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Functional organization of cutaneous reflex pathways during locomotion and reorganization following peripheral nerve and/or spinal cord lesions

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Functional organization of cutaneous reflex pathways during locomotion and reorganization following peripheral nerve and/or spinal cord lesions

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Résumé

L'activité de marche chez l'animal et chez l'homme résulte de l'intéraction dynamique entre 3 grands systèmes: le générateur central du patron de marche dans la moelle épinière (CPG), qui organise temporellement et spatialement l'activité des muscles des membres, les structures supraspinales pour le contrôle volontaire et la modulation de la marche et les retours sensoriels issus des membres en mouvements qui permettent des ajustements fins du patron de marche dans un environnement inégal. Toute lésion centrale ou périphérique modifie l'état de la circuitrie locomotrice et la récupération de la marche repose vraissemblablement sur une optimisation des informations (descendantes et/ou périphériques) épargnées par la lésion. Mes travaux ont visé à étudier une partie des réorganisations qui s'opèrent dans le système suite à une lésion centrale et/ou périphérique et plus particulièrement au sein des voies cutanées chez le chat.

Les réflexes cutanés ont été enregistrés dans différents muscles des pattes postérieurs en réponse à des stimulations du nerf Tibial (Tib) dans différentes phases du cycle locomoteur. Après l'acquisition des réponses réflexes chez chaque chat à l'état intact, plusieurs protocoles de lésion ont été utilisés dans différents groupes d'animaux et la nature des changements au sein des voies cutanées évaluée et quantifiée chez ces mêmes chats. Ainsi nous avons pu évaluer les changements de transmission dans la voie cutanée du nerf tibial après 1) une lésion complète de la moelle épinière au niveau thoracique bas (T13), 2) une dénervation du nerf innervant les muscles extenseurs de la cheville Gastrocnemius Lateral et Soleus (LGS), 3) une dénervation du LGS suivie après récupération de la marche d'une lésion complète de la moelle épinière et 4) une lésion complète de la moelle épinière suivie après récupération d'une lésion du LGS. De façon remarquable, nous avons pu montré que des modifications de l'organisation des reflexes cutanés issus du Tib, et leur modulation au cours du cycle de marche, accompagnaient la récupération de la marche. Pris dans leur ensemble nos données indiquent que ces changements sont

spécifiques aux différents changements d'état de la circuiterie spinale (spinalisation vs dénervation du LGS à l'état intact ou post-spinalisation). Néanmoins, la récupération d'une marche spinale chez des animaux ayant récupéré de la dénervation du LGS avant d'être spinalisés était grandement entravée et la récupération plus ou moins incomplète. Cette dernière observation indique que les mécanismes de plasticité dans la moelle épinière et les capacités d'adaptation diffèrent en fonction de la séquence temporelle des lésions périphériques et centrales et suggère que les retours sensoriels en provenance du nerf LGS sont nécessaires pour le développement normal d'une locomotion spinale.

Pris dans leur ensemble nos résultats suggèrent 1) que les réflexes cutanées peuvent contribuer à la récupération de la marche après des lésions centrale et/ ou périphériques, 2) que des mécanismes spinaux et supraspinaux peuvent contribuer à compenser la dénervation du nerf LGS lorsque la moelle épinière est intacte, et 3) les retours sensoriels sont extrêmement importants pour l'expression d'une locomotion spinale.

Mots-clés : Plasticité, locomotion, compensation, dénervation, spinalisation, recouvrement fonctionnel, générateur central du patron de marche

Abstract

During locomotion, the spinal locomotor central pattern generator (CPG), which generates the basic locomotor rhythm, dynamically interacts with descending supraspinal inputs and sensory feedback from the periphery. Performing different lesions of the spinal cord and/or peripheral nerves alters the 'state' of the spinal locomotor circuitry and influences how the CPG interacts with supraspinal and peripheral sensory inputs. Following a lesion, the spinal CPG optimizes remaining inputs to maximize remnant locomotor capabilities. A method amenable to investigate the reorganization of sensorimotor pathways and the role of cutaneous feedback in locomotor compensation is to evoke and record cutaneous reflexes during locomotion in the same animal before and after lesioning a given structure.

Cutaneous reflexes, evoked by stimulating the tibial (Tib) nerve at the ankle were recorded during locomotion before and after sectioning the left lateral gastrocnemius-soleus (LGS) nerve in cats with an intact spinal cord (i.e. intact state) and after a complete spinalization (i.e. spinal state) in adult cats. In the intact and spinal states cats compensated for the loss of LGS muscles. The locomotor adaptation was associated with an increased burst activity in several hindlimb muscles bilaterally and a change in the gain of cutaneous reflexes in the intact and spinal states. This suggests that a change in the transmission of cutaneous reflex pathways could offset the loss of proprioceptive inputs at a spinal level. However, there were differences in reflex changes between intact and spinal states, suggesting that supraspinal inputs are also implicated.

To determine the reorganization of cutaneous reflexes following the loss of supraspinal inputs, Tib nerve reflexes were recorded during locomotion before and after a complete spinalization. The gain of cutaneous reflexes was modified in all muscles studied and in specific phases of locomotion, which could have important implications for the recovery of walking following spinal cord injury. To determine if cutaneous reflexes were similarly organized after spinalization in a situation where

sensory feedback was modified before spinalization we denervated the left LGS in the intact state and following a period of recovery the complete spinal transection was made. It was shown that the expression of spinal locomotion was negatively influenced when a LGS denervation was performed before spinalization. The reorganization of cutaneous reflexes observed in "normal" spinal cats was different in these "denervated-spinal" cats suggesting that spinal plasticity differs in both cases. It also indicates that sensory feedback from the LGS nerve is required for the normal "development" of spinal locomotion.

Overall, the results suggest that 1) changes within cutaneous reflex pathways are involved in the recovery of locomotor functions following lesions to the spinal cord and/or peripheral nerves, 2) that spinal and supraspinal mechanisms mediate the locomotor compensation after a LGS denervation in the intact state, and 3) that sensory feedback is critical for the expression of spinal locomotion.

Keywords: Plasticity, locomotion, compensation, denervation, spinalisation, functional recovery, central locomotor pattern generator

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Abbreviations

APR	Automatic postural response
C	Conditioned
CCS	Caudal cutaneous
CNS	Central nervous system
CoM	Centre of mass
CoP	Centre of pressure
CS	Conditioned stimulus
CPG	Central pattern generator
D	Down
DHPG	Dihydroxyphenylglycine
dlsc	Dorsolateral spinal cord
DPc	Deep peroneal cutaneous
E	Extensor
EDL	Extensor digitorum longus
EMG	Electromyography
EPSP	Excitatory post-synaptic potential
FDB	Flexor digitorum brevis
FDL	Flexor digitorum longus
G	Guard
GABA	Gamma-aminobutyric acid
HRP	Horse-radish peroxidase
IPSP	Inhibitory post-synaptic potential
L	Lumbar
LGS	Lateral gastrocnemius-soleus
LPL	Lateral plantar
MEP	Motor evoked potentials
MG	Medial gastrocnemius

MPL	Medial plantar
N	Negative
NWR	Nociceptive withdrawal reflex
P	Positive
PAD	Primary afferent depolarization
PBSt	Posterior biceps-semitendinosus
PSP	Post-synaptic potential
PT	Pyramidal tract
RF	Receptive field
RN	Red nucleus
S	Sacral
Saph	Saphenous
SP	Superficial peroneal
St	Semitendinosus
T	Thoracic
TA	Tibialis anterior
Te	Test
Tib	Tibial
TMS	Transcranial magnetic stimulation
US	Unconditioned stimulus
WGA	Wheat-germ agglutinin

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Chapter 1 - General introduction

Overview

Locomotion is a complex task requiring dynamic sensorimotor interactions at multiple levels of the nervous system (Rossignol et al. 2006; Grillner 1981; McCrea 2001). At the core is the spinal locomotor central pattern generator (CPG), which interacts with descending inputs from supraspinal and propriospinal sources and with sensory feedback from the periphery. This basic spinal circuitry for locomotion is genetically determined since kittens after a complete transection of the spinal cord will develop walking capabilities without ever having walked (Grillner 1973; Forssberg et al. 1980b; Forssberg et al. 1980a). In adult animals, removing descending commands from supraspinal structures (e.g.. spinal lesions) or eliminating sensory feedback from the periphery (e.g. nerve sections, deafferentation) removes inputs to the spinal locomotor CPG but does not abolish its rhythm-generating capacity because locomotion can be re-expressed following complete transection of the spinal cord (Shurrager and Dykman 1951; Barbeau and Rossignol 1987; Lovely et al. 1990; Chau et al. 1998a; de Leon et al. 1998) and only transient deficits are observed after peripheral denervations (Wetzel et al. 1973; Pearson et al. 1999; Carrier et al. 1997; Bouyer et al. 2001; Bouyer and Rossignol 2003b; Bouyer and Rossignol 2003a; Tachibana et al. 2006). However, after such lesions a new equilibrium must be reached between the spinal CPG and remnant sources of inputs to maximize remaining locomotor capabilities (Rossignol 2006).

The functional changes occurring within descending pathways and in spinal sensorimotor pathways from the periphery after lesions to the spinal cord or to peripheral nerves during locomotion are largely unknown (Rossignol 2006; Rossignol et al. 2008). Evoking and recording cutaneous reflexes during locomotion before and after spinal or peripheral nerve lesions are clearly amenable to investigate changes within spinal sensorimotor pathways and to evaluate the contribution of cutaneous inputs in the functional recovery of locomotion following such lesions. It was shown long ago that cutaneous inputs from the paws are not necessary for generating the basic locomotor rhythm since removing all cutaneous nerves from the hindlimbs in

cats (Sherrington 1910; Duysens and Stein 1978) or infiltrating the dorsum or foot pads (Engberg 1964; Forssberg et al. 1977; Prochazka et al. 1978) with a local anaesthetic did not impede locomotion on level surfaces. Consequently, the role of cutaneous afferents during locomotion was for a long time underestimated. However, removal of cutaneous inputs could simply imply that the nervous system can select alternative inputs to compensate during treadmill locomotion with little demands for adjustments, namely from the foot.

That the skin of the foot did little for locomotion was puzzling because cutaneous inputs from the hindlimb have a well-defined somatotopic organization within the dorsal horn of the spinal cord up to the cortex and the foot is more densely represented than any other skin region of the hindlimb within the dorsal horn (Brown and Fuchs 1975; Brown P.B. et al. 1992). Moreover, within a dorsal root ganglia there are more cutaneous than proprioceptive afferents (Loeb 1981). This could indicate that a greater density of cutaneous afferents is necessary for the exteroceptive exploration of the surrounding environment and/or that cutaneous inputs have a more prominent or equally important in movement control compared to proprioceptive information. Over the past 30 years, recording, stimulating, or inactivating cutaneous afferents have highlighted the important role of cutaneous inputs from mechanoreceptors of the foot during locomotion.

The following chapters present data related to adaptive mechanisms, such as changes in muscle bursts and cutaneous reflex pathways, after partial denervation of ankle extensors to determine if the loss of proprioceptive information leads to an increase in cutaneous inputs. Cutaneous and proprioceptive inputs have complementary roles during locomotion and it is possible that the loss or reduction in one leads to an increase in the other (Duysens and Pearson 1976; Prochazka 1996). The mixed nerve supplying the left lateral gastrocnemius-soleus (LGS) muscles was cut in the intact (i.e. an intact spinal cord) and spinal (i.e. after spinalization) states during locomotion and cutaneous reflexes were recorded before and after the

denervation in the same cats to determine how cutaneous reflexes may participate in the recovery of locomotion (chapters 3 and 4). Performing the same denervation in the intact and spinal states will help in determining if adaptive mechanisms are similar when supraspinal inputs are available.

There is also a paucity of information with regards to changes in reflexes following a complete spinal transection in a locomotor condition. To determine how sensorimotor pathways (i.e. reflexes) are reorganized after the loss of supraspinal inputs we recorded cutaneous reflexes during locomotion before and after spinalization in the same cats (Chapter 5). Previous studies have shown that performing a muscle or cutaneous denervation before spinalization impaired the 'normal' expression of spinal locomotion (Carrier et al. 1997; Bouyer and Rossignol 2003b) and it is possible that sensorimotor pathways are reorganized differently after a complete spinal transection if a peripheral nerve lesion is performed before spinalization. To address this, we performed a denervation of the left LGS in otherwise intact cats and following a period of recovery a complete transection of the spinal cord was made. Cutaneous reflexes were recorded before and after the spinalization to determine if changes in reflexes normally observed in spinal cats (i.e. Chapter 5) are similar in cats that received a denervation prior to spinalization (Chapter 6).

In a first time, we discuss the importance of cutaneous afferents from mechanoreceptors of the foot during hindlimb locomotion and the potential role of these inputs in the functional recovery of locomotion following lesions to the spinal cord and/or to peripheral nerves.

Cutaneous nerves, afferents and mechanoreceptors of the foot

Cutaneous nerves of the foot

Figure 1 illustrates the five major cutaneous nerves of the hindfoot and their receptive fields in the cat (Bernard et al. 2007). The tibial nerve (Tib) at the ankle

covers the majority of the plantar surface of the paw along with central and digital pads by giving rise to the medial (MPL) and lateral plantar (LPL) nerves, which respectively innervate the medial and lateral plantar surfaces of the foot. The Tib nerve also gives off a small mixed branch, the calcaneal nerve that innervates intrinsic foot muscles. The superficial peroneal nerve (SP) covers most of the dorsal surface of the foot. The caudal cutaneous sural nerve (CCS) carries inputs from the lateral aspect of the foot and calcaneus whereas the saphenous nerve (Saph) innervates the medial aspect of the foot. The cutaneous branch of the deep peroneal nerve (DPc) supplies digits 2 and 3.

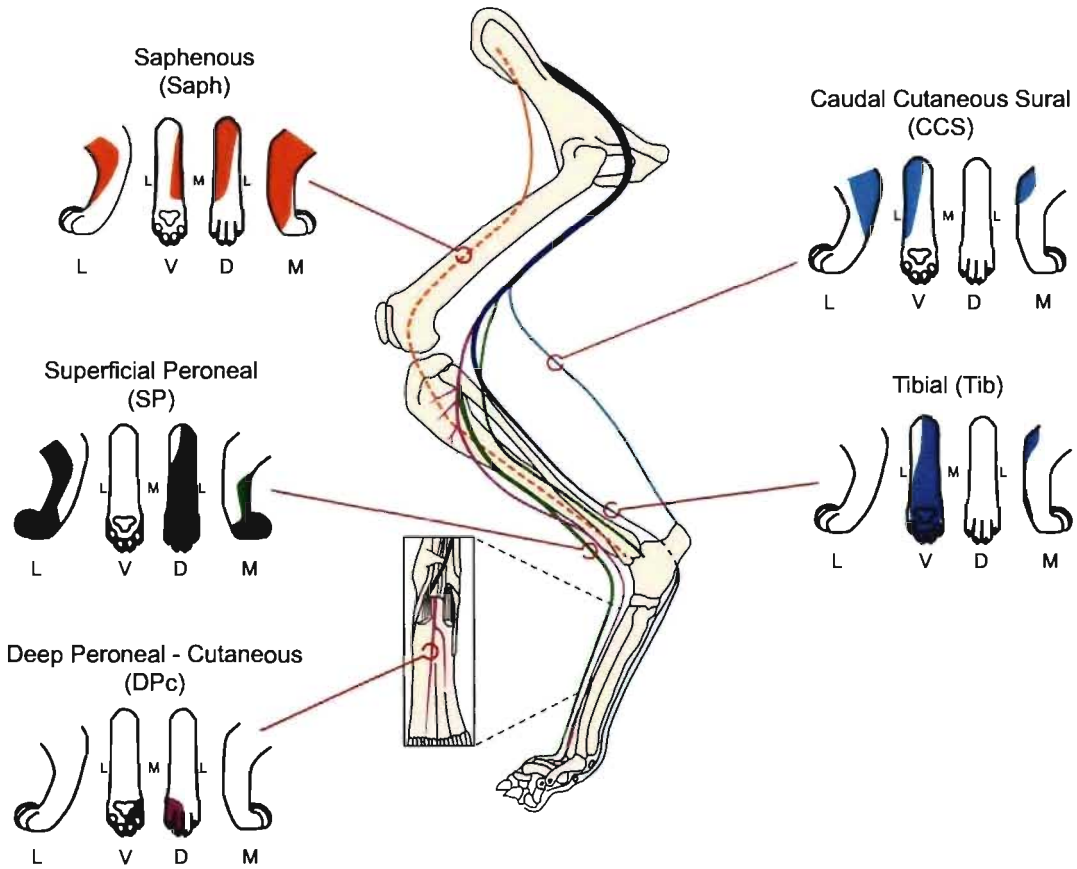

Figure 1. Cutaneous nerves of the left hindlimb and their receptive fields : superficial peroneal (SP, foot dorsum), tibial (Tib, plantar foot, central and digital pads), caudal cutaneous sural (CCS, lateral aspect of the paw and calcaneus), saphenous (Saph, medial aspect of the paw), and deep peroneal (DPc, cutaneous branch innervating digits 2 and 3). L, V, M, and D represent lateral, ventral, medial, and dorsal views of the paw.

Types of cutaneous afferents and mechanoreceptors of the foot

Cutaneous afferents can be categorized based on size and structure, myelinated versus non-myelinated, and according to conduction velocity [for reviews see (Perl 1992; Wilson and Kitchener 1996)]. Based on this classification scheme there are 3 main types of cutaneous afferents supplying the foot. Low-threshold mechanoreceptors of the foot are mainly subserved by Aβ type afferents, which are fast conducting and transduce mechanical stimuli such as touch, pressure, brushing and vibration. The Aδ fibres conduct more slowly and are primarily high threshold afferents that respond to noxious mechanical stimuli such as pinch and prick while a second type is low-threshold and is responsive to brushing hairs (D-hair). There are several sub-types of C afferents that transduce different types of pain modalities such as mechanical, thermal and chemical. For the purposes of this review we will focus on low-threshold cutaneous mechanoreceptors of the foot and their large myelinated Aβ afferents.

The foot of the cat and other mammals, including humans, contains different types of low-threshold cutaneous mechanoreceptors that respond to specific mechanical stimuli (Hunt and McIntyre 1960a; Hunt and McIntyre 1960b; Horch et al. 1977; Johnson 2001; Sanders and Zimmermann 1986; Leem et al. 1993; Perl 1992). It is generally accepted that in the body there are 4 major types of cutaneous mechanoreceptors innervating the glabrous skin (Johnson 2001; Kennedy and Inglis 2002; Perl 1992) and 6 types of cutaneous mechanoreceptors in hairy skin (Perl 1992; Burgess et al. 1968).

In the glabrous skin, two mechanoreceptors are slowly adapting units (SA1 and SA2), discharging throughout a maintained stimulus applied to the skin and two are rapidly adapting units (Pacinian and RA), discharging briefly at the onset and offset of the stimulus (Lynn 1971; Lynn 1969; Brown et al. 1980; Janig et al. 1968; Gray and Matthews 1951; Adrian and Umrath 1929; Malinovsky 1966; Janig 1971; Iggo and Ogawa 1977). Due to their slowly adapting nature SA1 and SA2 afferents

can inform the nervous system about maintained tactile and pressure sensations but have different discharge patterns. In the foot of the rat, SA2 units discharge regularly during constant force stimulation and are sensitive to skin stretch, whereas SA1 afferents discharge irregularly and do not respond to skin stretch (Leem et al. 1993). SA1 afferents are important for fine tactile discriminations because of their very small receptive fields ending in Merkel cells that are located superficially within the glabrous skin. On the other hand, SA2 afferents have larger receptive fields that are thought to terminate as Ruffini corpuscles, which are located more deeply. As mentioned, SA2 units in the hand and foot are very sensitive to skin stretch but also to the direction of stretch (Kennedy and Inglis 2002; Knibestol 1975; Johansson 1978; Birznieks et al. 2001). In other words the discharge rate for a given stretch is dependent on the applied direction of the tactile stimulus. Pacinian corpuscles are very sensitive to vibration in the 300-400 Hz range, are located deep within the foot pads and have large receptive fields whereas RA receptors are less sensitive, more superficially located, have smaller receptive fields and are best activated by vibration in the 5-40 Hz range (Janig et al. 1968; Lynn 1969; Leem et al. 1993; Iggo and Ogawa 1977). RA receptors appear of great importance in determining the direction of forces applied in the horizontal plane (Morasso and Schieppati 1999).

Hairs in the skin are also very sensitive receptors and based on their broad appearance are generally categorized as guard (G) hairs, which have thick and straight shafts, and down (D) hairs, which have thin and wavy shafts (Burgess et al. 1968). Based on receptive field sizes and mechanical sensitivity G and D hair receptors can be further sub-divided. For instance, G_1 hair receptors have larger receptive fields than G_2 and D hair receptors and while G_2 and D fibres respond readily to slow gentle hair movements G_1 receptors require rapidly moving stimuli. G_1, G_2 and D hair receptors are all rapidly adapting showing no maintained discharge in response to steady hair displacement. The hairy skin also contains receptors that are not associated with the hairs themselves. Type I and II receptors are slowly

adapting, have punctate receptive fields and do not respond to hair movement. These receptors have essentially the same properties as SA1 and SA2 receptors found in the glabrous skin. Pacinian corpuscles are also found in hairy skin and are identical to those found in glabrous skin.

Therefore, different types of mechanoreceptors in the glabrous and hairy skin of the foot can signal different parameters of a tactile stimulus, such as maintained pressure, direction of applied force, velocity of mechanical stimulation, minute contacts, and hair movement. As a result of these properties the skin of the foot acts as an exceptionally sensitive tactile sensor that should play an important role in foot placement and detection of loads during the various phases of locomotion.

Somatotopic and laminar organization within the spinal cord

Cutaneous afferents from mechanoreceptors of the foot project to the dorsal horn of the lumbosacral cord in a well-defined somatotopic organization (Brown P.B. et al. 1992; Woolf and Fitzgerald 1986; Brown and Fuchs 1975; Wall 1960; Wilson et al. 1986; Light and Durkovic 1984). For example, spinal interneurons with receptive fields (RFs) on the proximal limb are generally located in the lateral dorsal horn whereas those with distal RFs are usually more medial (Brown and Fuchs 1975). Thus, the foot is represented primarily in the medial dorsal horn from spinal segments L5 to S1 in the cat. The toes are represented in the medial dorsal horn in an orderly rostrocaudal sequence from toes 2 to 5 (Wilson et al. 1986). Receptive fields are usually small on the toes and increase progressively in size for skin regions that are located more proximally on the leg. For a given skin area at least some cutaneous afferents project to all areas of the dorsal horn representing that skin region (Brown and Fuchs 1975). Furthermore, cutaneous afferents make functional but weak synaptic connections rostral and caudal to the region where their receptive field is represented in the somatotopic map of the dorsal horn (Wilson and Kitchener 1996). As in other parts of the central nervous system (Haight 1972; Kruger et al. 1961;

Kuhn 1953; Mountcastle and Henneman 1952; Perl et al. 1962), the representation of the skin within the dorsal horn is not uniform across skin regions. The foot, particularly the toes, has a greater representation than the rest of the leg, reflecting a greater innervation density (Brown and Fuchs 1975; Brown P.B. et al. 1992). Moreover, using the retrograde anatomical tracer, horseradish peroxidase (HRP), it was found that cutaneous afferents from the foot diverge more widely mediolaterally within the dorsal horn than those from other regions of the leg (Millecchia et al. 1991). The greater representation and divergence of inputs from the skin of the foot underscores the importance of this information.

Cutaneous afferents also project to specific laminae within the dorsal horn of the spinal cord [for review see (Fyffe 1992; Wilson and Kitchener 1996)]. Projection patterns have been determined by mass transganglionic labelling of neuronal tracers, such as HRP and intra-axonal staining of functionally identified cutaneous afferent fibres. HRP is conjugated with different plant and bacterial lectins because free HRP is transported both by myelinated and unmyelinated primary afferents and does not permit a clear dissociation (Jessell et al. 1990). For example, HRP conjugated with the β-subunit of cholera toxin (HRP- β) is taken up only by myelinated afferents and can be used to map projections from Aβ and Aδ fibres whereas conjugation of HRP with the lectin wheatgerm agglutinin (HRP-WGA) labels terminals of unmyelinated C afferents. From these studies it has been shown that different classes of cutaneous afferents terminate in specific laminae (I-VI) of the dorsal horn (Figure 2). Myelinated afferents (Aβ) from cutaneous mechanoreceptors terminate mainly in laminae III and IV but also in laminae V-VI and the innermost part of lamina II, whereas those from cutaneous nociceptors (Aδ fibres) terminate in laminae I and V and the outermost part of lamina II (Fyffe 1992).

