needed during this relatively stable phase of the step cycle. During the more unstable swing phase, gating of the reflexes may be released to allow for appropriate balance compensation reactions. Therefore, the functional set may be gated not only to reflect the availability of the rail, but also to adjust for the relative contribution of each muscle involved in performing the task.

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We speculate that the observed context-dependent modulation of interlimb reflexes reflects the functional application of the phenomenon studied extensively by Lackner's group. It was clearly shown that increased postural sway which occurred when eyes were closed was effectively nullified by contact on the fingertips even at extremely low force levels (i.e. <1 N and below levels for mechanical support) (Jeka and Lackner 1994; Holden et al. 1994). Furthermore, it was shown that even 5-10 g of force can be effective and that the best effects are seen when the reference point is stationary (Lackner et al. 2001). We suggest that our data (where the largest effects were seen only in the earth-referenced context) extend these observations to integrated corrective responses during walking (also see Rietdyk and Patla 1998; Schneider and Capaday 2003).

Another possibility is that holding the rail represents a new motor task or program and the alterations in reflexes may be indicative of task-dependent, rather than context-dependent, differences in neural control. This seems unlikely since the facilitation in TB and PD reflex pathways was not observed when the arm was in the "rail-holding" position without holding anything. We believe it to be more likely that the observed changes associated with holding the rail were related to the stability provided by the rail during locomotion. This explanation could account for why reflexes in PD were amplified the most during the task where tripping was most likely, stair climbing, when the rail was held. In contrast, following the hypothesis that the observed changes were reflective of a change in the motor program, we would be unable to predict this specific response. Further to this point, Schneider and Capaday (2003) found that during an unfamiliar walking task (backward walking) there was an unexpectedly large H-reflex

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that occurred in midswing that was not present during forward walking. This large H-reflex was diminished immediately upon rail contact and was also similarly diminished over time with practice of backwards walking without rail contact. The authors concluded that this reflex was related to task uncertainties during backwards walking and that the reduction in the reflex that occurred with rail contact or with practice of the task was likely due to the increased postural confidence (Schneider and Capaday 2003). We suggest that the changes in cutaneous reflexes associated with rail contact in the present study are also related to alterations in stability and postural confidence.

5.4.2 Functional relevance

The facilitation of interlimb responses in TB and PD when an earth-referenced rail is held suggests that neural pathways to these muscles are gated to incorporate the rail into the automatic recovery strategy. That is, these enhanced reflexes may have a role in stumble correction, making use of the rail to restore stability. While this is likely of benefit to most people, it may be detrimental to those who rely on earth-referenced assistive devices such as a cane or walker. For instance, Batini et al. (2004) showed that when a cane was held during walking with large perturbations, subjects tended to keep holding onto the cane as they were falling instead of dropping it to grab onto a more stable object such as a rail. If interlimb reflexes are selectively gated to make use of an object that is perceived to provide stability, this conceivably may lead to an over-reliance on canes or walkers. This is an area of functional application that requires further study.

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CHAPTER 6: GENERAL CONCLUSIONS

6.1 Summary

The role for spinal CPGs contributing to rhythmic motor outputs has been suggested for humans and other animals (Dietz, 2003, Rossignol *et al*, 1996, Rossignol *et al*, 2006, Yang *et al*, 2004, Zehr and Duysens, 2004). However, the extent to which locomotor CPGs in humans have retained the characteristics found in other vertebrates has been subject to debate (Capaday, 2002, Dietz, 2002, Nielsen, 2003). Is the general organization of CPG networks conserved across species or has the evolution of bipedal walking been accompanied by changes in the neural control of locomotion? In non-human animals, the output of the pattern generator can be transformed into different rhythmic motor patterns (Hooper and DiCaprio, 2004, Smith *et al*, 1998) and CPGs for each leg have a considerable degree of autonomy (Forssberg *et al*, 1980, Foth and Bassler, 1985). The first objective of this thesis was to determine to what extent these characteristics of locomotor CPGs are retained in humans.