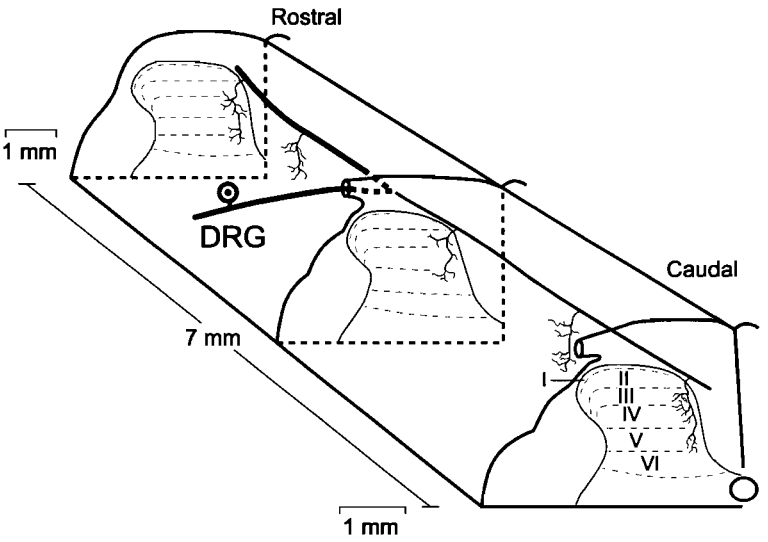

Figure 2. Projection of a cutaneous afferent within the dorsal horn of the spinal cord (laminas I-VI) in the cat. Cell bodies of cutaneous afferents are contained with dorsal root ganglia (DRG).

Cutaneous reflex pathways from the foot during locomotion

It is generally agreed that peripheral inputs serve to modify or correct the centrally generated pattern of muscle activity during locomotion (Rossignol et al. 2006; Rossignol 1996; Frigon and Rossignol 2006a). Stimulating different cutaneous nerves of the foot can influence the locomotor pattern by enhancing, prolonging or shortening ongoing activity. Fictive locomotion has been used to investigate the effects of particular afferents on the locomotor pattern because in this preparation there is no movement-related sensory feedback other than the nerve stimulated because the animal is paralysed (Grillner and Zangger 1979). For instance, stimulating the SP nerve during the early to mid-flexion phases of fictive locomotion in the cat increases the duration of ongoing flexor activity, delays the following extensor phase and onset of the next step cycle (Quevedo et al. 2005a; Schomburg et al. 1998). However, delivered during late flexion it can terminate the flexor burst and initiate extensor activity (i.e. reset of locomotor cycle to extension) (Quevedo et al.

2005a). Stimulation of the SP nerve during the early extension phase shortens or terminates ongoing extensor activity and causes a reset to flexion (Schomburg et al. 1998). Low-threshold stimulation of the Tib nerve, or direct stimulation of the skin of the foot sole, during the extension phase prolongs and enhances ongoing extensor activity, similar to group I afferents from ankle extensors (Guertin et al. 1995; Schomburg et al. 1998). On the other hand, stimulating the Tib nerve during the flexion phase terminates flexion and resets the step cycle to extension. Therefore, the effects of stimulation on the fictive locomotor pattern depend on the nerve stimulated and in what phase the nerve is stimulated (phase-dependence).

Organization of cutaneous reflex pathways

Cutaneous pathways are polysynaptic in that impulses carried by primary cutaneous afferents traverse at least one interneuron before contacting motoneurons. Indeed, there are no known monosynaptic cutaneous pathways to motoneurons (Fleshman et al. 1988). Most cutaneous pathways from the foot are minimally trisynaptic in the cat (Baldissera et al. 1981; Lundberg 1979; Lundberg 1975) but there are disynaptic linkages as well, such as from SP and MPL nerves to FDL motoneurons (Fleshman et al. 1988; Fleshman et al. 1984; Schmidt et al. 1989).

During locomotion in the intact cat, mechanical or electrical stimulation of the skin or cutaneous nerves of the foot evokes short- and longer-latency responses in hindlimb muscles that are modulated according to the phase of the step cycle (Duysens and Loeb 1980; Abraham et al. 1985; Loeb 1993; Pratt et al. 1991; Forssberg et al. 1975; Forssberg et al. 1977; Forssberg 1979). For instance, in the cat, electrical stimulation of cutaneous nerves during swing and end-stance elicits in ipsilateral (i.e. on the side of the stimulation) flexors, such as St and TA, two "positive" (P) or excitatory responses, with the first and second responses occurring at a latency of ~10 ms (P_1) and ~25-30 ms (P_2) after the stimulus, respectively. During stance, in ipsilateral extensors, such as VL and triceps surae muscles, the first

response is negative (N) or inhibitory with an onset of ~10 ms (N_1) followed by a longer-latency excitatory response at a more variable latency of ~25-45 ms (P_2 or P_3). Short- and longer-latency excitatory responses (P_1 and P_2, respectively) can also be evoked in ipsilateral extensors during the swing phase. Thus, although responses in flexors and extensors are generally more prominent during swing and stance, respectively, when these muscles are active, responses can be also be evoked in their 'non- locomotor' phase (e.g. excitatory flexor and extensor responses during stance and swing, respectively). In the contralateral hindlimb a single excitatory response in flexors and extensors at a latency of ~20-25 ms (P_2), which are more prominent during swing and stance, respectively, has been reported.

Although the pattern of reflex responses is well described in the cat during locomotion how these various pathways are organized at different levels of the nervous system is unclear. Obviously the most direct route from cutaneous afferents of the foot to fore- and hindlimb motor pools is by contacting interneurons within the spinal cord that then project to motoneurons. Cutaneous reflexes from the foot can pass through the spinal cord by contacting segmental interneurons based on the short-latency of responses in motoneurons and muscles and because reflexes can be readily evoked following a complete spinal transection (Forssberg 1979; Forssberg et al. 1975; Forssberg et al. 1977). However, supraspinal structures could also mediate or and/or contribute to the longer-latency responses (i.e. P_2 and P_3) evoked during locomotion. For instance, cutaneous reflex pathways from the foot can pass trough the brainstem (spino-bulbo-spinal) and cortex (transcortical) before contacting interneurons and/or motoneurons of the arms and leg, which are independent of descending commands (Christensen et al. 1999; Shimamura and Livingston 1963; Shimamura et al. 1991). The spino-bulbo-spinal reflex involves recurrent projections to the brainstem in a zone within the medulla oblongata, near the midline. There are two ascending pathways, a direct pathway to the bulbar reticular formation and an indirect pathway relayed by the lateral cervical nucleus. The descending pathways

from the bulbar reticular formation are within the reticulospinal tracts (Shimamura and Kogure 1979) and mainly project to flexor motor pools (Shimamura and Akert 1965; Shimamura et al. 1967). Whether or not spino-bulbo-spinal pathways mediate longer-latency responses during locomotion in the cat (i.e. P_2 or P_3 responses) is unknown but could be assessed by recording cutaneous reflexes before and after spinalization in the same cat to determine if the P_2 response is affected.

The evidence for transcortical cutaneous reflex pathways from the foot is more indirect. In humans, transcranial magnetic stimulation (TMS) applied over the motor cortex increases the excitability of cortical cells, which can be used to generate motor-evoked potentials (MEPs) in muscles. To test whether cutaneous pathways ascend to the cortex before descending back to motoneurons a TMS pulse was delivered to the leg representation of the motor cortex after stimulating the sural nerve below the lateral malleolus at rest (Nielsen et al. 1997) and during walking in humans (Christensen et al. 1999). It was found that MEPs in TA were greatly facilitated if the sural nerve was stimulated 50-80 ms before applying TMS because responses in TA were greater than the algebraic sum of MEPs and cutaneous reflexes evoked alone, providing evidence that part of the sural nerve pathway traverses the cortex before contacting TA motoneurons in the lumbar spinal cord.

Inhibitory and excitatory connections

In ipsilateral and contralateral motoneurons, cutaneous nerve stimulation can evoke excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs, respectively), indicating that cutaneous afferents have parallel projections to both excitatory and inhibitory last-order interneurons within the lumbosacral spinal cord (Edgley and Wallace 1989; Edgley and Aggelopoulos 2006; Burke et al. 2001; Quevedo et al. 2005b; Loeb et al. 1987). To illustrate this point, responses evoked in multiple hindlimb motoneurons ipsilateral to SP nerve stimulation will be described during the flexion phase of fictive locomotion in anesthetised decerebrate cats with

intact spinal cords (Quevedo et al. 2005b). In this study, stimulation of the SP nerve evoked short-latency EPSPs in ipsilateral FDL and TA motoneurons, which were followed by IPSPs. In EDL, however, initial responses were generally long-lasting IPSPs. Contrary to ankle flexor (TA and EDL) and FDL motoneurons, the initial SP-evoked EPSP was not cut short by an inhibition in PBSt motoneurons, which could persist throughout the stimulation. In sartorius motoneurons short-latency IPSPs could be followed by EPSPs. In ankle extensor motoneurons during fictive locomotion, short-latency EPSPs were generally evoked. In the absence of fictive locomotion, however, SP stimulation is largely inhibitory to ankle extensors. The range of excitatory and inhibitory PSPs in different muscles indicates that there are disynaptic, oligosynaptic and polysynaptic inhibitory and excitatory linkages.

Although the pattern of responses is better known in motoneurons ipsilateral to the stimulation, short-latency inhibitory and excitatory responses can also be evoked in motoneurons of the contralateral limb in anesthetised animals at rest (Edgley and Aggelopoulos 2006; Edgley and Wallace 1989). Crossed inhibitory responses are generally of smaller amplitude and occur at a slightly longer latency (~ 1ms) than those evoked on the ipsilateral side. On average, crossed excitatory responses occur at a slightly longer-latency than inhibitory responses but there can be disynaptic relays as well, which explains why some inhibitory responses show positive inflections. Curiously, crossed inhibitory or excitatory responses evoked by cutaneous afferents have not been reported during fictive locomotion. During locomotion in the intact cat, although inhibitory and excitatory responses can be evoked in ipsilateral muscles only crossed excitation has been shown in multiple flexor and extensor muscles (Duysens and Loeb 1980). It is possible that inhibition is present in contralateral muscles during locomotion, which could be an important mechanism for coordinating the hindlimbs following a perturbation.

What emerges from studies of cutaneous reflexes during locomotion apart that any given cutaneous nerve from the foot evokes responses in multiple muscles of

both hindlimbs is 1) that evoked responses are nerve and location specific and 2) that there is inter-individual variability in cutaneous reflexes.

Nerve and location specificity

During locomotion, contacting the dorsum of the foot evokes a coordinated reflex response that withdraws the limb away from the stimulated skin area (i.e. foot dorsum). Likewise an object touching the lateral surface of the foot also generates a withdrawal response but activates a slightly different subset of muscles to move the limb away from the stimulated skin region. These site-specific responses, originally described as local sign (Duysens 1977; Creed and Sherrington 1926; Sherrington 1910; Hagbarth 1952), serve to remove the limb away from a perturbation in an appropriate direction. Numerous studies have shown that stimulation of different skin areas or cutaneous nerves evokes different reflex responses from one muscle to another at rest (LaBella et al. 1989; Nakajima et al. 2006) and in the same phase of intact and fictive locomotion (Van Wezel et al. 1997; Zehr et al. 1997; Zehr and Stein 1999; Komiyama et al. 2000; Moschovakis et al. 1991; Schmidt et al. 1988; Pratt and Loeb 1991; Pratt et al. 1991). Responses are also modulated according to the phase of locomotion, which is discussed later on.

Nerve and/or location specificity is evident during intact and fictive locomotion. For instance, stimulation of the sural nerve during swing evokes a middle-latency (15-20 ms) excitatory response in sartorius (hip flexor/knee extensor) whereas stimulation of the saphenous nerve in the same phase evokes an inhibition of sartorius at a similar latency (Pratt and Loeb 1991; Pratt et al. 1991). As another example, stimulation of the SP nerve elicits P_1 and P_2 responses in FDL and FDB but sural stimulation evokes only P_2 responses in FDL and only P_1 in FDB (Loeb 1993). The plantar surface of the foot, particularly, appears to be composed of discrete regions that evoke specific responses in different leg muscles. For instance, in one study, middle latency reflex responses (70-110 ms) were evoked in several hindlimb

muscles by stimulating different skin areas of the plantar surface of the foot while sitting and standing (Nakajima et al. 2006). Stimulating the heel produced an excitatory response in soleus whereas stimulating the medial and lateral portions of the forefoot yielded inhibitory responses. Conversely, in TA, stimulating the medial forefoot produced a large excitatory response while heel stimulation evoked an inhibition. Thus afferents from different skin areas within the same nerve can evoke different response patterns from one muscle to another.

Perhaps the best example of nerve and/or location specificity is the extensive work by Schouenborg and collaborators on the modular organization of the nociceptive withdrawal reflex (NWR) [for reviews see (Schouenborg 2002; Schouenborg 2003; Schouenborg 2007). The NWR is comprised of "modules", with each module receiving sensory input from excitatory and inhibitory receptive fields from a specific skin area. Each module controls a single or a few synergistic muscles (Schouenborg 2002). The RFs are closely related to the movement produced by the output muscles. For example, in the rat, the excitatory RF for the gastrocnemii muscles is focalized on the heel of the foot sole whereas the RF of TA is located in the plantar forefoot and digits (Schouenborg and Weng 1994). The mechanical action of the gastrocnemii is to extend the ankle shifting foot contact to the forefoot and toes to remove the RF from the noxious stimuli. Likewise the TA flexes the ankle shifting foot contact to the heel, also removing the RF from the stimuli. Therefore, RFs on the skin are linked to specific modules within the spinal cord that serves to move the limb in specific directions.

Inter-individual variability of cutaneous reflexes during locomotion

Cutaneous reflexes during locomotion can differ from one individual or animal to another (Loeb 1993; Zehr et al. 1997; Haridas et al. 2005; Loeb 1999). For example, the extensor digitorum longus (EDL), an ankle flexor/toe extensor, is invariably recruited in the E1 phase during unperturbed locomotion (Philippson 1905;

Rossignol 1996). However, SP stimulation evokes responses in EDL that vary substantially from one cat to another. For instance, in one cat, stimulation of the SP nerve evoked P1 and P2 responses throughout the step cycle, whereas in another cat excitatory responses were mostly confined to the E1 phase (Loeb 1993). Several muscles from the cat hindlimb display these idiosyncratic responses from one cat to another, although cutaneous reflexes from one day to another within the same cat are very consistent. It is likely that this inter-animal variability persists after complete transection of the spinal cord indicating that differences in reflex circuitry between cats are present within the spinal cord and do not depend on differences in descending commands from supraspinal centres. Because of the individuality of cutaneous reflex responses from one animal to another during locomotion a main methodological concern raised by Loeb (Loeb 1993) is that pooling data from multiple animals is not appropriate to make comparisons from one situation to another; a concern that is not often discussed or considered.

Another important point raised by this inter-animal variability in reflex pathways is that spinal sensorimotor circuits are not entirely genetically determined and can be shaped by experience and training. In one study, mechanical actions of ankle muscles were altered by surgically crossing or transferring tendons in the right leg of kittens and cutaneous reflexes were evoked by stimulating the SP nerve once these cats reached adulthood (Loeb 1999). In cats with a persistent change in muscle action, locomotor EMG patterns were as would be expected of their normal recruitment patterns but cutaneous reflexes were asymmetric between hindlimbs indicating that some components of the spinal locomotor CPG are malleable. In a series of experiments, Schouenborg and colleagues investigated whether the NWR, evoked by stimulating cutaneous afferents of the foot, is shaped by experience during development in the rat (Levinsson et al. 2002; Schouenborg 2002). As stated earlier the NWR system has a modular organization (Schouenborg 2002). At birth, the modules are maladaptive, often causing movements towards the noxious stimuli. In

the first three weeks after birth, the reflex circuitry from receptive fields to modules is shaped by experience whereby erroneous connections are eliminated or reduced and appropriate connections are strengthened and become proportional to withdrawal efficiency (Holmberg and Schouenborg 1996; Waldenstrom et al. 2003). Therefore, spinal sensorimotor circuits are malleable during development and input-output properties of cutaneous reflex pathways are modified by experience, which could explain why different responses are evoked in the same muscle with the same nerve stimulation in two separate animals. Differences in spinal sensorimotor circuits can also explain the wide range of changes in reflex pathways between individuals following lesions of varying kinds although this has not been explored.

Phase- and state-dependent modulation

A hallmark of cutaneous reflexes during locomotion is that they are modulated according to the phase of the step cycle but also with respect to the state [for extensive reviews see (Rossignol et al. 2006; Duysens et al. 2004; Zehr and Stein 1999)]. Phase- and state-dependent modulation is critical to ensure that inputs from the periphery are functionally and contextually relevant during locomotion.

Phase-dependent modulation

Modulating responses according to the phase of the step cycle is a requirement for the smooth progression of locomotion (Zehr and Stein 1999; Rossignol et al. 2006). For instance, if flexion of the limb was evoked during stance the animal would lose its balance and locomotion would be disrupted. Consequently, sensory inputs from the periphery must be gated throughout the step cycle.

The concept of phase-dependent modulation is well illustrated by reflex 'reversals' during locomotion. Reflex reversals occur when the same cutaneous stimulation delivered during different phases of locomotion recruits antagonist muscles (Forssberg et al. 1975; Forssberg et al. 1977; Andersson et al. 1978; Duysens

1977; Duysens and Pearson 1976; Duysens and Stein 1978; Forssberg 1979; Yang and Stein 1990; Duysens et al. 1980; Duysens et al. 1990). The first evidence of this came by stimulating the dorsum of the paw either electrically or mechanically in chronic spinal kittens (Forssberg et al. 1975). Stimulating the foot dorsum during swing increased flexor activity whereas the same stimulation during stance enhanced extensor activity, thus the term phase-dependent reflex reversal. Phase-dependent reflex reversals can also occur in crossed pathways. In cats, stimulating the skin on the lateral surface of the ankle or on the plantar foot of the left leg generated responses in the right medial gastrocnemius in most phases of the step cycle, however around the end of the stance phase of the right leg responses in MG, an ankle extensor, were abolished and responses in the right TA, an ankle flexor appeared (Duysens et al. 1980).

Reflex reversals have also been described as a change in the 'sign' of a response at different phases of locomotion; in other words, when excitatory responses replace inhibitory responses, or vice-versa, at the same latency in some phases of the step cycle. For example, in humans, the middle latency response (P_2: 80-120 ms) in the ankle flexor TA evoked by stimulating the Tib nerve during swing is excitatory whereas at the transition from swing to stance it becomes inhibitory (Yang and Stein 1990; Duysens et al. 1990; Duysens et al. 1992; Duysens et al. 1996). The presence of both inhibitory and excitatory responses at the same latency in TA is thought to stem from parallel inhibitory and excitatory pathways from the Tib nerve to TA motor units (De Serres et al. 1995).

State-dependent modulation

Responses are also modulated according to the 'state' of the spinal sensorimotor circuitry and for our purposes a 'state' will refer to a physiological mode (Burke 1999). For example, going from rest to walking requires the spinal sensorimotor circuitry to enter a different state. Different states can be created in

several ways during locomotion. For instance, walking and running represent different states as do forward and backward walking, and incline and decline walking. In order to be functionally relevant, the spinal locomotor network must enter different physiological modes requiring an appropriate modulation of cutaneous inputs. For example, contacting an obstacle with the dorsum of the foot during forward walking elicits a timed activation of several hindlimb muscles, involving activation of hip and knee flexors and extensors of the ankle, which allow the limb to be lifted over the obstacle (Forssberg 1979; Wand et al. 1980) The ankle flexor TA is briefly excited followed by a period of inhibition and a delayed prolonged excitation (Buford and Smith 1993; Prochazka et al. 1978; Wand et al. 1980). However, if the foot contacts an obstacle while walking backwards the same muscular activation would not be appropriate to lift the foot over the obstacle. Instead contacting an obstacle during backward walking initially evokes hip and ankle flexion with reduced knee flexion, which is followed by knee and ankle flexion. Extension of the limb then completes the backward swing phase (Buford and Smith 1993). Thus, in both forward and backward walking contacting an obstacle generates a movement that takes into account the nature (in this case the direction) of locomotion and produces an appropriate compensatory reaction.

Cutaneous reflexes are also modulated differently during quiet standing, walking, and running (Komiyama et al. 2000; Duysens et al. 1993). Generally cutaneous reflexes evoked by stimulating the skin of the foot are increased during running or walking compared to standing in humans (Duysens et al. 1993). Conversely, the soleus H-reflex, the electrical analogue of the stretch reflex, is reduced going from standing to walking to running (Zehr and Stein 1999) indicating that reflexes stemming from muscle and cutaneous receptors are controlled differently when switching from one state to another.

Performing lesions at various levels of the nervous system cord can also create different 'states' and recording cutaneous reflexes before and after lesions in

the same animal can provide important clues as to the organization and reorganization of different reflex pathways during locomotion (Burke 1999). Holmqvist and Lundberg studied changes in reflexes with lesions at different levels of the nervous system in anesthetized animals (Holmqvist et al. 1960; Holmqvist and Lundberg 1959) but to date only two studies have used this approach to determine changes in cutaneous reflexes in a locomotor condition (Bernard et al. 2007; Bretzner and Drew 2005a). Importantly, because reflex pathways can differ from one individual to another during locomotion (Loeb 1993), state-dependent modulation is better assessed by recording reflexes during locomotion in the same animal before and after a lesion.

Control of cutaneous pathways from the foot

During the step cycle cutaneous reflex responses can scale with the level of background EMG activity (Matthews 1986) but in several cases there is a clear dissociation indicating that pre-motoneuronal mechanisms gate the activity of cutaneous reflex pathways. Vertebrates, contrary to invertebrates (Sillar and Skorupski 1986) cannot gate the excitability of cutaneous pathways directly at the peripheral receptor. Instead, modulation of cutaneous reflexes is accomplished by groups of spinal interneurons within the dorsal horn that receive inputs from a variety of sources and are directly gated by the spinal locomotor CPG.

It has been elegantly demonstrated that sensory feedback and supraspinal inputs are not necessary for generating the basic locomotor rhythm (Brown 1911; Grillner and Zangger 1979; Grillner 1981), which is evoked by a spinal locomotor CPG. However, the spinal CPG is constantly bombarded with inputs from supraspinal and peripheral structures during normal locomotion and must be capable of controlling the efficacy of transmission in sensory pathways and descending commands and must be capable of processing that information on a moment-by-moment basis to select which inputs are most functionally relevant for ongoing

locomotion. Several studies have suggested that the spinal locomotor CPG directly gates the activity in cutaneous reflex pathways during fictive locomotion (Gossard et al. 1989; Gossard et al. 1990; Andersson et al. 1978; Schmidt et al. 1988; Schmidt et al. 1989; Moschovakis et al. 1991; Quevedo et al. 2005a) by controlling the activity of interneurons interposed in cutaneous reflexes through pre- and post-synaptic mechanisms.

Presynaptic inhibition of large myelinated cutaneous afferents in the dorsal horn is thought to be mediated by last-order interneurons that use gamma-aminobutyric acid (GABA) as its neurotransmitter (Eccles et al. 1963; Jimenez et al. 1987). These interneurons are located within laminae III and IV (Jankowska et al. 1981) and diminish the efficacy of neurotransmitter release from the pre-synaptic cell [for a review of this topic see (Rudomin and Schmidt 1999)]. A few studies demonstrated that the excitability of primary cutaneous afferent terminals was modulated during the fictive locomotor cycle (Baev 1980; Baev and Kostyuk 1982). To determine how these terminals were gated, intracellular recordings of primary cutaneous afferents from SP and Tib nerves were made during fictive locomotion (Gossard et al. 1989). Two peaks of primary afferent depolarization (PAD) were recorded during the fictive step cycle, a large one in the flexion phase and a smaller one during extension. The authors suggested that the CPG could diminish the excitability of cutaneous pathways during locomotion, particularly in the flexion phase, by activating presynaptic inhibitory mechanisms.