Previously, it has been suggested that different directions of human rhythmic movement, like forward and backward walking and arm cycling, are regulated by common neural mechanisms (Duysens *et al*, 1996, Lamb and Yang, 2000, Zehr and Hundza, 2005). However, these tasks were also similar in muscle activity and/or joint kinematics. Is neural control still conserved across three locomotor tasks that were different in terms of EMG amplitude, joint kinematics, and stability demands? In fact, there were considerable similarities in the neural control of level walking, incline walking, and stair

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climbing (Lamont and Zehr, 2006 - Chapter 2), suggesting that the underlying neural mechanisms involved in coordinating walking could be modified to also coordinate other locomotor tasks. There were also task-specific differences between some tasks (e.g. in TA activity during swing of stair climbing versus level walking) that may be indicative of a specific adaptation to the mechanical and stability constraints unique to each task. Overall, this suggests a common neural control of these different locomotor tasks, with spinal CPGs interacting with afferent feedback and supraspinal input to modify the motor output to meet specific task and environmental demands (Zehr, 2005). Further similarities between bipedal human and multipedal animal CPGs for locomotion were identified in Chapter 3 (Yang *et al*, 2005). The stepping behavior of infants on a split-belt treadmill resembled that reported for other animals (e.g. cats, stick insects) under similar conditions (Forssberg *et al*, 1980, Foth and Bassler, 1985, Halbertsma, 1983, Kulagin and Shik, 1970), demonstrating that the coupling relationship among the pattern generators in humans has retained the flexibility found in other terrestrial animals.

Arm movement during human locomotion has not received as much attention as leg movement, thus the second objective of this thesis was to investigate how the arms interact with each other and with the legs to coordinate locomotion. Accumulating evidence supports the hypothesis that the arms are controlled in part by CPGs that resemble those for the legs (Zehr *et al*, 2004). However, one notable difference is that CPGs for the arms are even more autonomous than CPGs for the legs. Indeed, the only evidence for neural coupling between the arms during arm cycling was observed during a very asymmetric task in which one arm cycled twice as fast as the other, and even then,

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crossed-effects were only observed in ongoing EMG and not in reflex amplitudes (Chapter 4). The relatively weak coupling may reflect the relative independence of the arms during bipedal locomotion. Since the arms are not intrinsically linked to the production of gait, the state of the contralateral arm is probably not an important consideration in determining the response to a perturbation on the ipsilateral side. The crossed-effects in ongoing EMG may be a mechanism to limit asymmetrical muscle activity, which, if extended to walking, may affect stability. In fact, stability was shown to be a powerful factor in determining transmission in interlimb reflex pathways between the arms and legs (Haridas *et al*, 2006, Lamont and Zehr, 2007 – Chapter 5). In response to stimulation of the sural nerve at the ankle, reflexes in posterior deltoid and triceps brachii were greatly facilitated when a hand rail was held, particularly during less stable locomotor tasks like stair climbing (Lamont and Zehr, 2007 - Chapter 5). Since a hand rail can be used to restore balance after a disturbance, reflexes in the arms may be gated to reflect their enhanced role in the automatic recovery strategy.

The mechanisms of the observed changes in cutaneous reflexes during movement are not known with certainty. Since we found that reflexes are largely modulated independently from background EMG during rhythmic movement, we suggest that at least some of the gating of the responses occurs via spinal CPGs. This could be accomplished via a reorganization of reflex pathways in which those pathways that are operating during static postures (e.g. standing, sitting) are suppressed and other spinal interneurons are recruited during locomotion. Furthermore, presynaptic inhibition of transmission from afferents may regulate the gain of reflexes and contribute to the selection of specific

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reflex pathways during locomotion (reviewed in McCrea, 2001). If there is a CPG-related gating of reflexes, we might expect changes in interneuronal excitability across the movement cycle, which may result in differences in reflex latency (Quevedo *et al,* 2005). However, we found that the latency at which the reflexes peaked remained relatively constant, and few differences across task or contexts were noted upon statistical analysis. Perhaps single-cell recordings may reveal more cycle-dependent differences in reflex latencies. This issue requires further investigation.