An elegant series of experiments was performed to investigate inhibitory mechanisms within cutaneous reflex pathways controlled by a spinal locomotor CPG in Xenopus embryos during fictive swimming (Sillar and Roberts 1988; Roberts and Sillar 1990; Sillar and Roberts 1992b; Sillar and Roberts 1992a; Li et al. 2002; Li et al. 2003b; Li et al. 2003a). Light tactile stimulation of the trunk or tail initiate or enhance swimming in Xenopus tadpoles by using a primitive reflex system that evokes contraction of the musculature on the opposite side (Clarke et al. 1984).

Several interneurons involved in this cutaneous reflex circuitry have been identified. For instance, cutaneous stimulation excites Rohon-Beard neurons that then monosynaptically excite commissural sensory pathway interneurons located in the dorsolateral spinal cord (*dlsc*) that project to and excite contralateral motoneurons (Roberts and Sillar 1990). These *dlsc* interneurons are phase-modulated (i.e. inhibited) during swimming (Sillar and Roberts 1992a; Li et al. 2001). The inhibition is mediated by glycine because it is blocked by strychnine, a glycinergic antagonist, whereas bicuculline, a GABA antagonist, had no effect (Sillar and Roberts 1992a; Soffe 1993). The interneurons mediating glycinergic inhibition are located more ventrally within the spinal cord and project monosynaptically to *dlsc* interneurons (Li et al. 2002). These interneurons control the activity in cutaneous reflex pathways via post-synaptic inhibitory mechanisms and could be part of the locomotor CPG. Thus, although post-synaptic inhibition is observed before the spinal cord is fully developed similar inhibitory mechanisms probably operate in the adult and could be conserved in higher vertebrates.

The efficacy of transmission in cutaneous reflex pathways can also be modulated by other peripheral afferents from muscle, joint, and cutaneous receptors (Eccles et al. 1963; Buss and Shefchyk 1999), the effects of which vary according to the phase of the step cycle by acting directly on other afferents or indirectly via the spinal locomotor CPG. In one study, stimulating SP or Tib nerves modulated the primary afferent depolarization (PAD), a measure of presynaptic inhibition, in Tib and SP primary afferents, respectively, during the fictive step cycle (Gossard et al. 1990). In both cases the strongest cutaneous-evoked PAD was observed during the extension phase. Because stance (i.e. the extension phase) is more dependent on sensory feedback from the periphery (Pearson 2007; Donelan and Pearson 2004), presynaptic inhibition of primary cutaneous afferents by other cutaneous inputs could serve to filter unimportant or counterproductive information while emphasizing more critical information (Schmidt 1971; Menard et al. 2002). Cutaneous inputs are also

very effective in modulating the excitability of other primary afferents during locomotion. For example, cutaneous inputs from the SP nerve can reduce the PBSt-evoked PAD in plantaris and LGS group I afferents throughout the fictive step cycle showing that cutaneous inputs can modulate the presynaptic inhibition mediated by muscle afferents (Menard et al. 2002). Sensory afferents of the same or different modalities are interconnected and a peripheral nerve lesion will no doubt influence the excitability of other reflex pathways.

Groups of interneurons interposed or projecting to cutaneous reflex pathways also receive inputs from supraspinal structures. Convergence of cortical inputs on cutaneous reflex pathways from the foot has been examined using the technique of spatial facilitation (Lundberg 1964) in anesthetised preparations at rest (Lundberg and Voorhoeve 1962; Lundberg et al. 1962; Pinter et al. 1982; Fleshman et al. 1988; Hongo et al. 1969; Baldissera et al. 1981) and during normal locomotion in the cat (Bretzner and Drew 2005b). In anesthetised preparations, cutaneous nerves are stimulated, which evokes a test (Te) volley and the resulting post-synaptic potential (PSP) is recorded in motoneurons. The test volley can be conditioned (C) by stimulating different supraspinal structures or other peripheral nerves at different conditioning-test (C-Te) intervals to assess convergence of C and Te volleys on motoneurons (Lundberg 1964). From these studies it was shown that stimulating the pyramidal tract (PT) or red nucleus (RN) facilitated sural nerve evoked short-latency EPSPs in several flexor motoneurons (Lundberg and Voorhoeve 1962; Hongo et al. 1969b; Baldissera et al. 1971; Pinter et al. 1982). Stimulating the PT and/or RN also facilitated short-latency EPSPs and longer-latency IPSPs evoked by sural nerve stimulation in MG motoneurons (Pinter et al. 1982) and those evoked by the SP nerve in flexor digitorum longus (Fleshman et al. 1988). However, despite these results, little is known as to the convergence of supraspinal inputs on interneurons interposed in cutaneous pathways during fictive locomotion.

The principle of spatial facilitation is similar in intact preparations except that cutaneous reflexes are recorded using EMG. In one study, cutaneous reflexes evoked by Saph, SP, or Tib nerve stimulation were conditioned by stimulating the hindlimb representation of the motor cortex during the swing phase of locomotion in otherwise intact cats (Bretzner and Drew 2005b). Stimulation of the contralateral motor cortex (the conditioning volley) modulated cutaneous reflex responses (the test volley), in several hindlimb muscles. Facilitation or depression could be elicited depending on the muscle, the cortical sites and the nerves stimulated as well as on the C-T interval used, indicating that cortical inputs converge on distinct interneuronal populations in the spinal cord implicated in cutaneous reflex pathways during locomotion. In humans, TMS has been used to study the effects of descending commands from the brain on cutaneous reflex pathways during walking (Keck et al. 1998; Pijnappels et al. 1998). Stimulating the leg representation of the motor cortex with TMS facilitated reflex responses evoked by sural nerve stimulation in TA. The facilitation varied according to the phase of the step cycle with the effect being greatest during early swing. Lesions to the spinal cord or peripheral nerves disrupt this control by generating adaptive (or maladaptive) changes in descending pathways and consequently the modulation of cutaneous reflexes is modified (discussed in more detail later on).

Therefore, interneurons interposed in cutaneous reflex pathways receive convergent inputs from different sources, which are gated by the spinal locomotor CPG that filters functionally important information from the skin during locomotion. The spinal CPG can control the activity of cutaneous reflex pathways through GABAergic presynaptic and glycinergic post-synaptic inhibitory mechanisms or by inputs acting on CPG elements, as is the case for group I inputs, which are modulated by monosynaptic, disynaptic and polysynaptic levels of the CPG (Rossignol et al. 2006).

The role of cutaneous afferents in locomotion

Potential functions of cutaneous afferents from the foot have been investigated using a variety of recording, stimulation and inactivation techniques and have provided much insight as to the role of inputs from the skin during locomotion.

Recordings of cutaneous afferent activity during locomotion at different levels of the nervous system

During locomotion contacting the ground activates a population of cutaneous mechanoreceptors of different types that transmit information to the spinal cord via cutaneous afferents coursing in different nerves. As forward progression continues different receptors become activated and signals to the spinal cord change. Walking on uneven terrain, in unpredictable environments, or unexpected perturbations also activates different receptors to varying degrees. The information from the skin of the foot entering the spinal cord depends on the number and types of cutaneous mechanoreceptors activated but as stated earlier upon entering the spinal cord this information is gated at all levels of the nervous system and by groups of spinal interneurons that are controlled by spinal and supraspinal networks [see also (Rossignol et al. 2006)].

Although the anatomical organization of cutaneous afferents and the particular information they transmit are relatively well known there is limited information regarding their activity during locomotion. To investigate discharge properties of single afferents, a floating fine wire electrode array was implanted in dorsal root ganglia of freely walking cats (Loeb et al. 1977; Loeb 1981). In one study, two cutaneous fibres from guard-hair-cell receptors of the foot were identified (Loeb et al. 1977). One unit signalled foot lift and contact whereas another unit was active during late swing. In another study, a cutaneous fibre was activated just before foot contact, presumably by stretching of the skin (Loeb 1981). Thus, even from scarce recordings, it is apparent that cutaneous mechanoreceptors of the foot signal

important events during the step cycle such as foot lift, foot contact and phase transitions. Cuff electrodes placed around whole nerves, such as Tib and SP, have also been used to record neural signals during locomotion in the cat (Popovic et al. 1993). A single peak of activity was observed in the Tib nerve when the animal contacted the ground while the SP nerve showed two peaks, a small peak when the paw contacted the ground and a larger peak when the paw was lifted off the ground. Therefore, activity in the Tib nerve, which innervates the plantar surface of the foot, is a good indicator of foot contact whereas activity in the SP nerve can signal foot lift. Neurons within different supraspinal structures with peripheral RFs on the skin of the paws also respond during specific phases of the step cycle with or without cutaneous stimulation (Chapin and Woodward 1982b; Palmer et al. 1985; Drew et al. 1996; Apps et al. 1995). For example, responses to cutaneous stimulation of SP and superficial radial nerves of most reticulospinal neurons are maximal during the swing phase, although some cells also discharge preferentially during stance (Drew et al. 1996). In the primary somatosensory cortex, about half of all recorded cells with cutaneous RFs on the plantar surface of the paw discharged strongly when the forepaw contacted the ground during unperturbed locomotion whereas the other cells showed weak or absent responses (Chapin and Woodward 1982a; Chapin and Woodward 1982b). With stimulation, responses were maximal just before and after foot contact in the cells that discharged at ground contact while in the group of cells that did not respond at ground contact facilitation was observed during early swing. In another study, large changes in motor cortical activity with stimulation of the plantar surface of the contralateral forelimb paw were produced during mid-swing while the lowest activity was observed during late swing and the first half of stance (Palmer et al. 1985). However, without stimulation the majority of motor cortical units with cutaneous RFs on the palmar surface of the paw were most active at the end or beginning of stance and swing, suggesting that these cells during unperturbed walking are involved in phase transitions (Palmer et al. 1985). In the cerebellum,

responses to cutaneous stimulation of the superficial radial nerve (i.e. dorsum of forepaw) are maximal just before contacting the ground (Apps et al. 1995), suggesting that this input to the cerebellum is important for foot placement.

Therefore, these results suggest that cutaneous inputs during unperturbed walking provide important cues related to foot contact, foot lift and phase transitions. It also appears that in every supraspinal structure separate groups of cells respond preferentially to perturbations during a specific part of the step cycle.

Inactivation and stimulation studies

As stated earlier in the introduction cutaneous inputs are not required for generating the basic locomotor pattern on a treadmill although there are subtle changes in the locomotor pattern if these inputs are removed or diminished (Bouyer and Rossignol 2003a; Varejao and Filipe 2007). Inputs from the skin of the foot, however, appear of paramount importance as task difficulty increases. After complete cutaneous denervation of the hindpaws, cats were unable to walk across a horizontal ladder, a task easily performed before denervation, because they could not properly place their feet on the rungs. After 3-7 weeks, cats recovered the ability to perform the horizontal ladder walk but they adopted a new strategy tending to grip the rungs with a claw-like shape of the paws. This indicated that cutaneous inputs from the feet normally provide the sensory cues necessary to adjust paw placement on a step-by-step basis during difficult locomotor tasks.

Perhaps the better know function of cutaneous afferents of the foot is in responding to perturbations. As stated earlier on, mechanical or electrical stimulation of cutaneous afferents from the foot evoked reflexes during locomotion. The activation of cutaneous reflex pathways enables the nervous system to make fast, appropriate, and reliable movements to compensate for changes in the environment. Mechanical stimulation of the foot dorsum during the swing phase of locomotion generates a coordinated reflex, the stumbling corrective reaction, in several leg

muscles bilaterally allowing the perturbed limb to progress over the obstacle during forward and backward walking in cats and humans (Forssberg 1979; Prochazka et al. 1978; Wand et al. 1980; Buford and Smith 1993; Zehr and Stein 1999; Schillings et al. 1996; Zehr et al. 1997; Lam et al. 2003; Eng et al. 1994; Buford and Smith 1993; Duysens et al. 1996). The response disappears if the skin of the foot is anaesthetized (Forssberg et al. 1977; Wand et al. 1980) and besides the SP nerve, stimulation of no other nerves of the foot or muscles at present can reproduce the complex pattern of responses characteristic of the stumbling corrective reaction (Quevedo et al. 2005a). Therefore, the main functions of cutaneous inputs from the foot during locomotion are to control placement of the foot on a step-by-step basis and to correct limb trajectory following a perturbation. Naturally, this also requires maintenance of equilibrium and several lines of evidence indicate that cutaneous inputs from the foot are intimately involved in static postural control and most probably in the postural control of locomotion.

The role of cutaneous afferents in posture

The act of walking can hardly be considered without the proper maintenance of balance and consequently the control of locomotion and posture is deeply intertwined. Visual, vestibular, proprioceptive, and exteroceptive information all contribute to postural control and the weighting of each source probably depends on the specific context (Peterka 2002; Horak and Macpherson 1996; Mergner and Rosemeier 1998; Morasso and Schieppati 1999). During locomotion the control system must be capable of 1) maintaining proper balance on a moment-by-moment basis (i.e. control of postural sway) and 2) effecting rapid postural responses following a perturbation (Misiaszek 2006). The role of cutaneous afferents from the foot in postural control have also been investigated using a variety of techniques such as stimulating or inactivating the skin or cutaneous nerves and have provided much

insight as to the role of cutaneous inputs for the proper maintenance of balance at rest and during locomotion.

Control of posture on a moment-by-moment basis

The role of cutaneous afferents on a moment-by-moment basis during quiet standing or locomotion has been investigated using a variety of inactivation techniques such as hypothermia (Magnusson et al. 1990; Perry et al. 2001; Thoumie and Do 1996), ischemic (Horak et al. 1990; Diener et al. 1984; Mauritz and Dietz 1980) and pharmacological (Meyer et al. 2004b) anesthesia of the feet. These techniques, however, vary in their ability to selectively target cutaneous sensation. In these studies subjects perform standing or walking tasks and changes in the centre of pressure (CoP), such as displacement and velocity, are measured during control and anesthetised conditions. Most studies in humans have shown that anesthetising the sole of the feet increases postural sway during quiet standing and changes the strategy of balance control (Kavounoudias et al. 1998; Horak et al. 1990).

In one series of experiments the foot sole was anesthetised using alternating-pulse iontophoresis of a lidocaine/epinephrine solution, which targets the end-organs of cutaneous receptors while leaving muscle and joints afferents intact (Meyer et al. 2004b). Balance, measured by shifts in the center of pressure (CoP) or mass (CoM), was tested during bipedal stance with eyes closed or opened, and unipedal stance with eyes open with and without anesthesia of the foot sole. Bipedal stance was unaffected with eyes open, but with eyes closed or during unipedal stance CoP velocity increased. Thus cutaneous afferents contribute to the maintenance of balance on a continuing basis but vision and probably other sensory signals (i.e. vestibular, proprioceptive) can compensate for the loss of plantar sensitivity in these relatively undemanding balance tests. It is also probable that inactivation studies underestimate the contribution of cutaneous afferents because in these instances the

control system would probably emphasize inputs originating from other receptors to maintain proper balance (Meyer et al. 2004a).

Direct simulation of the foot sole during quiet stance, which mimics motion of the CoP, was also used to study the contribution of cutaneous afferents to the control of posture (Kavounoudias et al. 1998; Kavounoudias et al. 2001; Maurer et al. 2001; Roll et al. 2002). For example, low amplitude high frequency mechanical vibration was applied to different regions of the foot soles in human subjects during quiet standing with eyes closed (Kavounoudias et al. 1998) or opened (Kavounoudias et al. 2001). In both cases, vibration applied to the soles produced a postural sway but the direction depended on the spatial pattern of stimulation. This led the authors to suggest that the foot soles are a "dynamometric map" that compute tactile information from various plantar areas to provide a sense of body position allowing the CNS to make proper postural adjustments.

Gait termination, which is the transient state before coming to a complete stop during walking, involves feed-forward and feedback mechanisms to control proper foot placement, the increase in the breaking force of the forward leg, and the reduction in the propulsive force from the trailing limb (Jaeger and Vanitchachavan, 1992). If the CoM moves outside the support base, a compensatory step is taken (Hase and Stein 1998). The role of cutaneous afferents from the foot sole in gait termination was assessed with or without vision (Perry et al. 2001). In this study, vision was blocked at gait termination with special glasses and foot sole sensation was occluded with hypothermic anesthesia. Whereas vision is involved in initially slowing the CoM when the signal for gait termination is given, cutaneous sensation from the foot soles appears important in slowing down the CoM immediately following contact Thus, proper foot placement is controlled by a combination of visual and cutaneous inputs that provide feed-forward information about CoM movement.

In addition, middle latency (80-120 ms) cutaneous reflexes from the foot are actively controlled when walking in an unpredictable environment (Haridas et al. 2005). In this study cutaneous reflexes were evoked by stimulating the Tib or SP nerves during different phases of the step cycle and under different walking conditions. Applying forward or backward pulls to subjects using a padded belt attached to the hips created an unpredictable environment. Reflexes were not evoked during the pulls but between them and subjects were informed prior to unstable walking sessions of the forthcoming perturbations. It was shown that cutaneous reflexes were modulated differently when walking in an unpredictable environment compared to normal walking indicating that feedforward mechanisms also control the excitability of cutaneous reflexes.

Control of posture following a perturbation

Following a perturbation, inputs from the periphery, such as those from the skin and muscles, provide sensory feedback that can rapidly induce postural changes or compensatory stepping to counteract the disturbance by simultaneously activating muscles throughout the body (Macpherson 1988; Henry et al. 1998; Perry et al. 2000). These automatic postural responses (APRs), which occur at latencies of 40-60 ms in cats and 80-120 ms in humans are tuned to the direction of the perturbation and do not require visual or vestibular inputs (Ting and Macpherson 2004). Somatosensory receptors in the periphery have been shown to mediate APRs because abolishing large diameter afferents in cats (Stapley et al. 2002) or peripheral neurophathies in humans (Bloem et al. 2002; Bloem et al. 2000; Inglis et al. 1994) increases the latency of APRs. In one study, pyridoxine (Vitamin B_6), which destroys large calibre sensory afferents but leaves the motor innervation and central nervous system neurons intact (Xu et al. 1989; Windebank et al. 1985), was injected in cats to investigate the nature of APRs following perturbations (Stapley et al. 2002). Cats were trained to stand on a force platform that could deliver linear translations in 12

equidistant directions in the horizontal plane and trigger APRs in recorded muscles (Macpherson et al. 1987). In intact cats (i.e. without pyridoxine), APR latencies ranged from ~40-65 ms but after pyridoxine injection latencies increased to 91-222 ms and perturbations evoked falls or stepping in nearly half the trials. Although the specific type of afferent destroyed by pyridoxine could not be ascertained, the study showed that large diameter afferents, such as those from skin and muscle, are involved in APRs and that without these rapid postural adjustments the animal falls frequently. Large afferents within the cutaneous saphenous nerve were particularly sensitive to pyridoxine.

Although muscle proprioceptors are often ascribed a more prominent role in APR production, Ting and Macpherson suggested that based on their own results and a more thorough examination of the literature that cutaneous receptors are also strongly involved (Ting and Macpherson 2004). For example, previous studies showed that postural EMG responses were similar when rotations or translations were in opposite directions, which produced diametrical changes in ankle angle, muscle length, and CoP excursions (Henry et al. 1998; Carpenter et al. 1999). It was thus concluded that muscle and joint proprioceptors in these particular instances could not provide the signal responsible for shaping the directional component of APRs (Ting and Macpherson 2004). To further address this, postural perturbations were induced using rotations and translations of the support surface that caused similar CoM displacements with respect to the feet but different initial passive kinematic and kinetic effects. Matched CoM destabilizing rotations and translations evoked similar APRs in EMG activity indicating that both types of perturbations use the same neural strategy stemming from a common input signal. Muscle and joint proprioceptors were eliminated as a potential source of APRs in this case because changes in kinematics, such as joint angles could not encode the direction of perturbation. The key variable shaping the directional tuning of the APRs was determined to be the initial change in ground-reaction force angle, which is the ratio of shear and loading

forces. The authors concluded that the most likely inputs responsible for detecting changes in force angle are cutaneous receptors in the foot sole, which can sense force and are directionally tuned (Birznieks et al. 2001; Kennedy and Inglis 2002; Morasso and Schieppati 1999).

Plasticity of cutaneous pathways

In previous sections it was shown that cutaneous afferent pathways receive convergent inputs from other peripheral afferents, from spinal networks, and from supraspinal structures. Consequently, it would be anticipated that removing or enhancing some of these inputs influences the activity of cutaneous reflexes at rest and during locomotion.

Inactivation studies

Injury to any component of the nervous system simultaneously impacts neural networks at multiple levels of the nervous system [for reviews see (Navarro et al. 2007; Kaas and Collins 2003; Wall et al. 2002; Frigon and Rossignol 2006b; Chen et al. 2002; Jones 2000).

The skin of the foot's well-defined somatotopic and laminar organization in the dorsal horn of the spinal cord has been used to investigate plastic reorganization of somatosensory inputs from the periphery to the central nervous system after peripheral nerve injury [for reviews see (Wilson and Kitchener 1996; McMahon 1992)]. For example, lesioning peripheral nerves that supply the skin of the foot leaves cells in the medial dorsal horn without receptive fields but within a few days to weeks after denervation cells in the medial dorsal horn can acquire new receptive fields (Wilson and Kitchener 1996). However, whereas some studies have reported that cells in the medial dorsal horn acquire new receptive fields located on the thigh, lower back, or perineum in the cat and the rat (Devor and Wall 1978; Devor and Wall 1981a; Devor and Wall 1981b) it appears that somatotopic reorganization is more

limited, involving only areas of skin adjacent to the denervated area [for a discussion of this topic see (Wilson and Kitchener 1996; Wilson and Snow 1987). For example, following denervation of the skin supplying digit 3 spinocervical tract neurons in the medial dorsal horn that normally had receptive fields on digit 3 were responsive to tactile stimuli on digits 2 and 4 only (Wilson and Snow 1987). Therefore, although limited, the somatotopic organization within the dorsal horn of the spinal cord is not fixed in the adult and can be modified following a peripheral nerve lesion.

Chronic recording and stimulation procedures have also been used to evaluate changes in cutaneous reflexes from the foot following peripheral nerve injury at rest (Valero-Cabre and Navarro 2002) and during locomotion (Bernard et al. 2007) in the adult nervous system. During locomotion, cutaneous reflex responses evoked by stimulating the left Tib nerve gradually increased in some muscles after cutting adjacent cutaneous nerves of the foot in the left hindlimb of cats (Bernard et al. 2007). The motor threshold required to elicit these responses, also decreased. Moreover, there was no evidence that the Tib nerve acquired new receptive fields after denervating adjacent cutaneous nerves, indicating that increased reflexes from the Tib nerve were not due to a reorganization of receptive fields within the dorsal horn. There is evidence that corticospinal efficacy is enhanced following cutaneous denervation of the hindpaw (Bretzner and Drew 2005a) but whether it is implicated in changes in cutaneous reflexes following peripheral nerve lesions is unknown. Therefore, transmission in cutaneous pathways from the plantar surface of the foot is increased after removing adjacent cutaneous inputs. It could also be the case that removing proprioceptive inputs by sectioning a mixed peripheral nerve enhances transmission in cutaneous reflex pathways because exteroceptive and proprioceptive information are known to have similar effects on the locomotor pattern (Guertin et al. 1995; Rybak et al. 2006). As a result the loss in one source of information (i.e. proprioceptive) could be compensated by an increase in another source (i.e. cutaneous) (Duysens and Pearson 1976).

Complete or partial lesions of the spinal cord alters descending commands from structures rostral to the lesion and considerably changes cutaneous reflex pathways [for reviews see (Frigon and Rossignol 2006b; Hultborn 2003)]. As with other reflexes, responses in motoneurons or muscles evoked by stimulating cutaneous nerve are greatly reduced in the early days following spinal lesions. However, following this period of 'spinal shock' reflexes increase and become exaggerated. For example, in acute spinal cord injury, short- and longer-latency responses evoked in triceps surae muscles by stimulating the sural nerve were very weak but over time these responses greatly increased in anesthetised cats (Baker and Chandler 1987b; Baker and Chandler 1987a). In humans, conditioning effects from sural (Lebizec et al. 1983) and SP (Levin and Chapman 1987) nerves on the soleus H-reflex differed between normal and spinal cord-injured subjects, whereas conditioning effects from the sole of the foot were similar (Fung and Barbeau 1994; Knikou and Conway 2001), indicating that not all cutaneous pathways are similarly impacted by spinal cord injuries. Reflexes in arm muscles evoked by stimulating cutaneous afferents in the leg (i.e. interlimb reflexes) are also modified following spinal cord injury. For instance, after cervical spinal cord injury interlimb reflexes, evoked by stimulating the Tib nerve in the popliteal fossa, become more prevalent and occur at slightly earlier latencies (Calancie 1991; Calancie et al. 2005; Calancie et al. 2002; Calancie et al. 1996) compared to intact human subjects (Zehr et al. 2001). These studies show that cutaneous transmission is modified following spinal cord injury and continues to evolve over time.

However, as stated earlier, one of the main drawbacks in making comparisons between intact and lesioned groups is that cutaneous reflexes differ from one individual to another at rest and during locomotion. Recording responses in the same animal before and after a spinal lesion provides a more precise evaluation of changes in reflex pathways. One study used chronic recordings to assess changes in cutaneous reflexes after spinal lesions at rest (Valero-Cabre et al. 2004). The Tib

nerve was stimulated to evoke responses before and after a complete T9 spinal transection in rats (Valero-Cabre et al. 2004). Based on different onset latencies three responses mediated by different types of afferents were recorded (C1: Aαβ, C2: Aδ, C3: C fibres) before and after spinalization in ipsilateral and contralateral flexors. In the immediate days following injury the ipsilateral C1 component was largely unaffected whereas C2 and C3 responses disappeared. At 14 days post-spinalization C2 and C3 reappeared while C1 markedly increased compared to pre-spinalization. The results showed that different components of cutaneous reflexes could be modified independently after spinal lesion. Cutaneous reflexes evoked in the same animal before and after a partial or complete spinal lesion have not been evaluated during locomotion and such a paradigm could provide important clues regarding the functional reorganization of spinal sensorimotor pathways following the loss of descending (and ascending) information from the brain and brainstem.

Stimulation studies

Cutaneous reflex pathways can also be modified using classical conditioning (Durkovic 1975) and repetitive stimulation procedures (McVea and Pearson 2007). In a series of experiments, flexion reflexes, measured as the tension developed by the TA muscle, evoked by stimulating the saphenous nerve (CS; conditioned stimulus) were conditioned by stimulating the SP nerve (US; unconditioned stimulus) to determine if spinal sensorimotor circuits can undergo classical conditioning in the cat (Durkovic 1983; Durkovic 1975). Paired stimulation of CS and US produced a rapid facilitation of CS-evoked flexor reflexes in acutely spinalized decerebrate cats (Durkovic 1975). Backward conditioning (i.e. US preceding CS stimulation) also produced a reflex facilitation (Durkovic and Damianopoulos 1986) and it was later shown that forward (i.e. CS preceding US stimulation) and backward conditioning involved different spinal reflex pathways (Onifer and Durkovic 1988). These studies showed that spinal sensorimotor circuits mediating cutaneous reflexes could undergo

resilient changes in synaptic efficacy. However, the extent of conditioned reflex facilitation appears to vary substantially from one animal to another, which probably stems from inter-individual differences in neural connections.

More recently, it was shown that repetitively contacting the dorsum of the foot during the swing phase of locomotion in intact cats with a wooden rod led to a long-lasting hyperflexion at the knee of the perturbed limb during swing that persisted when the impediment was removed (McVea and Pearson 2007). The effects could last for more than 24 hours in some cats. Changes were context-dependent because the increased hyperflexion was not maintained when cats walked on a level walkway or on a series of offset pegs. Descending signals from cortical regions of the brain were deemed necessary for the persistent hyperflexion because of the context-dependency and because decerebrate cats did not exhibit any long-lasting changes. However, although limb trajectories are similar in treadmill and overground walking these still are two different states and it is likely that the activated spinal sensorimotor circuitry differs in the two conditions. It remains to be determined if repeatedly contacting the dorsum of the foot during swing can generate similar results in complete spinal cats. There is ample evidence that spinal networks can undergo long-lasting functional changes following different paradigms.

Therefore, using different experimental protocols the somatotopy and the transmission in cutaneous reflex pathways can be modified at rest and during locomotion. Long-lasting changes in the functional connectivity and strength of transmission within cutaneous reflex pathways from the foot could be an important compensatory mechanism for the recovery of locomotion following injury.

Cutaneous pathways and functional recovery of locomotion following spinal cord injury

As stated earlier, cutaneous pathways are altered following lesions to peripheral nerves or to the spinal cord. However, to what extent these changes are

implicated in the functional recovery of locomotion is unclear. Increases in cutaneous pathways could be critical in compensating for the loss of other peripheral nerves, as shown by (Bernard et al. 2007) but also following spinal cord injury.

It has been well established that adult cats can recover the ability to walk following a complete transection of the spinal cord (Barbeau and Rossignol 1987; Lovely et al. 1990). Treadmill locomotor training (Edgerton et al. 2004; Barbeau and Rossignol 1987; de Leon et al. 1998) and the administration of different pharmacological agents, such as clonidine (Barbeau et al. 1987; Chau et al. 1998b; Chau et al. 1998a), can facilitate the expression of spinal locomotion in adult cats. In cats and humans, locomotor treadmill training after spinal cord injury is thought to provide appropriate sensory cues consistent with walking (Frigon and Rossignol 2006b; Maegele et al. 2002; Rossignol et al. 2002; Rossignol 2000; Harkema 2001) and it is highly probable that inputs from the skin are crucial in the recovery process. For instance, completely denervating the paws in cats in the intact state elicits subtle changes in the locomotor pattern but in general locomotion is not greatly affected. However, after a complete transection of the spinal cord at the 13th thoracic segment (T13) these same cats permanently lose the ability to place the feet on the plantar surface despite several weeks of locomotor training (Bouyer and Rossignol 2003b). In normal spinal cats (i.e. without a denervation) plantigrade paw placement takes approximately 2-3 weeks to be fully expressed (Barbeau and Rossignol 1987; Belanger et al. 1996). If a small amount of cutaneous input (i.e. the cutaneous branch of the deep peroneal nerve) is left intact before spinalization, the ability to place the paw on the plantar surface will recover during spinal locomotion. Injecting local anesthetics into the cutaneous receptive field of the deep peroneal nerve abolished paw placement. In a similar vein, if a partial cutaneous denervation of the hindpaw is performed after spinalization (i.e. in the spinal state), proper plantar foot placement recovers but once the denervation is completed the ability to place the foot properly is permanently lost (Bouyer and Rossignol 2003b). Therefore, at least some

information from the skin of the foot is required for the expression and control of foot placement during spinal locomotion.

Treadmill locomotor training has also been used in human patients following spinal cord injury to restore walking ability (Wernig et al. 1998; Wernig et al. 1999; Wernig et al. 1995; Wernig and Muller 1992; Barbeau and Rossignol 1994; Fung et al. 1990; Wainberg and Barbeau 1985). In one paper, it was briefly reported that barefoot walking, which undoubtedly enhances cutaneous feedback from the sole of the foot, facilitated walking during the first few weeks of locomotor training in spinal cord-injured patients (Wernig and Muller 1992) but this was not discussed further. This suggested that providing cutaneous feedback in the recovery process following spinal lesions could ameliorate the recovery of locomotion after spinal lesions. To address this, the recovery of walking and swimming following partial spinal lesions was assessed in hatchling chicks to evaluate the contribution of cutaneous feedback in the recovery of locomotion (Muir and Steeves 1995). Because walking improved more rapidly than swimming the authors hypothesized that during walking the spinal CPG receives cutaneous and proprioceptive inputs related to foot contact and limb loading, which facilitates recovery, whereas during swimming inputs are primarily proprioceptive from the moving limbs. Moreover, providing a cutaneous stimulus to the foot during the extension phase of swimming in the days following the partial spinal lesion facilitated the recovery in hatchling chicks (Muir and Steeves 1995) and in a more recent study, in rats (Smith et al. 2006). Cutaneous stimulation may boost the general excitability of spinal circuits but could also provide a critical input to the extensor half of the locomotor CPG to regulate the extension phase whereas stimulation of the perineal region in cats, which facilitates the expression of spinal locomotion, is thought to provide a non-specific excitability to the spinal locomotor CPG (Barbeau and Rossignol 1987).

Cutaneous inputs can also control the excitability of other reflex pathways after spinal lesions. For example, after spinal cord injury the phase-dependent

modulation of H-reflexes found in intact subjects is lost (Yang et al. 1991). However, stimulating the medial plantar surface of the foot in incomplete spinal cord-injured patients during walking partially restored the normal modulation of soleus H-reflexes (Fung and Barbeau 1994) but whether this correlated with a better walking performance was not tested. Stimulating cutaneous nerves or mechanoreceptors of the foot can also prevent the loss of motor and/or sensory functions following injury. For instance, intermittent stimulation of cutaneous mechanoreceptors of the foot sole in rats, with the use of an inflating/deflating balloon, minimized atrophy and loss of strength of the soleus muscle during 14 days of hindlimb unloading (De Doncker et al. 2000) . This has important implications not only for prolonged unloading of the legs, such as spaceflight, but also on conditions that produce substantial muscular atrophy, such as spinal cord injury (Giangregorio and McCartney 2006), which no doubt interferes with locomotor recovery. Chronic stimulation of the Tib nerve or the skin of the foot sole by itself or in conjunction with stance training could strengthen muscles throughout the legs and facilitate the recovery of locomotion after injury.

Activity-dependent processes undoubtedly shape transmission in spinal sensorimotor pathways [for an excellent review see (Wolpaw and Tennissen 2001)]. Evidently, lesions to peripheral nerves and/or the spinal cord induce plastic changes in cutaneous reflex pathways but it is probable that some of these changes are detrimental. Consequently, normalizing or shaping the excitability of cutaneous reflex pathways with appropriate training is thought to promote recovery of motor functions (Cote and Gossard 2004; Cote et al. 2003; Frigon and Rossignol 2006b). In one study, the effects of locomotor training on changes in cutaneous reflex pathways from the foot after complete spinalization were assessed during fictive locomotion (Cote and Gossard 2004). One group of cats received treadmill training until a stable spinal locomotion with full hindquarter support was expressed (trained group) whereas in another group, cats were spinalized but not trained (untrained group). The effects of stimulating the CCS, MPL, and SP nerves on multiple hindlimb muscles were

evaluated at rest and during fictive locomotion in both groups 3-5 weeks after spinalization. Step training modified the activity in some cutaneous reflex pathways, particularly from the MPL to MG, an ankle extensor. In fact, plasticity in cutaneous pathways from the foot was more robust than in group I pathways from muscle receptors in the same cats (Cote et al. 2003).

Therefore, cutaneous inputs appear to be of particular importance for the recovery and proper expression of locomotion following spinal cord injury and making use of cutaneous pathways in conjunction with appropriate and specific training could greatly facilitate the recovery process.

Summary

To summarize it is now clear that information from the skin is important in the control of locomotion (i.e. foot placement, obstacle avoidance) and postural aspects of locomotion. Cutaneous reflexes can be used to probe the functional reorganization of spinal sensorimotor pathways following injury to different neural structures. It is likely that a functional reorganization of the cutaneous reflex circuitry is critical for the recovery of locomotion after lesions to peripheral nerves and/or to the spinal cord. Cutaneous pathways are ideally suited to mediate the functional recovery of locomotion following injury to the nervous system because they diverge extensively contacting several motor pools and exert powerful influences on the locomotor pattern.

Issues to consider

As we saw in the introduction, cutaneous inputs are involved in the control of locomotion but key questions remain. Does cutaneous feedback participate in the recovery of locomotor functions following lesions to the spinal cord and/or peripheral nerves? Is the normal phase-dependent modulation of cutaneous reflexes retained following lesions? Does the spinal cord have intrinsic mechanisms that can mediate

changes in cutaneous reflex pathways following spinal and peripheral nerve lesions or are descending supraspinal inputs involved or required? Are changes in cutaneous reflex pathways after a complete spinal transection dependent on past experiences (i.e. a lesion before spinalization)? Are there cutaneous reflex pathways left to uncover during intact locomotion in the cat? The following chapters report experiments aimed at answering these questions and should add to our understanding of the functional organization and reorganization of cutaneous reflexes during locomotion after spinal and/or peripheral nerve lesions.

In all studies described in the following chapters, reflexes were initially evoked and recorded in the intact state during locomotion because as discussed in the introduction, cutaneous reflexes differ from one cat to another, which limits the amount of information that can be drawn from making comparisons between groups of intact and groups of lesioned cats.

Question 1: Is cutaneous feedback increased following a muscle denervation in the intact state?

Recently, it was shown that cutaneous reflexes, evoked by stimulating the Tib nerve during locomotion, were increased in the days following denervation of adjacent cutaneous nerves of the foot, indicating that remaining cutaneous inputs can be enhanced following the loss of sensation from the skin (Bernard et al. 2007) but could cutaneous inputs also compensate for the loss of proprioceptive inputs during locomotion? To address this question, reflexes evoked by stimulating the Tib nerve at the ankle were recorded during locomotion in the same cat before and after sectioning the left LGS nerve (Chapter 2). Cutaneous and proprioceptive inputs are thought to have complementary roles in the control of locomotion (Prochazka 1996) and stimulation of the Tib nerve or group I afferents from ankle extensors have similar effects on the fictive locomotor pattern (Guertin et al. 1995). Consequently,

the loss of proprioceptive inputs from ankle extensors could lead to an increase in the transmission in cutaneous afferents of the Tib nerve.

Question 2: Is cutaneous feedback increased following a muscle denervation in the spinal state?

There is evidence that supraspinal mechanisms are involved in the locomotor compensation following a peripheral nerve lesion (Bretzner and Drew 2005a) and that adaptive mechanisms share similarities in the intact and spinal states following a muscle or cutaneous denervation (Bouyer et al. 2001; Bouyer and Rossignol 2003b; Carrier et al. 1997), which suggests a strong spinal contribution. However, the isolated spinal cord has a limited adaptive capacity following peripheral nerve lesions because plantar paw placement is lost following a complete cutaneous denervation in the spinal state whereas in the intact state placement of the foot is only temporarily affected (Bouyer et al. 2003). Thus, supraspinal structures can select other cues following a cutaneous denervation in the intact state. In chapter 3, we performed a lesion of the left LGS but in the spinal state (i.e. after spinalization) to determine if adaptive mechanisms (i.e. changes in cutaneous reflexes and locomotor bursts) are similar or different to those found in the intact state (i.e. Chapter 2). Similar changes would indicate that mechanisms intrinsic to the spinal cord are important or sufficient in effecting reflex changes whereas differences would suggest that supraspinal inputs are also involved.

Question 3: How are cutaneous reflexes reorganized during locomotion following a complete spinal transection?

The re-expression of locomotion after complete transection of the spinal cord involves a functional reconfiguration of spinal sensorimotor circuits during fictive locomotion, which are shaped by activity-dependent processes (Cote and Gossard 2004; Cote et al. 2003), but no studies have specifically addressed this by recording

reflexes in the same animal before and after spinalization in a locomotor condition. It is unknown whether there is a general increase in reflexes after spinalization, as is the case at rest, or if cutaneous reflexes retain a phase-dependent modulation with changes being most prominent in parts of the step cycle where they could be most functionally relevant for the expression of spinal locomotion. Therefore, Tib nerve reflexes were evoked in the intact state and after obtaining stable recordings a complete spinalization was made (Chapter 4). Cats were then trained on a motorized treadmill and recordings once a stable spinal locomotion was expressed.

Question 4: What is the consequence of performing a partial denervation of ankle extensors before a complete spinalization on the expression of spinal locomotion?

A few studies, using different peripheral denervations, have shown that the expression of spinal locomotion is influenced if a peripheral nerve lesion is performed before the spinalization (Bouyer and Rossignol 2003b; Carrier et al. 1997). The reasons for this are unclear. It could be that sensory feedback from the periphery provides a non-specific excitability to the spinal locomotor network that is required for the normal 'development' of spinal locomotion. However, this does not explain why deficits such as the inability to place the foot, and hyperflexions of the denervated limb are respectively observed during spinal locomotion when cutaneous (Bouyer and Rossignol 2003b) and ankle flexor (Carrier et al. 1997) nerves were lesioned in the intact state. In Chapter 5, we investigated some of the underlying mechanisms by recording reflexes before and after spinalization in cats that had a denervation of the left LGS before spinalization (i.e. cats used in chapter 2), to determine if the functional reorganization of spinal sensorimotor pathways following spinalization is the same as in non-denervated cats (i.e. Chapter 4).

Question 5: Have responses evoked by stimulating cutaneous nerves been overlooked in the literature?

Over the course of the studies described in Chapters 2-5 we examined reflex responses during locomotion in the intact state in multiple hindlimb muscles bilaterally. It was found that excitatory responses, evoked by stimulating the Tib or SP nerves, in contralateral ankle extensors were frequently preceded by a short period of inhibition. Crossed inhibition has been described in anesthetized cats (Aggelopoulos et al. 1996; Edgley and Aggelopoulos 2006; Curtis et al. 1958) but not during a locomotor condition. Thus, we decided to investigate these responses in more detail because crossed inhibition could be an important mechanism for interlimb coordination by adjusting the timing of locomotor bursts bilaterally following a perturbation (Chapter 6).

Chapter 2 - Article 1. Plasticity of reflexes from the foot during locomotion after denervating ankle extensors in intact cats

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Abstract

Although sensory feedback is important in regulating the timing and magnitude of muscle activity during locomotion few studies have evaluated how it changes after peripheral nerve lesions. To assess this, reflexes evoked by stimulating a nerve before and after denervating other nerves can be quantified to determine changes. The aim of this study was to investigate consequences of denervating ankle extensor muscles, the lateral gastrocnemius and soleus (LGS), on reflexes from the plantar foot surface evoked by stimulating the Tibialis (Tib) nerve. Three cats (n = 3) were trained to walk on a treadmill and chronically implanted with electrodes in 14 hindlimb muscles bilaterally to record electromyographic (EMG) activity. A stimulating cuff electrode was placed around the left Tib nerve (Tib) nerve at the ankle to evoke reflexes. Several control values of EMGs, limb kinematics, and Tib nerve reflexes were obtained during locomotion for at least three weeks before the left LGS nerve was cut. We found that the locomotor EMG bursts of several muscles was altered, with a large increase in amplitude in the early days post-neurectomy followed by a gradual decrease towards intact values later on. There were changes in the stimulated locomotor EMG bursts (Tib nerve reflexes) of ipsilateral flexors and extensors and of contralateral ankle extensors, which dissociated from changes in baseline locomotor EMG (e.g. non-stimulated bursts during reflex trials). The functional significance of these changes in muscle activity and reflex pathways on the recovery of locomotion after denervating ankle extensors is discussed.

Keywords: plasticity, reflex, locomotion, neurectomy

Introduction

The nervous system is capable of considerable adaptive plasticity following peripheral nerve lesions (Rossignol 2006). For instance, cats with muscle nerve sections demonstrate initial deficits during locomotion but recover relatively rapidly, highlighting the remarkable ability of the nervous system, and even the isolated spinal cord, to quickly compensate for these losses (Pearson et al. 1999; Bouyer et al. 2001). As a result, denervations have been used to study compensatory mechanisms within the nervous system involved in offsetting the loss of motor and/or sensory nerves during locomotion (Bouyer et al. 2001; Pearson et al. 1999; Carrier et al. 1997; Bouyer and Rossignol 2003b; Bouyer and Rossignol 2003a; Wetzel et al. 1973; Whelan et al. 1995; Abelew et al. 2000; Cope et al. 1994).

To investigate this adaptive plasticity Pearson and colleagues, in a series of seminal experiments, performed lesions of mixed nerves that innervate some ankle extensors [lateral gastrocnemius (LG), soleus, plantaris] (Misiaszek and Pearson 2002; Pearson et al. 1999; Pearson and Misiaszek 2000; Pearson et al. 2003). In the days following the ankle extensors neurectomy, an increased ankle flexion (yield) during early stance and a decrease in maximal ankle extension at end stance were observed on the denervated side during locomotion. With time these deficits returned towards intact values, which was attributed to an increased activity of the medial gastrocnemius (MG), the primary remaining ankle extensor (Pearson et al. 1999). Functional recovery was not associated with the release of trophic factors from the lesioned nerve since injecting botulinum toxin into the LG, soleus, and plantaris, preventing transmission across the neuromuscular junction without damaging the nerve, produced similar deficits and an increased MG activity (Misiaszek and Pearson 2002). Moreover, functional recovery was use-dependent since cats whose denervated leg was immobilized for six days after the denervation did not have an increased MG activity and ankle yield resembled non-immobilized cats once the splint was removed (Pearson et al. 1999). Large calibre afferents were deemed indispensable since performing a similar neurectomy in pyridoxine-treated cats, which is thought to selectively and permanently destroy large sensory afferents,

prevented ankle yield from returning to normal and MG activity was either unaffected or changed slightly (Pearson et al. 2003). Thus, large sensory afferents from the moving denervated leg are required for recovery.

Indeed, modified sensory feedback has been demonstrated after partially denervating ankle extensors (Fouad and Pearson 1997; Whelan et al. 1995; Whelan and Pearson 1997). For instance, MG group I afferents had enhanced transmission to interneurons within the intermediate nucleus of lumbar segments L6/L7 (Fouad and Pearson 1997) and stimulating the MG nerve at group I strength had an accrued effectiveness in prolonging the stance phase (Whelan et al. 1995; Whelan and Pearson 1997) in decerebrate cats after partially denervating ankle extensors. It was thus hypothesised that transmission in group I reflex pathways from MG increased to reinforce the activity of this muscle after an ankle extensors neurectomy. This hypothesis was also largely based on the observations that additional stretch (e.g. increased ankle yield) and loading (e.g. increased force production) imposed on MG after the neurectomy would generate greater feedback from group Ia and Ib afferents, respectively, and because the post-contact EMG of MG, thought to be mediated in part by sensory feedback (Gorassini et al. 1994; Hiebert and Pearson 1999), increased in the early days post-neurectomy (Pearson et al. 1999). However, when tested more directly it was shown that MG group I pathways did not reinforce MG activity after denervating its close synergists. For example, the magnitude of homonymous and heteronymous group Ia excitatory post-synaptic potentials recorded intracellularly in MG motoneurons were unchanged within 1 week of sectioning the LG-soleus (LGS) nerve (Fouad and Pearson 1997; Whelan et al. 1995). Furthermore, the increased MG activity recorded in spinal cats during locomotion did not parallel changes in MG muscle length after denervating the LGS, signifying that enhanced group Ia feedback did not mediate the increase (Bouyer et al. 2001). In another study, increased amplitude of stretch reflexes scaled with changes in the activity of MG after a LGS neurectomy, indicating that the increase in stretch reflex amplitude was due to an increased motoneuronal activity and not mediated by increased fusimotor drive or Ia afferent transmission (Gritsenko et al. 2001). Therefore, changes in MG group I

reflex pathways do not appear to reinforce MG activity and are probably involved in another aspect of functional recovery after an ankle extensors neurectomy.

However, since sensory feedback is critical to functional recovery it could be that several reflex pathways are modified after partially denervating ankle extensors. In the present study, to evaluate whether changes occur in other reflex pathways, we stimulated the Tibialis (Tib) nerve at the ankle, which evokes responses in multiple hindlimb muscles during locomotion (Duysens and Stein 1978; Abraham et al. 1985; Loeb 1993; Pratt et al. 1991), before and after a LGS neurectomy. The Tib nerve was chosen since denervating ankle extensors increases the amplitude and duration of the yield at the ankle and reduces maximal ankle extension at end-stance (Pearson et al. 1999), in effect modifying how the plantar surface of the paw interacts with the ground during locomotion. It would thus be anticipated that transmission in reflex pathways from pressure-sensitive afferents of the paw would be altered. Moreover, it has recently been shown that Tib nerve reflexes are enhanced following denervation of other skin afferents supplying adjacent cutaneous territories of the paw (Bernard et al. 2007), indicating that a remaining cutaneous nerve can alter its activity to compensate for the sensory loss of other skin inputs. Since proprioceptive and cutaneous afferents are thought to have complementary roles during locomotion it is possible that the loss or reduction in one leads to an increase in the other (Duysens and Pearson 1976). Increased transmission in cutaneous afferents from the Tib nerve could offset the loss of proprioceptive information incurred by sectioning the LGS nerve. Consequently, we hypothesized that Tib nerve reflexes would be modified following the loss of ankle extensors to promote functional recovery. Preliminary results have been published in abstract form (Frigon et al. 2006).