Overall, this thesis has shed light on the neural control of the arms and legs during rhythmic movement. It has highlighted a number of similarities in the control of leg movement during locomotion in animals and humans, suggesting that a number of characteristics of lower/hind limb pattern generators are conserved across species. The results have also identified key differences between the neural control of the arms and legs, which may be representative of the independence of the arms during bipedal gait. Finally, a functional role for interlimb reflexes in the arms has been postulated to aid in stability during gait.

6.2 Future Directions

6.2.1 Neural Control of Different Forms of Locomotion

Recent evidence suggests that there a few basic neural signals produced by a locomotor pattern generator and shaped appropriately by descending inputs and sensory feedback to regulate a number of rhythmic motor tasks in humans (Zehr, 2005). Certainly, support for shared neural control has been reported for various locomotor tasks, including

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forward walking, backward walking, incline walking, stair climbing, cycling, and recumbent stepping (Duysens *et al*, 1996, Lamb and Yang, 2000, Lamont and Zehr, 2006 – Chapter 2, Stoloff *et al*, 2007, Zehr *et al*, 2007). On this basis, it has been proposed that learning during one of these tasks could be generalized and transferred to other locomotor tasks. For example, in a rehabilitation context, basic components of walking could potentially be retrained via practice of recumbent stepping (Stoloff *et al*, 2007) or cycling (Brown *et al*, 2005). Some evidence for such generalization of motor learning has been shown for visuomotor tasks and arm movements (Kawato, 1999) as well as for the control of perceived walking direction in adults (Earhart *et al*, 2001, Earhart *et al*, 2002). However, it has been reported that adaptation during split-belt walking does not generalize to all other locomotor tasks; specifically, split-belt adaptation does not transfer between forward and backward walking but does transfer between forward walking and running (Choi and Bastian, 2005). Therefore, motor learning may be more specific than previously thought. What determines the generalizability of motor learning to other tasks is not clear and is certainly an area for future research.

Furthermore, while it is becoming increasingly clear that many rhythmic movements can be coordinated by common neural elements, the distinction between tasks that involve CPGs (e.g. walking) and those that do not (e.g. discrete leg extension) is not well defined. Conventional wisdom states that discrete movements have a defined beginning and end, whereas rhythmic movements are ongoing and cyclic in nature. Following this definition, differences in the neural control of discrete and rhythmic movement have been noted in brain activity patterns (Schaal *et al*, 2004) and in reflex modulation patterns

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(Zehr *et al*, 2007). However, at what point a discrete movement becomes rhythmic is not known. Examination of reflex modulation patterns during transitions between discrete, voluntary tasks and rhythmic tasks could shed light on this issue. For example, a rhythmic task like leg cycling could be broken down into a series of discrete segments with stops between each segment. The duration of these stops could be gradually decreased and/or the length of each discrete segment could be gradually increased until reflex modulation resembles that during continuous movement. Another factor that may influence CPG involvement is the degree to which cortical areas are active during a task. That is, CPG involvement may be diminished during locomotor tasks with a significant cognitive component, such as beam walking or walking across the rungs of a ladder. However, evidence from Chapter 4 of this thesis suggests that CPGs for the arms gate sensory input similarly during a very cognitive task (asynchronous arm cycling) and a more automatic task (bilateral arm cycling at 1 Hz). It was proposed that even though asynchronous cycling likely had a significant cortical component, these descending signals may have been relayed through spinal pattern generators to produce the arm cycling pattern. Whether this is also true for the lower limbs remains to be seen.

6.2.2 Bilateral coordination between the legs

There is a great deal of autonomy between lower limb CPGs in human infants, since different types of coupling (such as 2:1, 3:1), and opposite directions of stepping are possible simultaneously in both legs (Yang *et al*, 2005 - Chapter 3). To date, there have been no reports of similar types of asymmetric stepping during adult split-belt walking (Dietz *et al*, 1994, Reisman *et al*, 2005). This may be attributed to a number of factors,

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including the lower speed differentials used in adult studies (< 4-fold speed difference), possible intervention from higher centres in the nervous system in adults, and differences in body weight support in adult and infant studies. However, recent reports from Bastian and colleagues have indicated that neurologically intact adults continue to step in an alternating 1:1 fashion up to a 10-fold difference in belt speeds (A.J. Bastian, personal communication, May 23, 2007); therefore, the lack of asymmetric stepping in adults cannot be solely attributed to lower speed differentials. The only report of asymmetric stepping in people older than 1 year of age was in a 4-year-old girl who had undergone a hemispherectomy 2 weeks prior to testing (A.J. Bastian, personal communication, May 23, 2007). At only a 2-fold speed differential on the split-belt treadmill, this girl performed 2:1 stepping automatically, without any instruction or prompting. No body weight support was provided. Interestingly, when this girl was retested 3-4 weeks post-surgery, an alternating stepping pattern was observed and she no longer performed 2:1 stepping on the split-belt treadmill. Bastian and colleagues (personal communication, May 23, 2007) found no instances of asymmetric stepping in children after stroke; however, all of these children were at least 1 month post-stroke. There have also been no reports of asymmetric stepping in adults after stroke or cerebellar damage (Morton and Bastian, 2006, Reisman *et al*, 2007).

Overall, these preliminary findings suggest that the autonomous nature of leg CPGs in humans is only revealed when there is extremely little input from cortical areas, such as in infants or shortly after hemispherectomy. Since strict 1:1 coordination between the legs is restored within weeks of hemispherectomy, this may be a learned behaviour that is

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of significant importance in coordinating bipedal gait, perhaps to help maintain stability. It would be of interest to see if children and adults less than 1 month post-stroke also show asymmetric stepping on the split-belt treadmill. It would also be interesting to examine how this strict coordination between the legs develops with age. Considerable improvement in walking proficiency and lower limb kinematics (e.g. foot trajectory and pendular mechanism during stance) occurs within the first few months of unsupported locomotion (Cheron *et al*, 2001a, Cheron *et al*, 2001b, Dominici *et al*, 2007, Ivanenko *et al*, 2004, Ivanenko *et al*, 2005). Perhaps strict 1:1 interlimb coordination also develops over the first few months of walking, indicating that nervous system maturation and the practice of independent walking may act as a functional trigger of gait maturation (Ivanenko *et al*, 2005). Finally, if the lack of autonomy between lower limb CPGs in adults is due to descending control, then theoretically adults with complete spinal cord injury should perform asymmetric stepping when supported over a split-belt treadmill regardless of the amount of time post-injury. However, this hypothesis would be difficult to test since these subjects require therapist assistance for stepping, which would probably influence the coordination.

6.2.3 Bilateral coordination between the arms

Chapter 4 identified crossed-effects due to contralateral activity during an asynchronous arm cycling task. When the contralateral arm was cycling at a higher frequency (2 Hz), EMG in the ipsilateral arm (cycling at 1 Hz) was significantly increased above that in the other ipsilateral 1 Hz cycling tasks in most muscles. However, the underlying neural

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mechanism of this crossed-effect in ongoing muscle activity is difficult to extrapolate from this study.