Methods

Animals and general procedures

Three adult cats of either sex (weight 3.0-7.0 kg) were first selected based on their ability to walk for prolonged periods on a treadmill and trained for a few weeks at their preferred speed (0.35-0.5 m/s). Cats were subsequently implanted with

chronic electrodes for EMG recordings and nerve stimulation, allowed to recover from the implantation, and baseline values of EMGs, Tib nerve reflexes, and kinematics were then recorded. After stable control data were obtained, a neurectomy of the left LGS nerve was performed and recordings resumed, at the same treadmill speed, 24 hours later and at different times thereafter to plot changes over time. The number of days studied after the neurectomy differed between cats (cat 1 = 43 days; cat 2 = 116 days; cat 3 = 54 days). A total of 78 recording sessions were made.

The experimental protocol was in accordance with the guidelines of the animal Ethics Committee of the Université de Montréal. All surgical procedures were performed under general anesthesia and aseptic conditions. Prior to surgery, cats were injected with an analgesic (Anafen 100 mg; subcutaneously) and pre-medicated (Atravet 0.01 ml/kg, Glycopyrrolate 0.05 ml/kg, Ketamine 0.1 ml/kg; intramuscularly). Cats were then intubated and maintained under gaseous anesthesia (isoflurane 2%) while heart rate and respiration were monitored. After surgery, an analgesic (Buprenorphine 0.05 ml/kg) was administered subcutaneously. An oral antibiotic (cephatab or apo-cephalex, 100 mg/day) was given for the 10 days following surgery.

EMG

Since Tib nerve stimulation during locomotion evokes responses in multiple hindlimb muscles, chronic electromyographic (EMG) electrodes were implanted bilaterally in the following: semitendinosus (St: knee flexor/hip extensor), anterior part of sartorius (Srt: hip flexor/knee extensor), vastus lateralis (VL: knee extensor), lateral gastrocnemius (LG: ankle extensor/knee flexor), medial gastrocnemius (MG: ankle extensor/knee flexor), soleus (ankle extensor), and tibialis anterior (TA: ankle flexor). Recording from a large subset of muscles enabled us to determine if a LGS neurectomy generated changes in several muscles or was limited to synergistic ankle extensors. A pair of Teflon-insulated multistrain fine wires (AS633; Cooner wire, Chatsworth, CA) was directed subcutaneously from head-mounted fifteen pin

connectors (Cinch Connectors; TTI Inc., Pointe-Claire, Canada) and sown into the belly of each muscle for bipolar EMG recordings. The neurectomy was performed several days following chronic electrode implantations to ensure that EMG recordings were consistent for a prolonged period. Over the course of the study some EMG recordings were lost in each cat due to some unknown reasons (cat 1: right MG; cat 2: left Srt, left VL; cat 3: left MG, right TA) as determined by the disappearance of EMG signals. EMG was bandpass filtered (100-3000 Hz) and amplified (gains of 0.5-50K) using two Lynx-8 amplifiers (Neuralynx, Tucson, Arizona). EMG data were digitized (5000 Hz) using custom-made acquisition software. Locomotor EMG bursts were recorded during non-stimulated trials to compare changes in muscular activity before and after the neurectomy. Burst duration, amplitude, and timing relative to foot contact of locomotor EMG were calculated using custom software. Normalized mean amplitude was defined as the area under the rectified EMG burst divided by its duration and expressed as a percentage of the average control value. The EMG amplitude for extensors of both hindlimbs were also divided into two periods similar to a previous study (Pearson et al. 1999). The first period comprised the initial 120 ms of the burst, and the second included the entire integrated area following ground contact. Kinematic and EMG data were synchronized using an SMPTE time code generator (Evertz, time code master 5010).

Neurectomy

After establishing stable control (EMG bursts and Tib nerve reflexes) recordings during locomotion ($\geq$ 33 days) the left LGS nerve was cut by carefully separating the nerve from its surrounding tissue in the popliteal fossa (Figure 1). Capping the proximal end with flexible vinyl polysiloxane (Reprosil; Dentsply International, Milford, DE) was used to prevent nerve regeneration. No sham operations were included in this study since it was shown (Pearson et al. 1999) that using similar surgical procedures but leaving the LGS nerve intact did not produce deficits in locomotion. Contrary to a previous study (Pearson et al. 1999) but like

others (Bouyer et al. 2001; Gritsenko et al. 2001) the plantaris nerve was left intact to minimize the damage done and because both types of denervations produce similar deficits. The anaesthesia and surgery for the neurectomy lasted under an hour and cats were tested the next day to allow sufficient recovery. Post-mortem analysis confirmed that the LGS nerve was cut and did not regenerate in each cat.

Kinematics

Kinematics of the left hindlimb were captured during treadmill locomotion using a Panasonic digital 5100 camera (1/1000 s shutter speed, 30 frames/s or 60 de-interlaced fields/s = time resolution of 16,7ms) and a Sony RDR-GX315 DVD recorder. Small reflective markers were placed over prominent bony landmarks at each joint of the left hindlimb including the iliac crest, greater trochanter, lateral epicondyle, lateral malleolus, metatarso-phalangeal (MTP) joint, and the tip of the fourth toe. Joint angles (hip, knee, ankle, MTP) were reconstructed off-line using custom-made software with a resolution of 60 fields/s from 10-20 step cycles.

Tibial nerve stimulation

A chronic stimulating electrode composed of bipolar wires (AS633; Cooner wire, Chatsworth, CA) embedded in a polymer (Denstply International) cuff (Julien and Rossignol 1982) was placed around the left Tib nerve at the ankle adjacent to the Achilles' tendon (Figure 1). The Tib nerve was stimulated (Grass S88 stimulator) at varying intensities using constant current through an isolation unit during locomotion with a single 1 ms pulse at a constant time (100 ms after onset of St burst) to determine the threshold for obtaining a small yet consistent short-latency (~ 10 ms) response in TA. Stimulation current was then set at 1.2 times this threshold to evoke consistent yet non-noxious reflexes in several hindlimb muscles. During testing sessions, a stimulus was given once every three cycles at pseudo-random times to elicit responses at different epochs of the step cycle for a total of approximately 120 stimulations. Once reflexes were qualitatively and quantitatively reproducible from one session to another for a few weeks the same stimulation current was used for the

remainder of the study and assumed to remain constant (see discussion). Reflexes were evoked before and at different times after the LGS neurectomy. M-wave amplitude from intrinsic foot muscles innervated by the Tib nerve could have been recorded and maintained at a similar size throughout the study to confirm the consistency of stimulation. However, since we were interested in how reflexes from the foot change as a result of altered interactions between the paw and the surface of the treadmill we did not want to add any extraneous factors related to implanting small intrinsic foot muscles.

The methodology for quantifying reflexes is outlined in Figure 2. The EMGs were grouped into stimulated or control (non-stimulated) trials. The step cycle was divided into 10 phases by synchronizing the cycle to the onset of a flexor burst (St). Averaged responses of EMGs with or without stimulation during reflex testing were separated into these 10 bins according to the time they were evoked in the cycle. An average of at least 50 non-stimulated cycles provided a template of baseline locomotor (blEMG) during the step cycle, and from the 120 stimulations approximately 10 reflex responses were grouped in each of the 10 phases. This provided about 10 reflexes in each of the 10 phases superimposed on the blEMG. Onset and offset of reflexes, delineated as a prominent negative or positive deflection away from the blEMG, were determined using pre-defined latencies as guidelines (Duysens and Stein 1978; Abraham et al. 1985; Loeb 1993; Pratt et al. 1991) such as P1 or N1 (~8-10 ms) and P2 ($\geq$ 25 ms), where "P" and "N" denote positive (excitation) and negative (inhibition) responses, respectively. For extensors, onsets and offsets were manually determined since P2 latency varied at different phases of the step cycle (see Figure 2), whereas for ipsilateral flexors, time windows were fixed: St: P1 = 10-25 ms, P2 = 25-55 ms; TA: P1 = 10-30 ms, P2 = 30-55 ms). To measure reflex amplitude, the EMG from onset to offset was rectified and integrated and divided by the blEMG occurring in the same phase of the step cycle. This enabled us to compare changes in reflexes after the neurectomy independent of changes in the level of EMG activity (Matthews 1986; Duysens et al. 1993). To illustrate the phase-dependent modulation of reflexes during the step cycle, reflexes

for all days are expressed as a percentage of the maximal control value occurring in one of the 10 phases.

Statistics

To determine statistical differences after the LGS neurectomy, data from 3 separate control trials were pooled and compared with data recorded on each of the many post-neurectomy days using a one-way ANOVA followed by Dunnett's post hoc test for many comparisons against a control group if significant changes were detected by the ANOVA (Bouyer et al. 2001). Reflexes were analysed identically except that each response in each phase of the step cycle was treated independently. For example, N1 responses evoked in the fifth bin of the step cycle in the left MG in the intact state were compared against N1 responses evoked in the fifth bin of the step cycle in the left MG for each and every day after the neurectomy.

Results

Reflexes evoked by stimulating the Tib nerve, were used to determine if sensory feedback from the foot is altered after a LGS neurectomy. Changes in locomotor EMG bursts and reflexes were recorded during locomotion in several hindlimb muscles before and after the neurectomy. It was found that both EMG and Tib nerve reflexes were altered during locomotion post-neurectomy in multiple muscles and that changes in reflexes dissociated from those in the EMGs, indicating a modified gain of reflex pathways.

Locomotor EMG of several hindlimb muscles

Figure 3 shows rectified locomotor EMG bursts for selected muscles in the 3 cats in the intact state and at 2, 14, and 40-45 days post-neurectomy. As can be seen, the EMG of several hindlimb muscles in all cats was increased after the neurectomy while others were largely unaffected. In most cases, changes peaked at 2 days post-neurectomy before returning to intact values thereafter. For example, large increases in EMG for MG occurred 2 days after the neurectomy before returning towards intact

values (cats 1 and 2). Substantial increases in muscle activity could also be observed for other extensors including the left VL (cats 1 and 3) and the right VL (cat 1). More modest increases in EMG were also seen 2 days after the neurectomy in contralateral ankle extensors (cat 2). Changes in locomotor EMG were also apparent for some ipsilateral flexors such as the St, with the occurrence of an additional burst during stance (cats 1 and 3), and in TA at 2 days post-neurectomy (cats 2 and 3).

Figure 3 also shows that within a step cycle the time structure of the locomotor pattern (burst onsets/offsets expressed relative to left St burst onset) was not affected by the neurectomy. Although, there were small significant differences in step cycle duration in each cat post-neurectomy, there were no clear trends (not shown). For instance, in cat 1, step cycle duration was only significantly different ($p \leq 0.05$) at 8 (-70.77 ± 22.28 ms) and 14 (-68.17 ± 22.28 ms) days post-neurectomy. In cat 2, step cycle duration was significantly different ($p \leq 0.05$) at 2 (-61.60 ± 19.75 ms) days post-neurectomy but at no other day, including 1 day after. In cats 1 and 2, step cycle duration changed by less than 10 ms the day after the neurectomy. In cat 3, step cycle duration was significantly different ($p \leq 0.05$) at 1 (-83.92 ± 8.20 ms), 2 (-27.26 ± 8.35 ms), 5 (-61.61 ± 8.35 ms), 8 (-28.01 ± 8.35 ms), 12 (-34.86 ± 8.35 ms), 14 (-37.91 ± 8.35 ms), and 27 (-40.12 ± 8.20 ms) days post-neurectomy but not ($p \geq 0.05$) at 6, 19, 22, and more than 30 days post-neurectomy. Therefore changes in the timing and structure of the step cycle were not responsible for changes in reflexes described later on.

A more complete representation of changes in the mean amplitude of locomotor EMG for selected muscles is provided in Figure 4 for each cat up to 50 days post-neurectomy with each colored line illustrating a different muscle. In most extensors increases in EMG peaked at 2 days after the neurectomy before returning towards intact values thereafter. However, whereas activity of some muscles completely returned to intact levels (left MG, left VL, right VL in cat 1) in others the EMG could remain elevated for prolonged periods without ever fully returning to pre-lesion values (left MG in cat 2; right MG in cat 3) and in a few cases the EMG even started to increase a second time following the initial large increase and sharp

reduction (left VL in cat 3). In all cats, the LGS neurectomy produced considerable changes in locomotor EMG in multiple hindlimb muscles but the subset of muscles affected could vary from one animal to another.

Initial and post-contact components of the locomotor EMG

It was previously shown that the centrally generated initial component of the MG EMG during locomotion followed a different time-course of increase than the post-contact, partially reflex-mediated component (Pearson et al. 1999). Figure 5 illustrates changes in the initial and post-contact components of the locomotor EMG, as denoted in Figure 2B, in selected hindlimb extensors for the 3 cats. In general, both the initial and post-contact EMG components saw a large increase in the first 2 days before declining thereafter. The left MG of cat 1 was the only instance where both components did not share similar profiles. In this muscle, the initial component did not significantly ($p = 0.204$) increase with time after the LGS neurectomy (up to 14 days) whereas the post-contact EMG peaked 2 days after the neurectomy before sharply declining. In cat 2, both the initial and post-contact components of the left MG significantly increased ($p \leq 0.001$) immediately after the neurectomy and then gradually decreased toward the intact value, although the relative increase in post-contact EMG was greater. In the left VL of cats 1 and 3, both the initial and post-contact components of the EMG significantly increased ($p \leq 0.001$) and peaked at 2 days before gradually towards intact values. In contralateral extensors, the right VL of cats 1 and 3 and the right soleus of cat 2 showed large significantly increases ($p \leq 0.001$) that peaked at 1 day post-neurectomy followed by a sharp decrease thereafter.

Left hindlimb kinematics

Figure 6A reconstructs the left hindlimb during swing and stance for cat 3 in the intact state and at 2 and 14 days post-neurectomy. The most apparent change in limb trajectory is an increased yield (amount of flexion) at the ankle joint during the stance phase. With time, the magnitude of yield returned towards intact values. Similar to a previous study (Pearson et al. 1999) deficits resulting from the LGS

neurectomy were quantified. Figure 6B shows changes in ankle yield for the 3 cats. Cat 3 had a large significant ($p \leq 0.001$) increase in ankle yield, which returned towards the intact value but remained significantly ($p \leq 0.001$) elevated for the remainder of the study. Cat 1 also had a significant ($p \leq 0.001$) increase in ankle yield but it returned to intact levels at 14 days ($p = 0.962$). In cat 2, although ankle yield changed significantly after the neurectomy ($p \leq 0.001$) post-hoc comparisons revealed that this was only significant at 2 days ($p \leq 0.05$) following the denervation but not at 1 day ($p = 1.000$). Figure 6C shows changes in maximal ankle extension during the latter half of stance in the 3 cats for the same days as in B. The magnitude of ankle extension significantly decreased in cat 3 ($p \leq 0.001$) in the week following the denervation whereas in cat 1 it significantly increased ($p \leq 0.01$). In cat 2 it was unchanged 1 day after the neurectomy ($p = 1.00$) before increasing on days 2 and 4 ($p \leq 0.01$) and returning to intact values at 7 days ($p = 0.68$). In summary then, in all cats there was a significant increase in ankle yield in early stance in the first few days after the neurectomy and in maximal ankle extension attained during the latter half of stance but the magnitude of these changes could vary from one cat to another.

Tib nerve reflexes

Figures 7 gives raw data for Tib nerve reflexes in the left MG of cat 1 (Fig. 7A) and the left St of cat 3 (Fig. 7B) in the intact state and for two selected days after the LGS neurectomy. In the left MG of cat 1 N1 responses increased in most phases of the step cycle but this increase scaled with the level of blEMG and was not significantly different, except a significant decrease ($p \leq 0.05$) in phase 0.7. The P2 responses did not significantly change ($p \geq 0.05$) 2 days post-neurectomy but at 8 days they were significantly increased in phases 0.3-0.6, up to 300% of the maximal control value above the level of blEMG. In the left St of cat 3, P1 and P2 responses were significantly increased above the level of blEMG ($p \leq 0.05$) 2 days post-neurectomy particularly from phases 0.4-0.7 when this muscle is normally inactive and by 28 days reflexes returned towards intact values.

Figures 8-10 provide a more detailed account of modifications in Tib nerve reflexes for selected muscles and days after the neurectomy in cats 1-3, respectively, expressed as a function of changes in blEMG. As can be seen from these figures, some Tib nerve reflexes were significantly altered in each cat at specific days post-neurectomy, indicated by the colored asterisks, in several muscles, although reflex changes for a given muscle could differ from one animal to another. In several instances, changes in Tib nerve reflexes were significant in only certain phases of the step cycle.

In cat 1 (Fig. 8), P1 responses of the left TA were increased and remained so for up to 21 days in phases 0.0-0.3, where the muscle is normally active, and 0.9 (Fig. 8A). The P2 responses were significantly different in phases 0.0 and 0.2 for all selected days after the neurectomy but not in other phases except for phase 0.4 at 2 days post-neurectomy (Fig. 8B). In the left St, P1 responses were increased predominantly in phases 0.4-0.7, when this muscle is inactive, for up to about 8 days following the neurectomy before returning to intact values (Fig. 8C). In general, P2 responses were decreased at 2 and 5 days in phases where the St muscle is active (0.8-0.2) before returning towards intact values later on (Fig. 8D). In the left MG, N1 responses were mostly unchanged after the neurectomy except for phases 0.6-0.7 where responses were decreased for some days (Fig. 8E). The P2 responses of the left MG were unchanged 2 days post-neurectomy before increasing in phases 0.4-0.7 at 8 days, when this muscle is active, before returning towards intact values thereafter (Fig. 8F).

In cat 2 (Fig. 9), P1 responses of the left TA were unchanged in the first 2 days post-neurectomy before increasing at 9 days, mostly in phases 0.0 and 0.2-0.5, before increasing in all phases at 36 and 50 days (Fig. 9A). Changes in P2 responses of the left TA were more variable showing no clear trends after the neurectomy (Fig. 9B). In the left MG, N1 responses were decreased in a few phases in the first 2 days post-neurectomy (Fig. 9C). Changes in P2 responses of the left MG were much more variable and only showed a decrease in the first 2 days in phase 0.5 of the step cycle. There were very large (over 800% of the maximal control value) increases in P2

responses of the right MG (Fig. 9E) and soleus (Fig. 9F) after the neurectomy, which were limited to phase 0.8 of the step cycle or early stance of the right leg. Changes were gradual and only became significant at 36 days post-neurectomy.

In cat 3 (Fig. 10), P1 responses of the left TA increased at 2 days post-neurectomy in phases 0.2 and 0.4 before increasing in most phases at 8 days and then returning toward intact values thereafter (Fig. 10A). Changes in P2 responses of the left TA were subtler and were confined to phases 0.0-0.1 and 0.4-0.6 (Fig. 10B). In the left St, P1 and P2 responses were increased in the first 2 days post-neurectomy, particularly in phases where the muscle is active, before returning to intact values thereafter (Fig. 10C). In the right MG, increases in P2 were observed at 2 and 8 days after the neurectomy but only in phase 0.8 (Fig. 10E). In the right soleus, increases in P2 were seen 1 day post-neurectomy in phase 0.7 and in phase 0.3 at 27 days (Fig. 10F).

Moreover, although each response is expressed as a function of the level of blEMG, in some cases increased Tib nerve reflexes after the neurectomy were observed in muscles that showed little change in locomotor EMG [e.g. P1 responses in the left St of cats 1 (Fig. 8C) and 3 (Fig. 10C); P2 responses in the right MG (Fig. 9E, Fig. 10E) and soleus (Fig. 9F, Fig. 10F) of cats 2 and 3, respectively]. In addition, the largest changes in reflexes could be seen at days where changes in locomotor EMG were not peaking but declining [e.g. P2 responses in left MG of cat 1 (Fig. 8F)]. Therefore, modifications in reflex pathways can be independent from those seen in EMGs.

Discussion

In the present study changes in the activity of several hindlimb muscles and in reflexes from the Tib nerve after an ankle extensors neurectomy in otherwise intact walking cats were investigated. It was found that a LGS neurectomy of the left leg produced changes in the activity of multiple hindlimb muscles and sensory feedback from the Tib nerve during locomotion. Reflex modifications dissociated from those at the motoneuron, inferred from EMG recordings, both in time and amplitude, and

thus represent a change in the gain of these pathways. How these various changes are involved in the functional recovery of locomotion is discussed in the following sections.

Technical considerations

When working with chronic animals it is imperative that recordings and stimulations remain stable for the duration of the study to draw meaningful physiological conclusions. Based on several observations, we feel confident that most recordings reflected a physiological change in muscle activity. For instance, EMG activity in several extensors increased considerably in the immediate days following the LGS neurectomy before gradually returning towards control values over several weeks (see Figs. 3 and 4). Moreover, reflex amplitude, at a given stimulus intensity, was modified in the days following the neurectomy but progressively returned to intact levels (Fig. 7) in some muscles, suggesting that stimulation intensity remained constant. If stimulation intensity were somehow modified, reflexes in all muscles would have changed similarly over time but the data show that modifications were most often limited to a particular muscle, to specific responses such as N1, P1, or P2, or to particular phases of the step cycle (see Figs. 8-10)

Reorganisation of muscular activity

Previous investigations had shown that functional recovery following an ankle extensors neurectomy was mediated by an increased activity of close synergists (Pearson et al. 1999; Bouyer et al. 2001). Although we found considerable increases in MG EMG, changes in the activity of several muscles of both hindlimbs, which paralleled and in some cases exceeded that of MG, were also observed (see Figs 3 and 4). Muscles frequently displaying an increased activity included the left and right VL (knee extensors) and contralateral ankle extensors. Augmented activity in extensors other than MG could reinforce weight support and propulsion during stance. In addition, the appearance of an additional burst in the left St during stance

in the early days after the neurectomy (see Fig. 3) could assist other extensors in supporting and propelling the denervated leg during stance since this muscle is also a hip extensor but normally only a flexor burst is recorded during level treadmill locomotion in cats (Rossignol 1996). Presumably, the activity of other muscles, such as hip extensors/adductors that were not recorded was also altered. Therefore, modified activity after a LGS neurectomy is not solely limited to close synergists but involve several muscles, as is the case with cutaneous denervations of the hindpaws (Bouyer and Rossignol 2003a; Bretzner and Drew 2005). Furthermore, although the EMG was increased in several muscles for all cats the subset of muscles involved could differ between cats probably stemming from interanimal differences in kinematic deficits incurred by the neurectomy (Fig. 6) and in neuronal connections (Loeb 1993). Thus cats share similar but also have unique adaptive strategies following a LGS neurectomy (Bouyer et al. 2001).

Changes in the locomotor EMG of most muscles followed similar time-courses and profiles. For instance, in cats 1 and 2 changes in muscle activity in all extensors peaked 2 days after the neurectomy before gradually returning towards normal values (Fig. 4). Although muscle hypertrophy after the neurectomy was not measured, studies have shown marked and progressive hypertrophy in a muscle after denervating its synergists (Degens et al. 1995; Walsh, Jr. et al. 1978; Whelan and Pearson 1997). As a result, the muscle can produce more force and less neural drive is required. However, in the early days after the neurectomy large increases in EMG activity could only have been mediated by modifications in neural drive to motoneurons since hypertrophy at the muscle takes approximately 10 days to develop (Degens et al. 1995).

Like previous studies (Bouyer et al. 2001; Gritsenko et al. 2001) changes in both initial and post-contact EMG components of most extensors shared similar profiles. A previous report demonstrated that the initial (first 120 ms) and late (100 ms centred on peak activity after foot contact) components of the MG EMG followed different profiles after denervating synergistic ankle extensors (Pearson et al. 1999). For example, the late component increased immediately after the lesion, which could

be followed by either an increase or a decrease thereafter, whereas the initial period increased more gradually after the neurectomy. We found large increases in post-contact MG activity, which peaked 2 days after the neurectomy before gradually returning towards intact values (see Fig. 5 cats 1 and 2). The initial component of MG EMG was more variable showing virtually no change in cat 1 for a period of two weeks, while cat 2 showed a similar profile as the post-contact EMG, albeit smaller in proportion. Thus, although there might be a slight dissociation between the two components for MG, in some animals, in other extensors the two periods behave similarly. Therefore, the immediate increase in post-contact EMG might in part be reflex-mediated but it can also be centrally generated, as is the early pre-contact EMG as previously suggested (Gritsenko et al. 2001; Pearson et al. 1999), and also be modified by transmission in reflex pathways, such as the Tib nerve pathway, to the locomotor CPG. A recent model formalizes how peripheral feedback from proprioceptive and cutaneous afferents can influence the locomotor CPG and the activity of several motoneuron pools (Rybak et al. 2006). The isolated spinal cord can govern the increased muscular activity (Bouyer et al. 2001) but in the intact state it is probable that both spinal and supraspinal structures are involved. Increased corticospinal efficacy has been demonstrated after a cutaneous denervation of the hindpaw in intact cats during locomotion (Bretzner and Drew 2005).

Plasticity in cutaneous reflex pathways

Plasticity in reflexes from plantar structures of the foot was observed in several muscles of both hindlimbs following the LGS neurectomy. At an intensity of 1.2x the threshold for a small but consistent short-latency response in the ipsilateral TA, responses were most likely mediated by low threshold afferents. Indeed, at this stimulation intensity responses evoked in TA are only slightly higher in stimulation intensity than the threshold for recording an afferent volley in the sciatic nerve and are thought to be generated by the largest diameter Aβ afferents (Loeb 1993). However, influences from group I or II afferents cannot be eliminated since the Tib nerve also supplies intrinsic foot muscles. The stimulation did not visibly alter limb

trajectory during locomotion, which is important because perturbations of the limb could introduce responses linked to the movement through proprioceptive reflexes. The pattern of responses, as opposed to amplitude, is typically invariant unless very high and clearly noxious stimulus intensities are used (Abraham et al. 1985; Loeb 1993). Therefore, responses are probably mediated by low-threshold cutaneous afferents.

It has been proposed that electrically stimulating low-threshold cutaneous afferents could activate the same afferent population normally recruited during movement (Lundberg 1979; Lundberg et al. 1987; Pratt et al. 1991). Evidently, electrically stimulation differs from natural activation since multiple afferents are recruited simultaneously (Wand et al. 1980), but nevertheless, responses evoked during the time these afferents are normally active during locomotion could provide important clues as to their normal function. In other words, since cutaneous afferents from the Tib nerve are normally active during ipsilateral stance (e.g. when the paw is contact with the ground) evaluating changes in Tib nerve reflexes during this period could provide some clues as to why they are altered after the LGS neurectomy. For example, the large increase in P2 responses in MG with time after the neurectomy, especially during the middle portion of stance, could reinforce MG activity during the propulsive portion of stance (Fig. 8F). In the left St, large increases in short-latency excitatory responses throughout stance in the early days post-neurectomy (Figs 8C and 10C) could reinforce hip extension for the purposes of weight bearing and propulsion during stance to offset the loss of ankle extensors, which are normally involved. Therefore, changes in reflex pathways mediated by low-threshold afferents from the Tib nerve could serve to modify or correct the centrally generated pattern of muscle activation and promote functional recovery.

Reflexes evoked by stimulation have been shown to increase progressively with the level of pre-existing voluntary activity, and thus remain proportionally constant with the level of background activity, giving rise to the concept of automatic gain control (Matthews 1986). In other words, an afferent volley will evoke a larger reflex if more motoneurons are active and vice-versa (this goes for excitation but also

for inhibition as shown by Matthews). In the present study, since changes in reflexes were in many cases independent of changes in locomotor EMG, pre-motoneuronal mechanisms must be involved in gating sensory feedback. The increase in force produced by knee and ankle extensors might increase the pressure on receptors of the foot and thus alter transmission in cutaneous afferents of the Tib nerve. Also, cutaneous pathways make polysynaptic connections within the spinal cord and a reorganisation of pre- and/or post-synaptic inhibition at these different relays could substantially change reflex amplitude. Changes in pre- and/or post-synaptic inhibition could result from altered input originating in spinal and/or supraspinal structures to interneurons involved in these processes (Gossard et al. 1989; Gossard et al. 1990). Lastly, collateral sprouting of afferents could re-wire interneuronal circuitry and modify reflexes, at least in the long term (Cameron et al. 1992; Koerber et al. 1994; Goldberger and Murray 1988).

In summary, whatever the mechanism(s) may be it is evident that reflex pathways from the Tib nerve are modified following a LGS neurectomy and that functional recovery involves a reorganised activity in several motoneuron pools. Adaptations to the loss of sensory and/or motor innervation caused by a neurectomy undoubtedly occur at multiple levels of the nervous system including reflex pathways from the periphery, locomotor generating networks within the spinal cord and supraspinal inputs from the brain and brainstem, which in turn alters the activity of several muscles.

Acknowledgments

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Figures

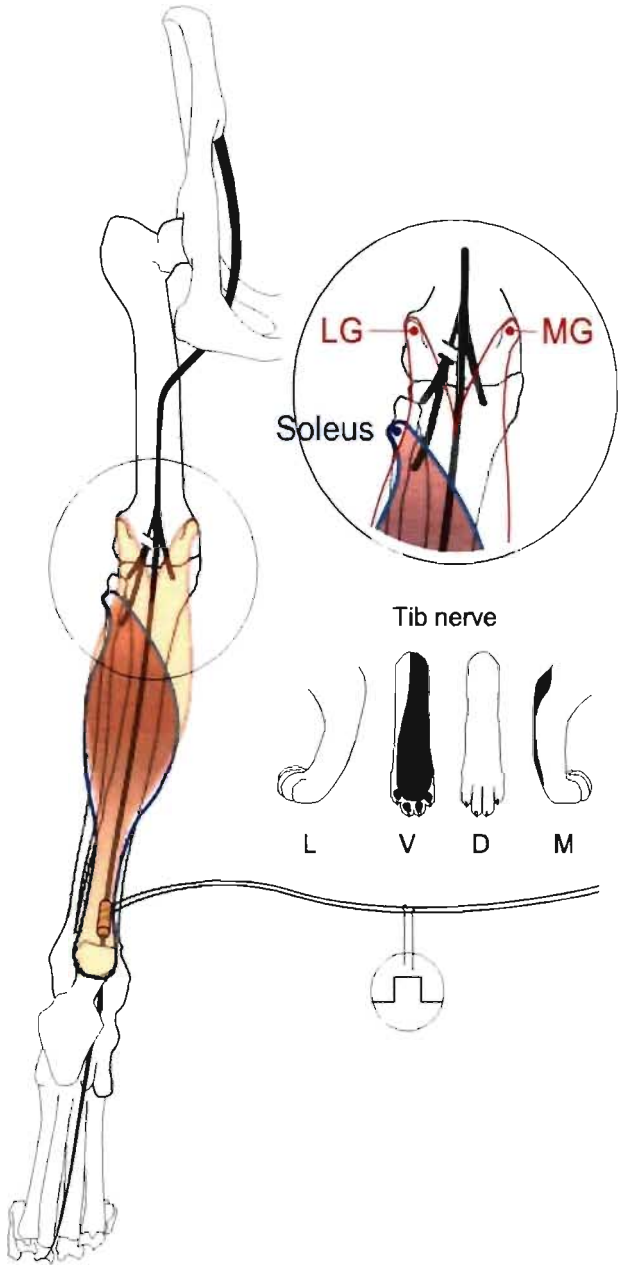

Figure 1. The common nerve to the lateral gastrocnemius and soleus (LGS) was cut just after it bifurcates away from the main branch of the Tib nerve leaving the innervation of the medial gastrocnemius (MG) intact (see insert). The Tib nerve at the ankle, which innervates the skin on the ventral and medial surface of the paw (black area), was stimulated with electrodes in a polymer cuff.

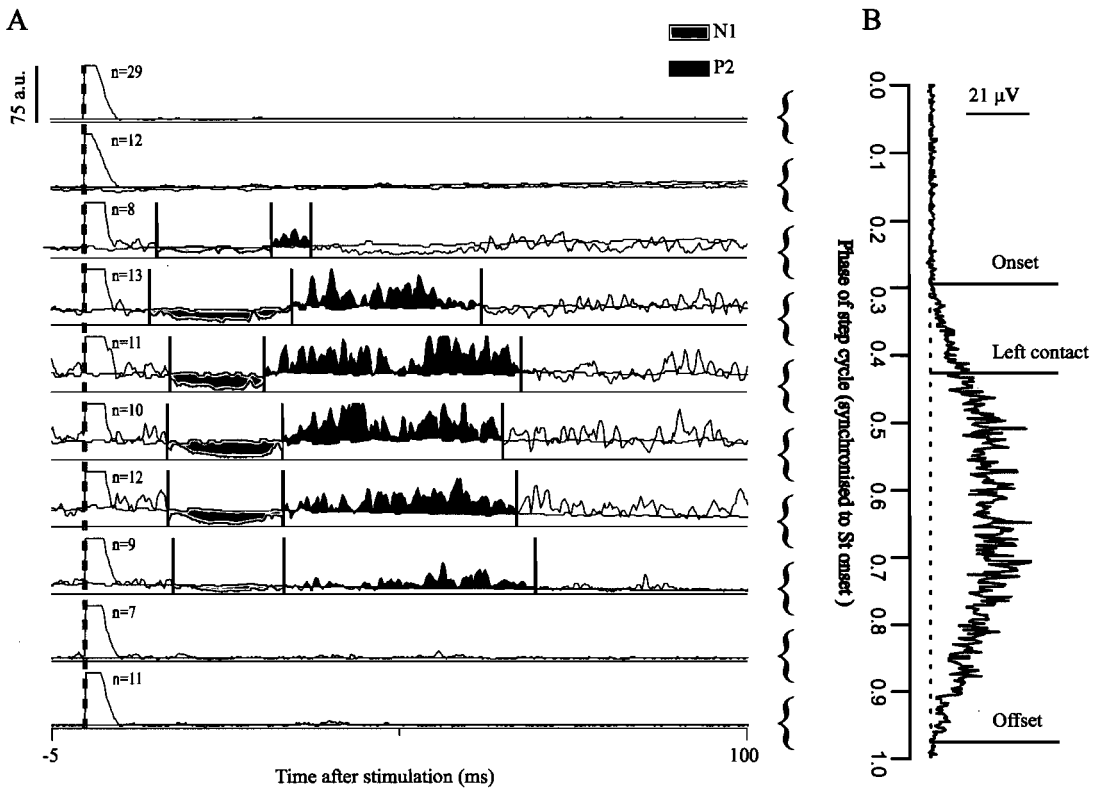

Figure 2. Measurements of reflexes and extensor burst activity. A) Reflexes evoked from the Tib nerve in various phases of the step cycle in the left MG of cat 1. The step cycle was separated into 10 phases (each trace represents a phase), from 0 to 1.0 respectively. For extensors, the onset and offset of responses was determined manually for each phase. The area below (N1; grey area) or above (P2; black area) the baseline locomotor EMG (blEMG) recorded during non-stimulated cycle was integrated to provide the amplitude in arbitrary units (a.u.) of inhibitory and excitatory responses, respectively. These responses were then divided by the blEMG occurring in the same part of the step cycle and expressed as a percentage of the maximal control value occurring in one of the 10 phases. B) Rectified and integrated locomotor EMG of the left MG normalized to the step cycle from 0 to 1 and synchronised to the onset of the left St. The initial component of the locomotor EMG consisted of the first 120 ms following burst onset whereas post-contact EMG included the portion from foot contact to burst offset.

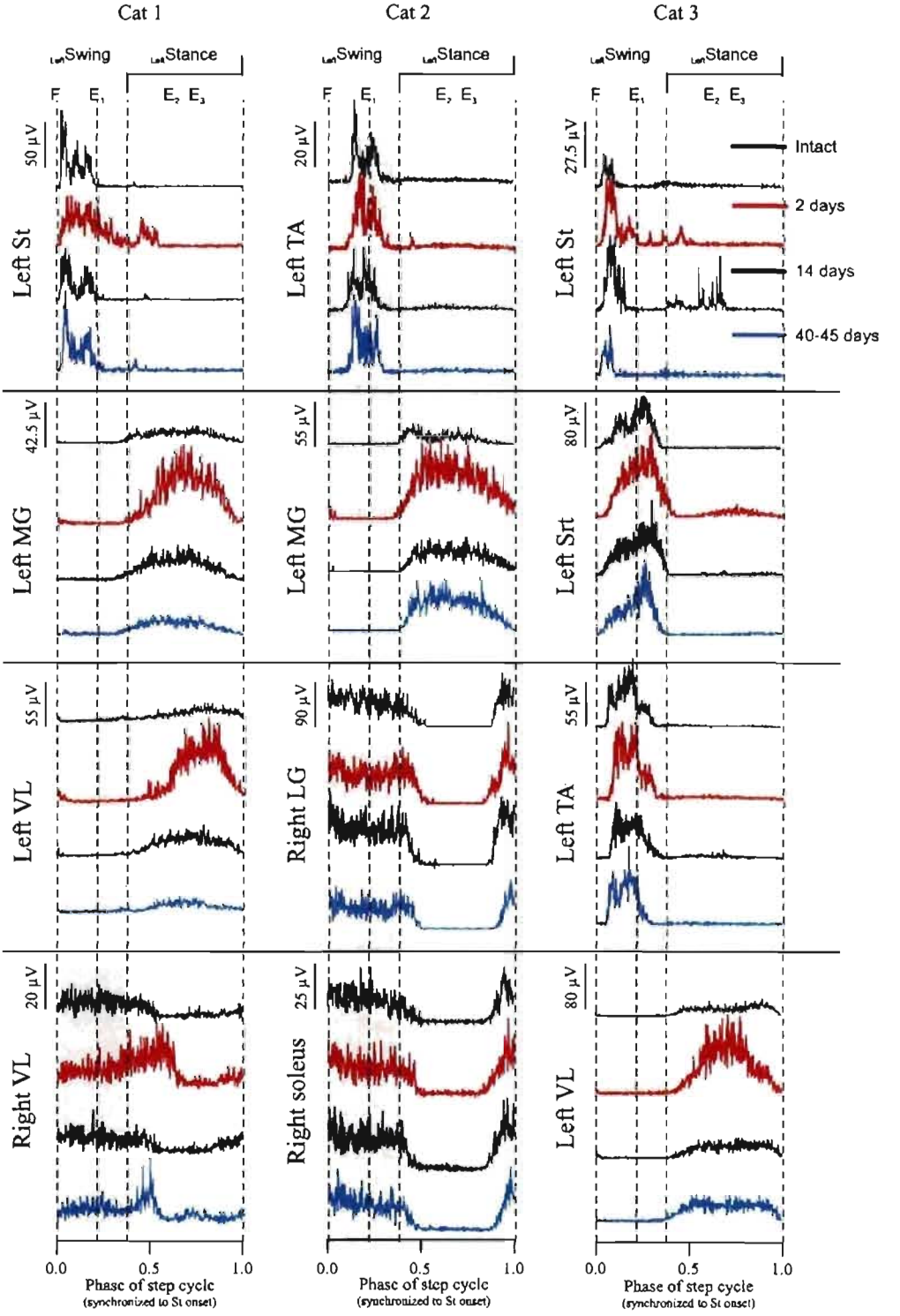
Cat 1
Cat 2
Cat 3
Swing
Stance
F
E1
E2, E3
Intact
2 days
14 days
40-45 days
Left St
Left TA
Left St
Left MG
Left MG
Left Srt
Left VL
Right LG
Left TA
Right VL
Right soleus
Left VL
50 µV
20 µV
27.5 µV
42.5 µV
55 µV
80 µV
55 µV
90 µV
55 µV
20 µV
25 µV
80 µV
0.0
0.5
1.0
Phase of step cycle
(synchronized to St onset)

Figure 3.

Figure 3. Rectified, averaged, single burst EMG traces for selected muscles and days after the neurectomy for cat 1 (left panels), cat 2 (middle panels), and cat 3 (right panels) during locomotion. The swing and stance of the left hindlimb were determined from kinematic analyses and denoted for each cat using Philippson's terminology (Philippson 1905) using flexion (F), first extension (E_1), and second and third extension (E_2, E_3) phases. The E_1 phase was determined using kinematic data. Each EMG trace is the average of approximately 20 bursts.

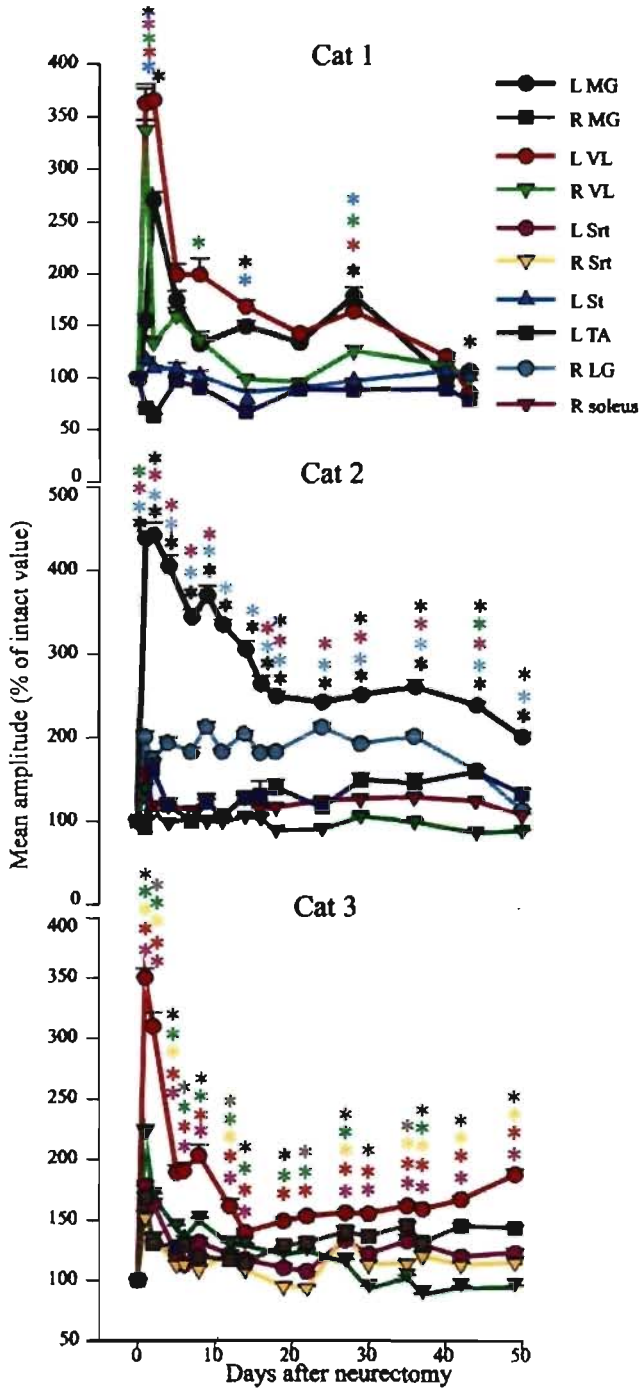

Figure 4. Changes in mean EMG amplitude for selected muscles during locomotion for up to 50 days post-neurectomy expressed as a percentage of the intact value. Each colored line represents a different muscle. Significant differences for the days after the neurectomy, for a given muscle, are indicated by an asterisk above the topmost line in the same color (p ? 0.05). Each data point is the mean ± SEM of approximately 20 locomotor bursts. There were significant ($p < 0.001$) increases in locomotor EMG activity in the left MG (cats 1 and 2), the left VL (cats 1 and 3), the right VL (cats 1 and 3) and some contralateral ankle extensors (cats 2 and 3).

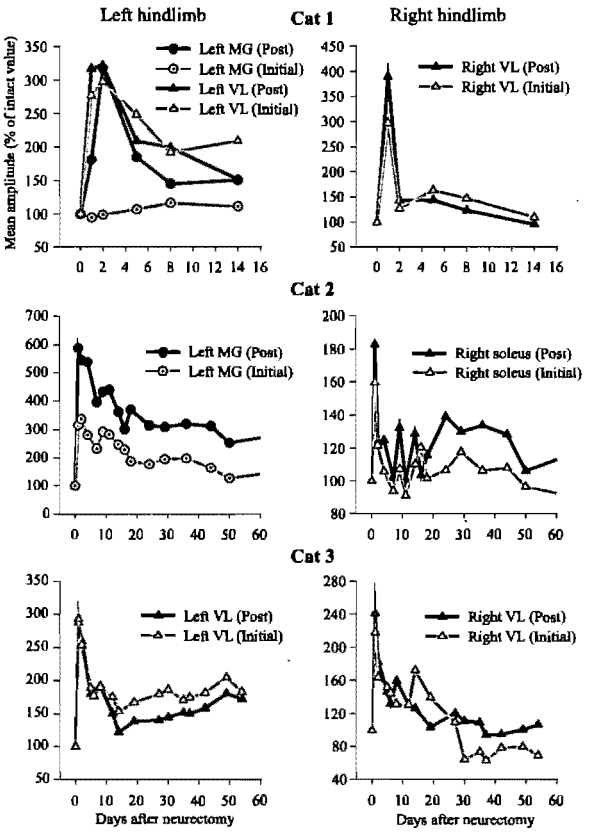

Figure 5. Initial and post-contact components of EMG in selected extensors for cat 1 (top panels), cat 2 (middle panels), and cat 3 (bottom panels) in the left (left panels) and right (right panels) hindlimb. Each data point is the mean ± SEM and is derived from approximately 20 locomotor bursts.

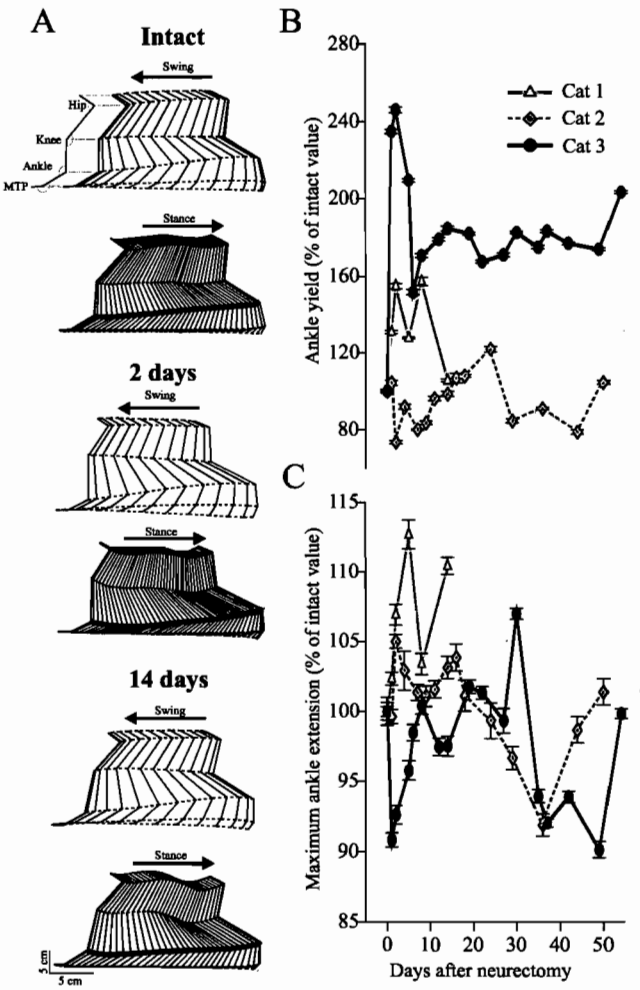

Figure 6. A) Stick figure of the left hindlimb in the intact state, and at 2 and 14 days post-neurectomy in cat 3. A representative step cycle is illustrated for each day. The thick black line represents the trajectory of the lateral malleolus during stance. Angles for the hip, knee, ankle, and MTP were measured as illustrated in the intact state during swing. B) Changes in ankle yield during early stance and in C) maximal ankle extension at end-stance for up to 14 (cat 1), 50 (cat 2), and 54 (cat 3) days after the neurectomy expressed as a percentage of the intact value. Each data point is the mean ± SEM of 10-20 step cycles.