Previous investigations of coordination between the upper limbs have focused on bimanual movements like finger-tapping or continuous circle drawing in the horizontal plane. During these tasks, it is clear that in-phase movements, in which homologous muscles are active together, are most stable (Bressler and Kelso, 2001, Swinnen, 2002, Tuller and Kelso, 1989, Varela *et al*, 2001). Temporal coupling between the arms is disrupted in callosotomy patients during continuous circle drawing movements (Kennerley *et al*, 2002) and when coupling between the upper limbs goes beyond basic in-phase coupling (Preilowski, 1972, Preilowski, 1975). Therefore, temporal aspects of bimanual coordination likely involve crossed pathways between cortical hemispheres.

However, there is evidence for CPG involvement during arm cycling in the transverse plane (Zehr *et al*, 2004), which may differentiate arm cycling from bimanual tasks like circle drawing. Since CPGs for the arms are proposed to be quite similar to those for the legs, coordination between the arms during cycling may also resemble that between the legs. In contrast to bimanual coordination, the legs tend to prefer an out-of phase coordination pattern (Kautz *et al*, 2002, Kawashima *et al*, 2005, Pang and Yang, 2001, Ting *et al*, 2000), which is adopted during the majority of locomotor tasks. Indeed, it has been postulated that inhibitory connections between swing generating half-centres prevent the co-occurrence of swing on each side during human locomotion (Pang and Yang, 2001), thus discouraging in-phase patterns of leg coordination. These inhibitory

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crossed connections are thought to be subcortical or even spinal, since human infants (Pang and Yang, 2001) and spinal cord injured patients (Kawashima *et al*, 2005) show a preference for out-of-phase coordination. Interestingly, infants as young as 2 post-natal weeks show out-of-phase coordination between the arms during the swimming reflex, which is evoked when infants are held horizontally in the water (McGraw, 1939). Therefore, out-of-phase coordination may indeed be the default or "preferred" mode of coordination between the arms during locomotor movements. However, in Chapter 4, out-of-phase coordination during arm cycling did not seem to be particularly stable, since the phase relationship drifted away from 180° as the frequency of bilateral cycling increased (see Figure 4.2C). Future work could investigate the mechanism of coupling during arm cycling in more detail by comparing the relative stability of in-phase and out-of-phase coordination during high frequency movement.

While Chapter 4 reported crossed-effects in ongoing muscle activity during asynchronous arm cycling, cutaneous reflex amplitudes were remarkably conserved regardless of the activity on the contralateral side. In other words, CPG gating of ipsilateral sensory inputs was quite independent of contralateral activity, despite attempts to introduce interference between the motor patterns on the two sides. It was proposed that this may be a reflection of the independence of the arms: typically the state of the contralateral arm is not important in determining a response to an external perturbation of the hand. However, if the arms were given a more direct role in locomotion, interlimb pathways between the arms may be facilitated. It has already been shown that interlimb reflex pathways between the legs and arms are facilitated when a hand rail is held during

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locomotion, thus offering the arms more of a functional role in maintaining stability (Lamont and Zehr, 2007 - Chapter 5). Perhaps a similar facilitation in interlimb pathways between the arms would be observed during activities like crawling, cross-country skiing, or Nordic hiking (i.e. hiking with poles), during which coordination between the arms is more important for propulsion and stability.

6.2.4 Interlimb coordination between the arms and legs

In Chapter 5, the facilitation of interlimb responses in arm extensor muscles when an earth-referenced rail is held suggests that neural pathways to these muscles are gated to incorporate the rail into the automatic recovery strategy, particularly during tasks in which the threat of tripping is higher (e.g. during stair climbing). What happens to these interlimb reflex pathways when the threat of tripping or falling is very high, for instance in people exhibiting balance impairment? Since interlimb reflex pathways are closely tied to stability (Haridas *et al*, 2006, Lamont and Zehr, 2007), these pathways may be chronically facilitated in people who are chronically unstable, thus resulting in a reliance or even an over-reliance on the arms for stability. Interestingly, there may be an increased tendency to rely on the arms for stabilization in people who are more unstable due to age or neurological impairment (Maki and McIlroy, 1997, Maki *et al*, 2000, McIlroy and Maki, 1996), but it is not known whether this is due to facilitation in interlimb reflex pathways.