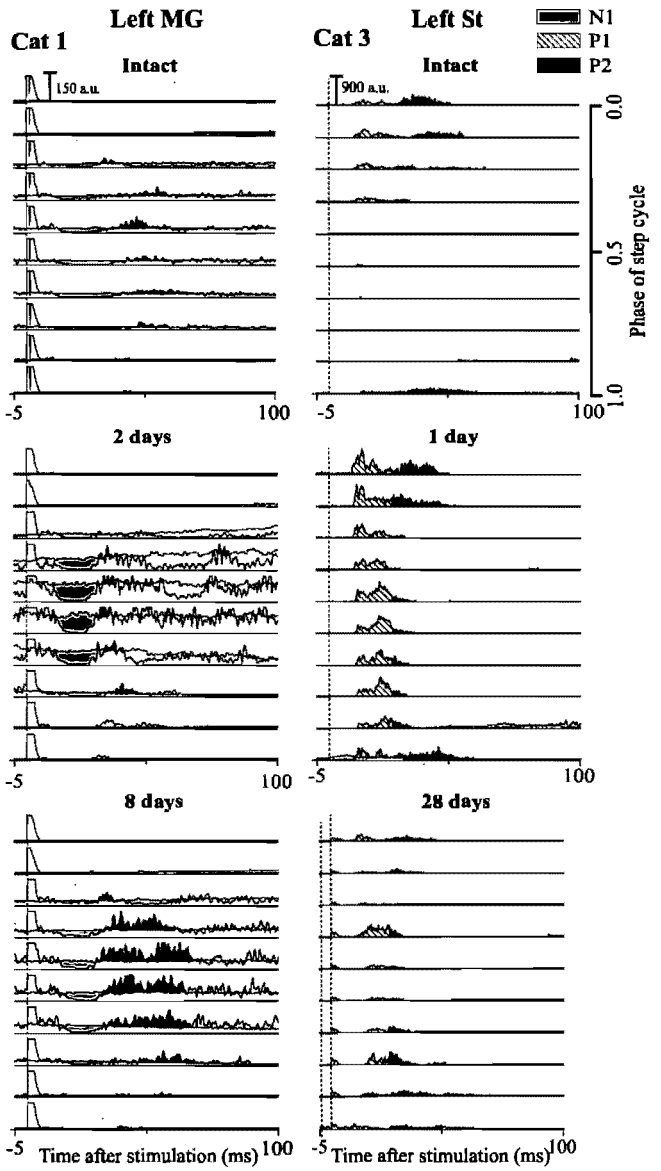

Figure 7. Averaged reflex responses before and after the LGS neurectomy for the left MG of cat 1 (left panels) and the left St of cat 3 (right panels) in the intact state and for selected days after the neurectomy. Values for a given muscle are at the same scale in arbitrary units (a.u.). The grey and hatched areas respectively denote short latency inhibitory (N1) and excitatory (P1) responses whereas the black area represents longer-latency excitatory (P2) responses. The first horizontal trace for a given day is phase 0.0 of the step cycle synchronized to the left St onset.

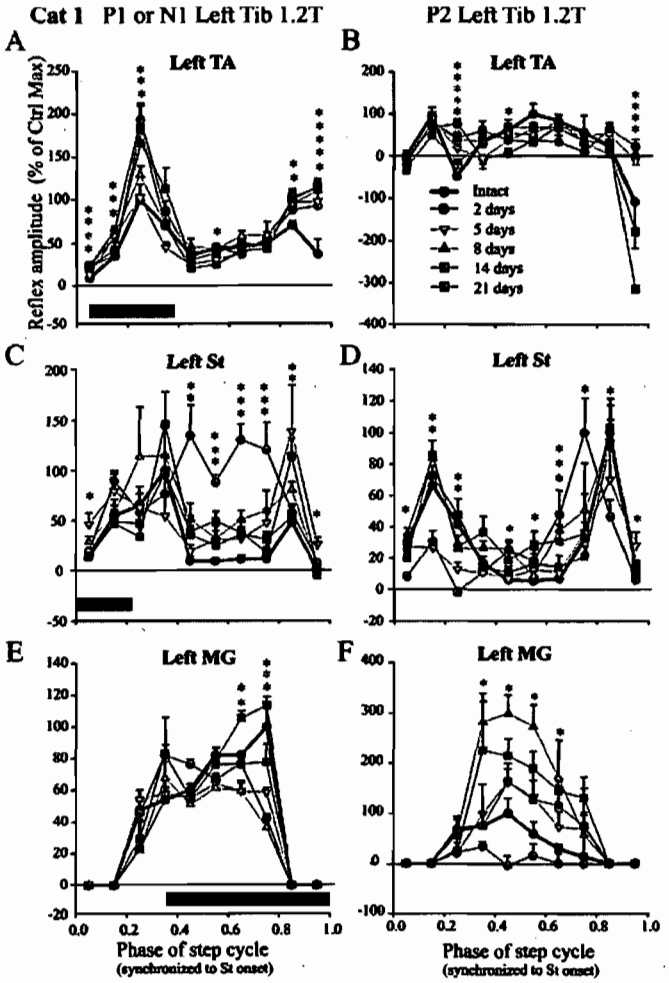

Figure 8. Phase plots summarizing changes in Tib nerve reflexes at 1.2T in cat 1. A, C, E: N1 or P1 responses measured in 3 muscles on the left side (TA, St, MG). Average of 3 control sessions (thick black line) and days 2 (red), 5 (green), 8 (light blue), 14 (dark blue), and 21 (dark pink) post-neurectomy are shown. Horizontal black rectangles represent the phase of activity of each muscle during locomotion in the intact state. B, D, F show P2 responses in the same muscles for the same days. Significant differences from the intact value during a given phase after the neurectomy are indicated by an asterisk of the same color as the day ($p < 0.05$). Each data point is the mean ± SEM of approximately 10 responses.

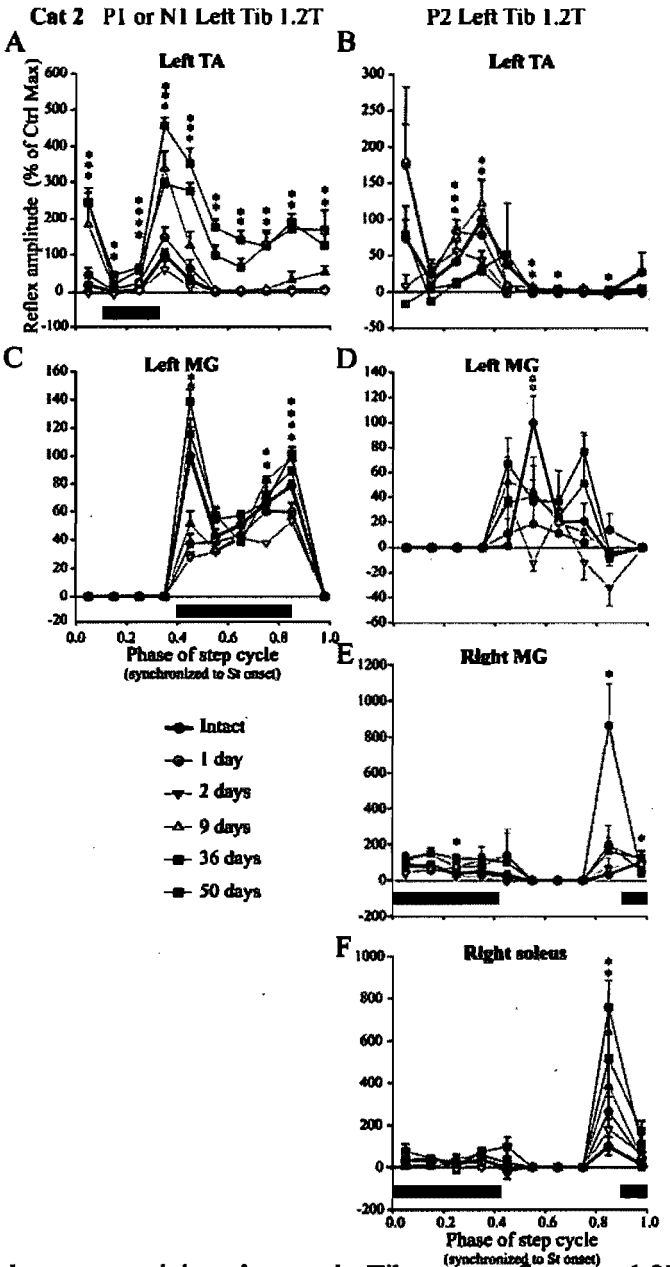

Figure 9. Phase plots summarizing changes in Tib nerve reflexes at 1.2T in cat 2. A, C: N1 or P1 responses measured in 2 muscles on the left side (TA, MG).. Average of 3 control sessions (thick black line) and days 1 (red), 2 (green), 9 (light blue), 36 (dark blue), and 50 (dark pink) post-neurectomy are shown. Horizontal black rectangles represent the phase of activity of each muscle during locomotion in the intact state. B, D, E, F show P2 responses in the left TA, left MG, right MG, and right soleus, respectively for the same days. Significant differences from the intact value during a given phase after the neurectomy are indicated by an asterisk of the same color as the day ($p < 0.05$). Each data point is the mean ± SEM of approximately 10 responses.

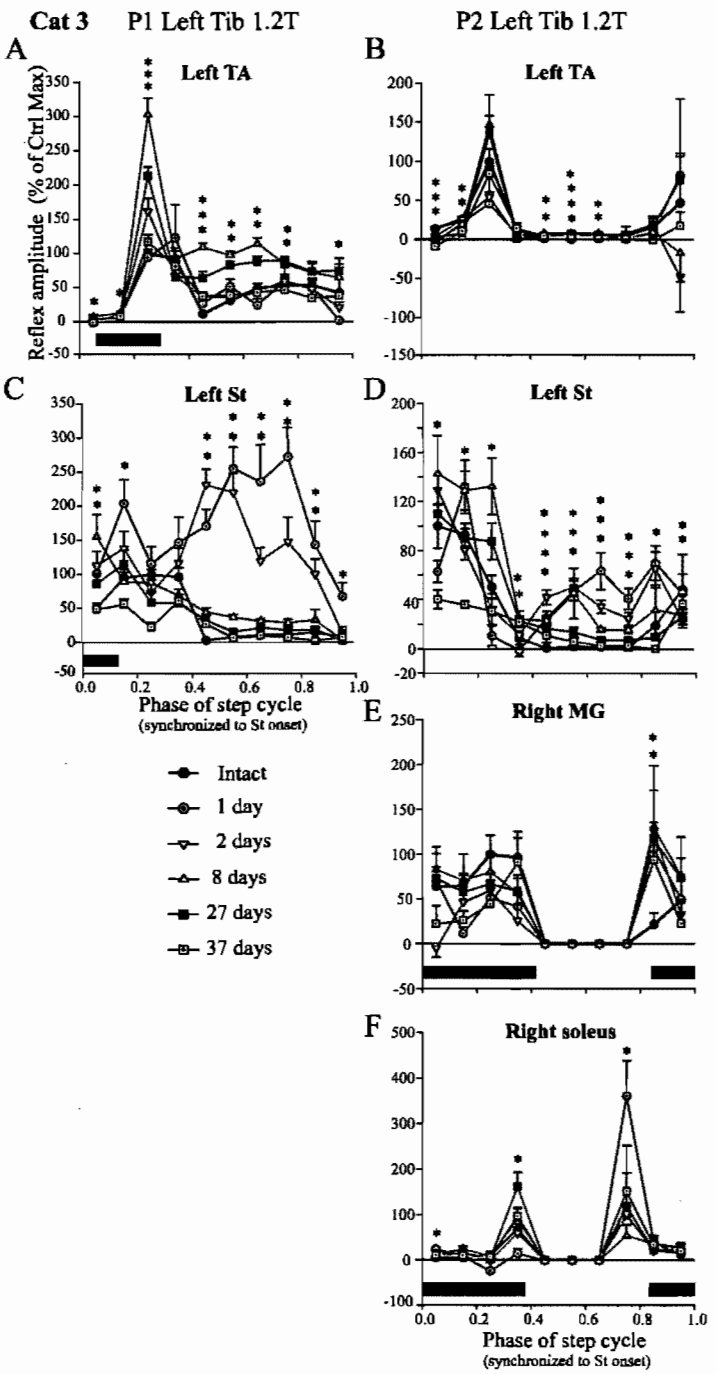
Cat 3
P1 Left Tib 1.2T
P2 Left Tib 1.2T
A
Left TA
B
Left TA
Reflex amplitude (% of Ctrl Max)
C
Left St
D
Left St
Phase of step cycle
(synchronized to St onset)
Intact
1 day
2 days
8 days
27 days
37 days
E
Right MG
F
Right soleus
Phase of step cycle
(synchronized to St onset)

Figure 10.

Figure 10. Phase plots summarizing changes in Tib nerve reflexes at 1.2T in cat 3. A, C: P1 responses measured in 2 muscles on the left side (TA, St). Average of 3 control sessions (thick black line) and days 1 (red), 2 (green), 8 (light blue), 27 (dark blue), and 37 (dark pink) post-neurectomy are shown. Horizontal black rectangles represent the phase of activity of each muscle during locomotion in the intact state. B, D, E, F show P2 responses in the left TA, left St, right MG, and right soleus, respectively for the same days. Significant differences from the intact value during a given phase after the neurectomy are indicated by an asterisk of the same color as the day ($p \leq 0.05$). Each data point is the mean ± SEM of approximately 10 responses.

Chapter 3 - Article 2. Adaptive mechanisms following partial denervation of ankle extensors in spinal cats during locomotion

By Alain Frigon and Serge Rossignol

Submitted to the Journal of Neurophysiology

Abstract

The recovery of locomotion following peripheral nerve lesions in the spinal state (i.e. after a complete spinal transection) indicates that the spinal cord has intrinsic adaptive mechanisms. To study some of these adaptive mechanisms the left lateral gastrocnemius-soleus (LGS) nerve was sectioned in 3 cats previously spinalized and the activity of multiple hindlimb muscles and reflexes, evoked by stimulating the tibial nerve at the ankle were recorded before and after the neurectomy during treadmill locomotion. Data obtained from these spinal cats were compared to data previously recorded in 3 cats that received an identical denervation but in the intact state (i.e. an intact spinal cord). There was an increased activity in multiple hindlimb muscles and several changes in reflex responses following a denervation in the spinal state, similar to what is found in the intact state. However, contrary to the intact state, reflex responses in the days following the denervation could be decreased compared to control values (i.e. before denervation). The present results demonstrate that the spinal cord possesses the circuitry to mediate the increased activity in hindlimb muscles and produce some of the changes in reflex pathways seen after a muscle denervation. Decreases in reflex responses post-denervation in the spinal state, however, indicate that other mechanisms, such as descending supraspinal inputs, are involved in the intact state. A higher percentage of reflex changes were noted in the spinal state compared to the intact state, which could reflect the greater dependency of sensory feedback from the moving hindlimbs during spinal locomotion.

Keywords: Plasticity, reflex, locomotion, denervation, spinalization

Introduction

The spinal cord possesses intrinsic mechanisms that enable the animal to adapt to a changing environment during locomotion. For example, when the legs of a spinal cat are placed on a split-belt treadmill operating at different rates each leg will match the speed of its respective belt (Forssberg et al. 1980). Moreover, contacting the foot dorsum during the swing phase of locomotion in spinal cats will generate a coordinated reflex response that allows the leg to overcome the obstacle (Forssberg 1979). This shows that the isolated spinal cord can process sensory inputs from the hindlimbs to meet the demands of the environment during locomotion.

The spinal cord can also adapt to changes induced in its internal environment. For instance, lesioning the mixed peripheral nerve supplying the lateral-gastrocnemius-soleus (LGS) in already spinal cats (i.e. after spinalization) produced an increased ankle yield during early stance but with time this deficit disappeared (Bouyer et al. 2001), as is the case in otherwise intact cats (Pearson et al. 1999). However, the spinal cord has a limited ability to adapt to changes induced in its internal environment because plantar paw placement was permanently lost after a complete cutaneous denervation of the hindpaws in a spinal cat (Bouyer and Rossignol 2003b), whereas in intact cats plantigrade placement can recover following the same denervation (Bouyer and Rossignol 2003a). This suggests that in the intact state (i.e. with an intact spinal cord) the spinal central pattern generator (CPG) for locomotion can make use of other inputs to properly place the paw whereas in the spinal state (i.e. following spinalization) the spinal CPG requires at least a modicum of input from the skin for plantigrade placement of the paw. Therefore, although intrinsic spinal mechanisms can offset to a certain extent the loss of sensory and/or motor innervation and effect changes leading to the functional recovery of locomotion the isolated spinal cord has its limitations.

In a recent study, reflex responses evoked by stimulating the tibial (Tib) nerve at the ankle, which is primarily cutaneous, were increased in the days following a LGS neurectomy or a partial unilateral cutaneous denervation of the hindpaw in otherwise intact cats, suggesting that remaining cutaneous inputs can be increased

following the loss of proprioceptive information (Frigon and Rossignol 2007). Whether or not cutaneous reflexes are modified following a similar denervation in the spinal state and participate in the locomotor compensation is unknown. Supraspinal inputs could be required to evoke changes in reflex pathways following a muscle denervation. In addition, in spinal and intact cats functional recovery following partial denervation of ankle extensors was attributed to large increases in the burst activity of remaining synergists (Pearson et al. 1999; Bouyer et al. 2001), such as the medial gastrocnemius (MG). However, in a more recent study it was shown that the activity of several leg muscles increased bilaterally following a LGS denervation (Frigon and Rossignol 2007). Does a similar increase in multiple muscles occur following a muscle denervation in the spinal state?.

Therefore, the purpose of the present study was 1) to determine if Tib nerve reflexes are modified after a LGS neurectomy in the spinal state and whether these changes are similar to those observed in the intact state (Frigon and Rossignol 2007) and 2) to evaluate if a LGS denervation produces increases in multiple hindlimb muscles bilaterally in the spinal state.

Methods

The general methodology is similar to that used in a previous study (Frigon and Rossignol 2007). The experimental protocol was in accordance with the guidelines of the animal Ethics Committee of the Université de Montréal. All surgical procedures were performed under general anesthesia and aseptic conditions. Prior to surgery cats were injected with an analgesic (Anafen 100mg; subcutaneously) and pre-medicated (Atravet 0.01 ml/kg, Glycopyrrolate 0.05 ml/kg, Ketamine 0.1 ml/kg; intramuscularly). Cats were then intubated and maintained under gaseous anesthesia (isoflurane 1-2%) while heart rate and respiration were monitored. After surgery, an analgesic (Buprenorphine 0.05 ml/kg) was administered subcutaneously. An oral antibiotic (cephatab or apo-cephalex, 100 mg/day) was given for 10 days following surgery.

Animals and general procedures

Three adult cats (2 males, 1 female) weighing between 3.5 and 5.0 kg were first selected based on their ability to walk for prolonged periods on a treadmill and trained for a few weeks at their preferred speed (0.4-0.5 m/s). Cats were then chronically implanted with electrodes for EMG recordings and nerve stimulation, allowed to recover from the implantation, and then values of EMGs and reflexes were recorded in the intact state. Figure 1 summarizes the sequence of events for each SD cat (Spinal-Denervated) from the time of spinalization at T13. Once stable spinal locomotion was achieved after a period of 26-41 days of treadmill training (black rectangle) control values were obtained for a few weeks (hatched area) and then the left LGS was denervated. Recordings resumed the day after the denervation up to 31 days (gray area). Each recording session lasted approximately 1 hour. Data from a previous study (Frigon and Rossignol 2007) in which a neurectomy of the left LGS was made in otherwise intact cats (ID: intact-denervated) were also used to make comparisons of the effects of a LGS denervation performed in the intact and spinal states. Cats ID1-ID3 correspond respectively to cats 1-3 in the previous study.

Implantation of electromyographic (EMG) electrodes, recording and processing

Chronic electromyographic (EMG) electrodes were implanted bilaterally in the following hindlimb muscles: semitendinosus (St: knee flexor/hip extensor), anterior part of sartorius (Srt: hip flexor/knee extensor), vastus lateralis (VL: knee extensor), lateral gastrocnemius (LG: ankle extensor/knee flexor), medial gastrocnemius (MG: ankle extensor/knee flexor), and tibialis anterior (TA: ankle flexor). A pair of Teflon-insulated multistrain fine wires (AS633; Cooner wire, Chatsworth, CA) was directed subcutaneously from head-mounted fifteen pin connectors (Cinch Connectors; TTI Inc., Pointe-Claire, Canada) and sown into the belly of each muscle for bipolar EMG recordings. EMG recordings were bandpass filtered (100-3000 Hz) and amplified (gains of 0.5-50K) using two Lynx-8 amplifiers (Neuralynx, Tucson, Arizona). EMG data were digitized (5000 Hz) using custom-made acquisition software. The duration, mean amplitude, and timing of the EMG

bursts during locomotion relative to left St burst onset were calculated using custom software. Mean amplitude was defined as the area under the rectified EMG burst divided by its duration and expressed as a percentage of the averaged intact value. Kinematic and EMG data were synchronized using an SMPTE time code generator (Evertz, time code master 5010). Over the course of the study some EMG recordings were lost in each cat, as determined by the disappearance of EMG signals.

Tibial nerve stimulation and reflexes

A chronic stimulating electrode composed of bipolar wires (AS633; Cooner wire, Chatsworth, CA) embedded in a polymer (Denstply International) cuff (Julien and Rossignol 1982) was placed around the left Tib nerve at the ankle adjacent to the Achilles' tendon. The Tib nerve was stimulated (Grass S88 stimulator) at different intensities during spinal locomotion (e.g. before the neurectomy) with a single 1 ms pulse at a constant time (100 ms after onset of St burst) to determine the threshold for obtaining small yet consistent short-latency (~ 10 ms) responses in the left TA. Stimulation current was then set at 1.2 times this threshold and once reflex responses were qualitatively and quantitatively reproducible from one session to another for a few weeks (≥ 22 days), the same current was used before and after denervation. In all testing sessions, a stimulus was given once every three cycles at pseudo-random times to elicit responses throughout the step cycle for approximately 120-200 stimulations. After the initial implantation substantive scar tissue forms around the stimulating electrode and stabilizes it and it is thus improbable that the afferent volley evoked at the stimulating electrode changed over time.

Reflexes were measured as detailed previously (Frigon and Rossignol 2007). The EMGs were grouped into stimulated or control (non-stimulated) trials. The step cycle was divided into 10 phases by synchronizing the cycle to the onset of the left St. Averaged responses of EMGs with stimulation were separated into these 10 bins according to the time they were evoked in the cycle. An average of at least 50 control cycles provided a template of baseline locomotor EMG (blEMG) during the step cycle. Onsets and offsets of responses, denoted as prominent negative or positive

deflections away from the blEMG, were determined manually using pre-defined latencies as guidelines (Duysens and Stein 1978; Abraham et al. 1985; Pratt et al. 1991; Loeb 1993) such as P1 or N1 (~8-10 ms) and P2 (≥ 25 ms), where "P" and "N" are positive (excitatory) and negative (inhibitory) responses, respectively. For ipsilateral extensors, onsets and offsets were manually determined since P2 latencies vary slightly at different phases of the step cycle, whereas for ipsilateral flexors and contralateral muscles, the same windows were used pre- and post-neurectomy. The stimulated EMG from onset to offset was rectified and integrated and divided by the blEMG to provide a measure of reflex amplitude. Thus significant changes in reflex amplitude are independent of changes occurring in the underlying EMG, which can change after the neurectomy. Reflex responses evoked during swing (data points at phases 0.5 to 0.35) and those evoked during stance (data points phases at 0.55 to 0.85) were averaged together to provide an average reflex response during swing and stance, respectively.