Facilitation of interlimb reflexes when an earth-referenced hand rail is held may also lead to an over-reliance on canes and walkers. In addition to mechanical stabilization, such

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assistive devices can provide haptic information that can be used in stabilizing the body (Jeka, 1997); however, a number of studies actually have linked use of assistive devices to an increased risk of falling (Campbell *et al*, 1989, Charron *et al*, 1995, Mahoney *et al*, 1994, Maki *et al*, 1994, Morse *et al*, 1987). If interlimb reflexes are selectively gated to make use of an object that is perceived to provide stability, this could conceivably cause individuals to persist in holding an assistive device that is perceived to provide stabilization, in lieu of grasping a more stable handhold (Bateni *et al*, 2004). In order to determine if this is the case, interlimb reflexes could be investigated in people with impaired balance, people who use a cane or walker, and age-matched controls. Additionally, the behavioural outcomes of these reflexes could be investigated by examining changes in arm kinematics and contact force on the rail as a result of the perturbation.

6.3 Conclusions

Overall, the results from this thesis suggest that transmission in interlimb and reflex pathways is closely tied to the relative importance of these pathways in the task being performed. For example, in Chapter 2 (Lamont and Zehr, 2006), suppressive cutaneous reflexes in TA were observed near swing-onset during stair climbing, whereas facilitatory reflexes were evoked at this phase during walking. This reflex reversal during stair climbing may prevent contact-evoked dorsiflexion at the stance-to-swing transition since this could result in tripping on the upcoming stair (if swing has been initiated) or collapse of the supporting ankle (if at end stages of stance). Chapter 3 (Yang *et al*, 2005) demonstrated that interlimb coupling between the legs was primarily expressed at the

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onset of swing-phase to prevent both legs from entering swing at the same time, which would be detrimental to forward progression. Chapters 4 and 5 (Lamont and Zehr, 2007) also showed that interlimb coordination was selectively expressed when it could be important for the stability of the movement (i.e. to equalize muscle activity on the two sides and to make use of an assistive device for support). Conversely, transmission in these pathways was limited when it was of little importance to the global motor pattern. For example, there was a lack of crossed-effects in cutaneous reflexes during asynchronous arm cycling (Chapter 4), perhaps because interaction between the arms is unnecessary in determining a response to an external perturbation of one arm. Furthermore, transmission in interlimb reflex pathways from the legs to the arms was limited when the arms had a minimal role in stumble correction (i.e. when a stabilizing object was not held – Chapter 5). Altogether, these results demonstrate the remarkable ability of the human nervous system to gate sensory inputs and interlimb communication so that only those responses relevant to the maintenance of forward progression (i.e. those that are related to maintaining equilibrium and the trajectory of the limbs that have a direct role in propulsion) are expressed during locomotion.

Is the coordination of locomotion in human bipeds like that of quadrupeds? It appears that some aspects of the neural control of locomotion are quite similar across species, particularly in terms of the control of the legs. Indeed, evidence for shared neural control of different tasks and for strong inhibitory connections between flexor half-centres has been presented in this thesis. However, some differences may also exist between humans and quadrupeds in the control of the arms/upper limbs. Humans have a great deal of

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independence between the arms, even during locomotor behaviours. An adaptation that may have been made as a result of this relative independence is the somewhat loose inter-arm coupling that we found in Chapter 4. Therefore, while the arms are thought to be coordinated in part by CPGs that are similar to those of the legs (and also to those of quadrupeds) (Zehr *et al,* 2004), specific changes have likely occurred as a result of differences in the role of the upper limbs in locomotion. In other words, while there are many similarities between human and quadrupedal locomotion, there are also a few differences that may be a result of adaptations in locomotor control to the unique characteristics of bipedal walking.

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