Spinalization and training

Spinalization procedures were identical to previous studies (Bélanger et al. 1996). In the early days after spinalization training consisted of having two experimenters move the hindlimbs over the motorized treadmill to simulate locomotion while the forelimbs were positioned on a fixed platform located ~ 3 cm above the belt. The skin of the perineal region was stimulated to facilitate stepping movements. A Plexiglas separator was placed between the limbs to prevent them from impeding each other because of increased adduction. Initially, the experimenter supported the hindquarters by lifting the tail. After spinalization, recording sessions resumed once a steady locomotor pattern was attained with the experimenter providing equilibrium by gently holding the tail (Barbeau and Rossignol 1987; Bélanger et al. 1996)

Neurectomy

The left LGS nerve was cut by carefully separating the nerve from its surrounding tissue in the popliteal fossa and the proximal end was capped with flexible vinyl polysiloxane (Reprosil; Dentsply International, Milford, DE) to prevent nerve regeneration (Frigon and Rossignol 2007). The anesthesia and surgery for the neurectomy lasted under an hour and cats were tested the next day to allow sufficient recovery from surgery. Post-mortem analyses confirmed that the LGS nerve was cut and did not regenerate in each cat.

Statistics

To determine statistical differences for locomotor EMG bursts data from the 3 last trials recorded before the neurectomy (control) were averaged and compared to each session after the neurectomy using a one-way ANOVA followed by Dunnett's post hoc test for many comparisons against a control group if significant changes were detected by the ANOVA (Bouyer et al. 2001; Bouyer and Rossignol 2003b). Reflexes were analyzed identically except that each average reflex response in swing or stance was treated independently. For example, the average reflex response during swing in the left St in the control state were compared against the average reflex responses during swing in the left St for all day after the neurectomy.

Results

There was little deficit in the quality of the locomotor pattern after denervating the left LGS in all spinal cats, as previously shown (Bouyer et al. 2001). Spinal cats had no difficulty in maintaining the same treadmill speed as before denervation the day following the neurectomy. There were, however, several changes in EMG bursts and reflex responses during locomotion in the days following the neurectomy.

Changes in locomotor EMG bursts after denervation

The bursts of activity in several muscles were investigated during locomotion in the spinal state before and after LGS denervation to determine if the neurectomy produced changes in multiple muscles, as is the case in the intact state (Frigon and Rossignol 2007). Figure 2 shows EMG bursts for selected muscles before (left panel) and 2 days after (right panel) neurectomy of the left LGS in cat SD1. The burst magnitude and/or duration of several muscles, particularly Srt bilaterally and the right VL were altered 2 days post-neurectomy in this cat. Before the neurectomy the Srt burst discharged during the swing phase but 2 days post-denervation there was a large burst during stance as well.

A more complete representation of changes in mean amplitude of locomotor EMG bursts for selected muscles is provided in Figure 3 for each cat up to 22 days post-neurectomy with each colored line illustrating a different muscle. In cat SD1 (Fig. 3, top panel), muscles of the right leg (Srt, VL, LG), contralateral to the neurectomy, had large increases in the first few days post-denervation, a return towards pre-denervation values thereafter, followed by a second increase around 13 days in some muscles. The left and right VL increased immediately after the neurectomy and remained elevated for the remainder of the study. The mean amplitude of other muscles in the left leg (St and Srt) changed little post-neurectomy. In cat SD2 (Fig. 3, middle panel) the left MG increased considerably post-denervation and remained so for the rest of the study. The activity of other muscles increased in the first few days post-neurectomy. Whereas activity returned towards pre-denervation values in some muscles (left Srt, left VL, right LG) in some (Right VL) it could start to increase again at approximately 13 days post-neurectomy. In cat SD3 (Fig. 3, bottom panels), the left Srt had a large increase, which remained elevated for the duration of the study. The left VL peaked 2 days post-neurectomy and remained elevated up to about 16 days post-neurectomy. The mean amplitude of other muscles (right LG, right MG) increased up to about 4 days post-neurectomy before decreasing and increasing again around 13 days after denervation. The left St was relatively unchanged post-neurectomy. Therefore, as in intact cats (Frigon and

Rossignol 2007), denervating the left LGS produced changes in the activity of multiple muscles bilaterally although the amount of changes in specific subsets of muscles could differ from one spinal cat to another.

Locomotor EMG bursts were also used to measure step cycle duration (Fig. 4) and the onset of activity of different muscles (Table 1) before and after the denervation. Regression analyses were plotted and the correlation coefficient (r) was calculated (Sigmaplot 9.0) to determine if step cycle duration changed over time after the denervation (Fig. 4). There was a tendency for step cycle duration to decrease over time after the neurectomy in cats SD1 and SD2 while in cat SD3 step cycle duration did not change considerably. The onset of several muscle bursts was also altered post-denervation (Table 1). For instance, in cat SD1 the burst onset of muscles of the right leg (i.e. contralateral to denervation) occurred earlier within the step cycle up to ~ 22 days post-denervation. In cat SD3 onset of EMG bursts bilaterally was unchanged in the first 2 days but started to occur earlier from 4-14 days before returning to pre-denervation values. In cat SD2 there were no changes in burst onset bilaterally except an earlier onset 2 days post-denervation in the left Srt, left VL, and right VL.

Changes in reflexes after denervation

The Tib nerve was stimulated before and after a LGS neurectomy in the spinal state to determine if reflex responses were altered following the denervation, as is the case in the intact state (Frigon and Rossignol 2007). Responses in the left St evoked by stimulating the left Tib nerve in cats SD1 and SD2 for the 10 different phases of the step cycle are illustrated in Figure 5 before and at 1 and 10 days post-neurectomy. In cat SD1 (Fig. 5A), P1 responses either decreased or were unchanged 1 day after the neurectomy as compared to the spinal control state and returned to pre-denervation values at 10 days (phases 0.05 and 0.25) or could be increased (phase 0.15). P2 responses were mostly absent throughout the step cycle except for phase 0.05 and were not greatly affected by the neurectomy compared to the spinal state. In

cat SD2 (Fig. 5B), P1 responses increased throughout the step cycle, particularly during the stance phase (i.e. hatched responses in phases 0.45-0.85).

Figures 6-9 show average reflex responses in flexors of the left leg such as St (Figs. 6-7) and TA (Figs. 8-9) during swing and stance before and for all days after denervation of the left LGS in the spinal (left panels) and intact (right panels) states.

In the left St during swing (Fig. 6), the pattern of changes in the average reflex responses differed between cats in the spinal state (Fig. 6sA-C). For example, in cat SD1 (Fig. 6A) reflexes were unchanged up to 10 days post-denervation before showing an increase at 13 days and returning to control values. In cat SD2 (Fig. 6B) reflex responses increased up to 15 days post-denervation before returning to control values. In cat SD3 (Fig. 6C) responses were increased at 2 days, returned to control values up to 15 days, at which point they remained increased. In the intact state (Figs. 6D-F) changes in average reflex responses also differed from one cat to another. In cats SD1 (Fig. 6D) and SD3 (Fig. 6F) reflex responses tended to increase in the days following the denervation and returned to control values, although these changes were not significant in cat SD1. In cat SD2 (Fig. 6E), responses were unchanged up to approximately 8 days post-denervation before showing some increases. Thus, cats SD2, ID1, and ID3 shared similar patterns of reflex changes while cats SD3 and ID2 were similar.

In the left St during stance (Fig. 7), the average reflex response also differed from one spinal cat to another (Figs. 7A-C). In cat SD1 (Fig. 7A) responses were decreased in the first 3 days post-denervation before returning to control values and peaking 13 days. In cat SD2 (Fig. 7B), responses were increased up to 10 days post-denervation before returning to control values. In cat SD3 (Fig. 7C), responses were unchanged up to about 5 days post-denervation before showing increases at 7 days or more. In the intact state (Figs. 7D-F) changes in reflex responses also differed between cats but in general responses were increased at 1-2 days post-denervation. From that point responses started to return towards control values (Figs. D&F). In cat ID2, responses fluctuated but tended to remain increased over time. Thus, cats SD2,

ID1, and ID3 shared similar pattern of reflex changes post-denervation while cats SD3 and ID2 tended to have responses that increased and remained so over time.

In the left TA during swing (Fig. 8), changes in the average reflex response followed a different pattern from one spinal cat to another (Figs. 8A-C). In cats SD1 (Fig. 8A) and SD3 (Fig. 8C) besides an increase at 3 days in cat SD3 responses tended to increase over time. In cat SD2 (Fig. 8B) however, responses peaked at 2 days post-denervation before gradually returning towards control values. In the intact state (Figs. 8D-F) responses peaked at around 10 days post-denervation in cats ID2 (Fig. 8E) and ID3 (Fig. 8F) before returning towards control values whereas in cat ID1 (Fig. 8D) there were no changes. Although there were similarities in the pattern of reflex changes within spinal (i.e. SD1 and SD3) and intact (i.e. ID2 and ID3), contrary to the left St, there were no similarities in reflex changes between cats in the spinal and intact states.

In the left TA during stance (Fig. 9) responses were generally decreased post-denervation in cats SD1 (Fig. 9A) and SD3 (Fig. 9C) whereas in cat SD2 (Fig. 9B) responses increased and peaked at 7 days post-denervation before returning to control values. In the intact state (Figs. 9D-F), responses were unchanged in all cats in the first few days post-denervation before peaking at around 8-10 days. After 10 days, responses tended to return toward control values. Thus, cats SD1 and SD3 shared a similar pattern of reflex changes whereas cat SD2 was similar to cats in the intact state showing an increase that was maximal approximately 1 week post-denervation before returning towards control values.

If data of average reflex response is pooled together for all days in SD cats and in ID cats we see that in the intact state 57 out of 108 days post-denervation in the spinal state differed from the control value (53%) whereas in the intact state 32 out of 96 days differed from the control value (33%). However, in the spinal state, of the 57 significant days, 16 of those were a decrease compared to the control value while in the intact state there were no decreases. Decreases compared to control values were primarily found in the left TA during the stance phase of spinal cats (Figs. 9A and C).

To determine if reflex changes paralleled those of the mean amplitude of locomotor bursts reflex responses were averaged during swing or stance and were plotted against the mean amplitude of selected muscles that showed the greatest change post-neurectomy (Fig. 10). Reflex responses were averaged during the period of activity of the muscle. For example, in the left MG, P2 responses from phases 0.45-0.85 (i.e. stance) were averaged. Like the mean amplitude, the average reflex response was expressed as a percentage of the control value (i.e. before the neurectomy). A regression analysis was plotted and the correlation coefficient was calculated (Sigmaplot 9.0) to determine the strength of the linear relationship between the average reflex response and the mean amplitude before and after the neurectomy. Figure 10 shows that there were no positive correlations between changes in the average reflex response and mean amplitude of locomotor bursts post-neurectomy for all selected muscles. The correlation coefficient (r) was weak for all spinal cats and in intact cats the correlation was negative in all cases. Therefore, changes in reflex responses do not positively correlate with changes in the mean amplitude of locomotor bursts.

Discussion

Although detailed kinematic analyses were not performed all spinal cats could walk with no difficulty the day following the denervation at the same treadmill speed, as previously shown (Bouyer et al. 2001). In the present study, the effects of performing a neurectomy of the left LGS on the locomotor bursts and reflex responses evoked by Tib nerve stimulation of several muscles were investigated in spinal cats and comparisons were made with data previously obtained in the intact state. Changes in the mean amplitude of locomotor bursts of multiple muscles bilaterally were found in the days post-denervation, as is the case in otherwise intact cats (Frigon and Rossignol 2007). Moreover, although there were some differences in the pattern of reflex changes following denervation when comparing cats in the spinal and intact states there were also some similarities. The results suggest that intrinsic spinal mechanisms are involved in increasing muscle activity in multiple

hindlimb muscles bilaterally and in effecting reflex changes following a neurectomy of the left LGS, although supraspinal and/or propriospinal inputs most certainly also contribute.

Post-denervation changes in EMG bursts

In a previous study, denervating the LGS in spinal cats produced an increased ankle yield during early stance, which disappeared within approximately 2 weeks (Bouyer et al. 2001). However, besides small kinematic deficits, spinal locomotion was largely unaffected, a clear demonstration that the spinal cord can compensate for motor and/or sensory loss. Data presented here and elsewhere (Bouyer et al. 2001; Bretzner and Drew 2005a; Frigon and Rossignol 2007) indicate that changes in the activity of multiple muscles mediate functional recovery following muscle or cutaneous denervations, although large changes are evident and most prominent in close synergists following lesion of a mixed nerve (Pearson et al. 1999; Bouyer et al. 2001; Tachibana et al. 2006). Modified activity in several muscles could reduce the demand for increased force generation imposed on remaining synergists, some of them by providing simple biomechanical advantages, until more long-term adaptive mechanisms develop (Bouyer et al. 2001), such as muscular hypertrophy (Walsh, Jr. et al. 1978; Degens et al. 1995; Whelan and Pearson 1997). As in intact cats (Frigon and Rossignol 2007), the subset of muscle showing increased activity following the denervation could vary from one spinal cat to another. Therefore, even though walking is different in intact and spinal cats (Bélanger et al. 1996), an increase in the activity of multiple muscles appears to mediate the functional recovery after partially denervating ankle extensors.

Changes in reflex pathways following denervation

As in otherwise intact cats (Frigon and Rossignol 2007) reflex responses evoked by stimulating the Tib nerve were modified following denervation of the left LGS. Although there were differences in reflex changes following the denervations between spinal and intact states, there were also some similarities. For instance, the

pattern of changes of reflex responses in the left St during swing (Fig. 6) and stance (Fig. 7) were similar in some cats in the intact and spinal states. The similarities in reflex responses following a denervation in the intact and spinal states indicate that spinal mechanisms can mediate changes in cutaneous reflex pathways following a muscle denervation.

On the other hand, differences in reflex changes after denervation in the spinal and intact states suggest that different mechanisms also subserve reflex changes in the intact and spinal states. For instance, reflex responses could be decreased compared to control values in the spinal state, primarily in the left TA during stance, whereas in the intact state no significant decreases were found. Descending supraspinal inputs are known to influence the excitability in cutaneous reflex pathways in anesthetized cats (Fleshman et al. 1988) and during intact locomotion (Bretzner and Drew 2005b). and following a cutaneous denervation of the hindpaw corticospinal efficacy can increase (Bretzner and Drew 2005a), suggesting that descending inputs from cortical areas are involved in functional recovery by increasing the excitability in spinal circuits. While these studies have not shown that supraspinal inputs are involved in modifying cutaneous reflex pathways following a denervation it has been demonstrated that the corticospinal tract is required in operant conditioning of the soleus H-reflex in rats (Chen and Wolpaw 1997; Chen et al. 2006). Thus, it is probable that supraspinal structures are involved in mediating changes in reflex pathways following a LGS denervation.

Mechanisms of recovery

Enhanced transmission in sensory afferents from the Tib nerve cannot explain increased burst activity in multiple muscles following the neurectomy because no positive correlations between changes in reflex responses, at least from the Tib nerve, and those of EMG bursts during locomotion were found in the intact or spinal states (Fig. 10). This also clearly shows that changes in reflex pathways from the Tib nerve dissociate from those occurring at the motoneuron, inferred by EMG recordings, and thus pre-motoneuronal mechanisms must be involved in mediating reflex changes

after a LGS denervation. However, this does not exclude that other reflex pathways mediate increased activity in hindlimb muscles. Pearson and colleagues (Pearson et al. 1999) hypothesized that group I afferents from remaining synergists (i.e. MG) could mediate increased homonymous burst activity because the MG muscle is more stretched and must produce more force after denervating the LGS. However, changes in muscle length following denervation of the left LGS in spinal cats did not parallel changes in the activity of the left MG (Bouyer et al. 2001) and in another study, stretch reflexes evoked in MG following denervation of synergistic ankle extensors simply scaled with the underlying muscle activity, demonstrating that the gain of MG group I pathways was unchanged (Gritsenko et al. 2001).

The most likely explanation for the increased muscle activity is an enhanced central drive from the spinal CPG (Pearson et al. 1999; Gritsenko et al. 2001; Frigon and Rossignol 2007), which can mediate increases to multiple muscles simultaneously. Changes in reflex pathways, including those from the Tib nerve, could provide important signals to the spinal CPG because sensory feedback from large diameter afferents is required for functional recovery following partial denervation of ankle extensors (Pearson et al. 2003). What is clear, however, is that the isolated spinal cord can compensate for the loss of sensory and motor innervation and induce changes in reflex pathways from the sole of the foot.

Functional significance

The present results showed that spinal mechanisms can generate increases in multiple hindlimb muscles bilaterally and produce changes in the gain of reflex pathways from the Tib nerve. Several changes in reflex responses were noted in the spinal state following a neurectomy of the left LGS suggesting that changes in the gain of cutaneous reflexes can compensate for the loss of two primary ankle extensors. A greater percentage of reflex changes were reported in the spinal state compared to the intact state, which could reflect the greater dependency of sensory feedback from the moving hindlimbs during spinal locomotion. The spinal cord possesses a rich and complex circuitry and should not be underestimated in efforts to

facilitate the recovery of motor functions following spinal cord injury. Changes in cutaneous reflex pathways appear of particular importance following a muscle denervation.

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Figures and table

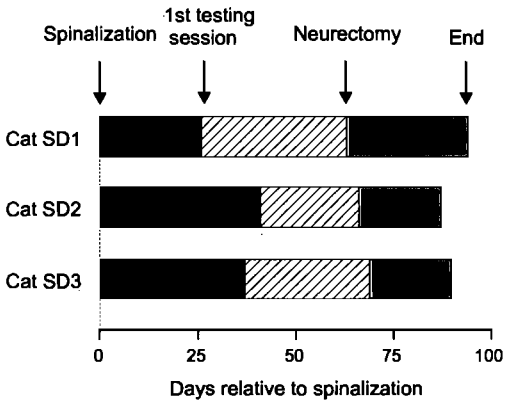

Figure 1. Sequence of events in SD cats (SD: Spinal-Denervation). Spinalization was initially made and recordings resumed once spinal locomotion was stable (1st testing session). After a few weeks of baseline recordings the left LGS was cut and recordings resumed the following day.

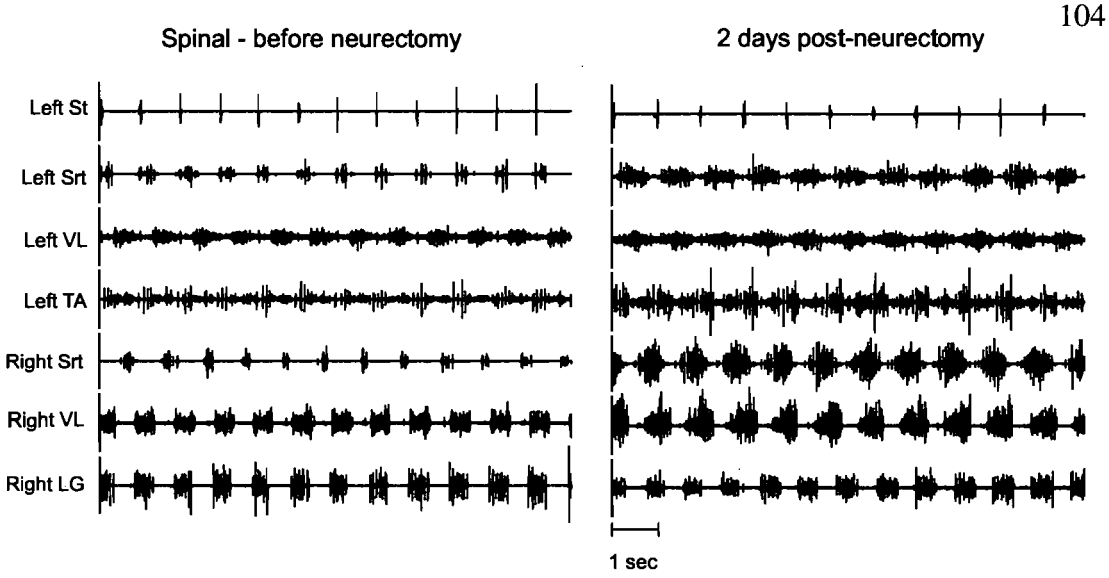

Figure 2. The locomotor EMG pattern of selected muscles for 10s before (left panel) and 2 days after neurectomy of the left LGS (right panel) in cat SD1.

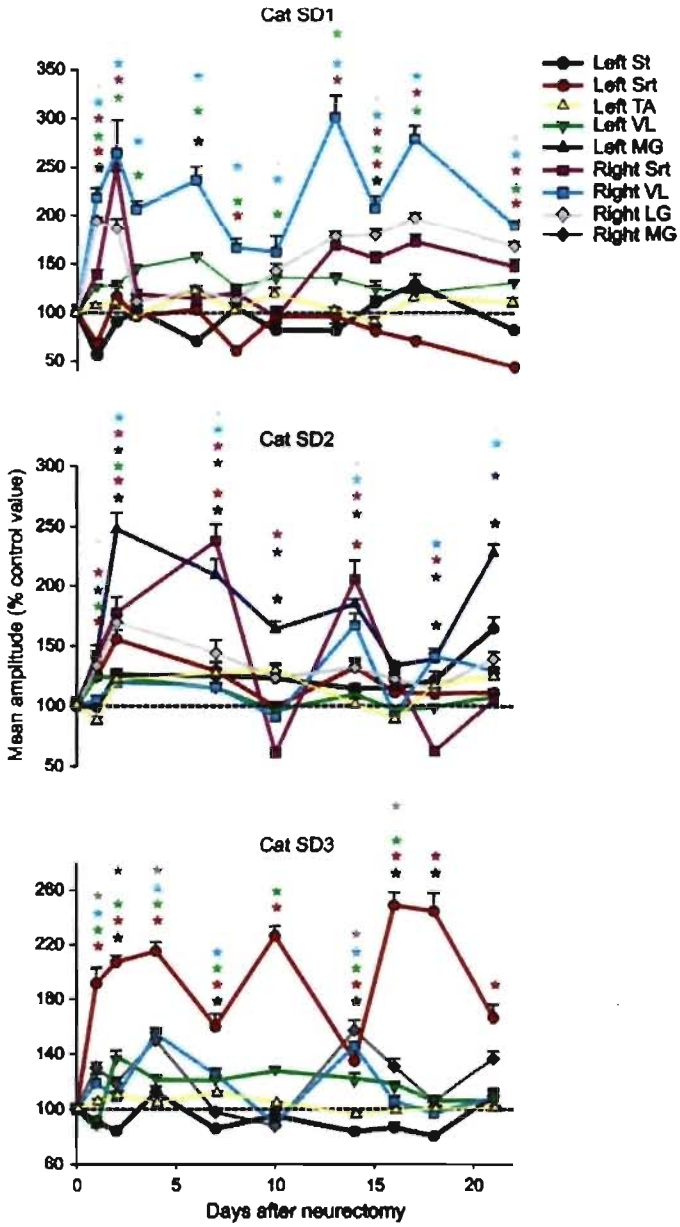

Figure 3. Changes in mean EMG amplitude for selected muscles during locomotion for up to 22 days post-neurectomy expressed as a percentage of the control value in cats SD1-3, from top to bottom, respectively. Each colored line represents a different muscle. Significant differences for the days after the neurectomy, for a given muscle, are indicated by an asterisk above the topmost line in the same color ($p < 0.05$). Each data point is the mean ± SEM of ~20 locomotor bursts.

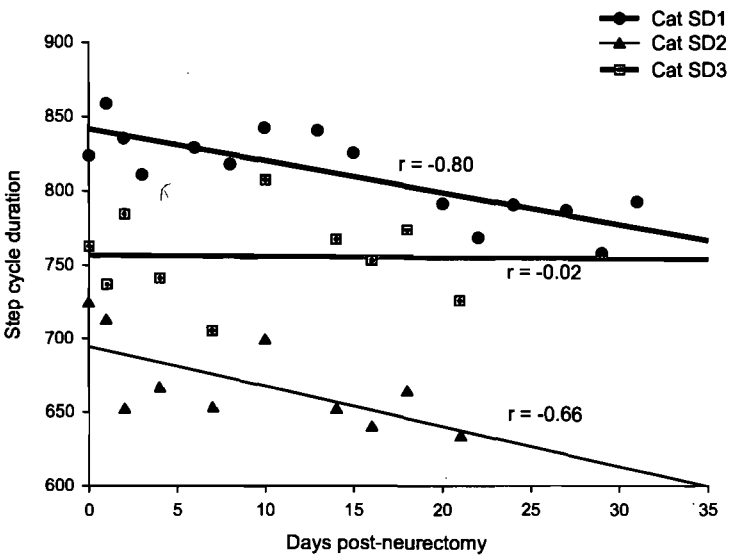

Figure 4. Step cycle duration before (time 0) and for all recorded days after the LGS neurectomy in each SD cat.

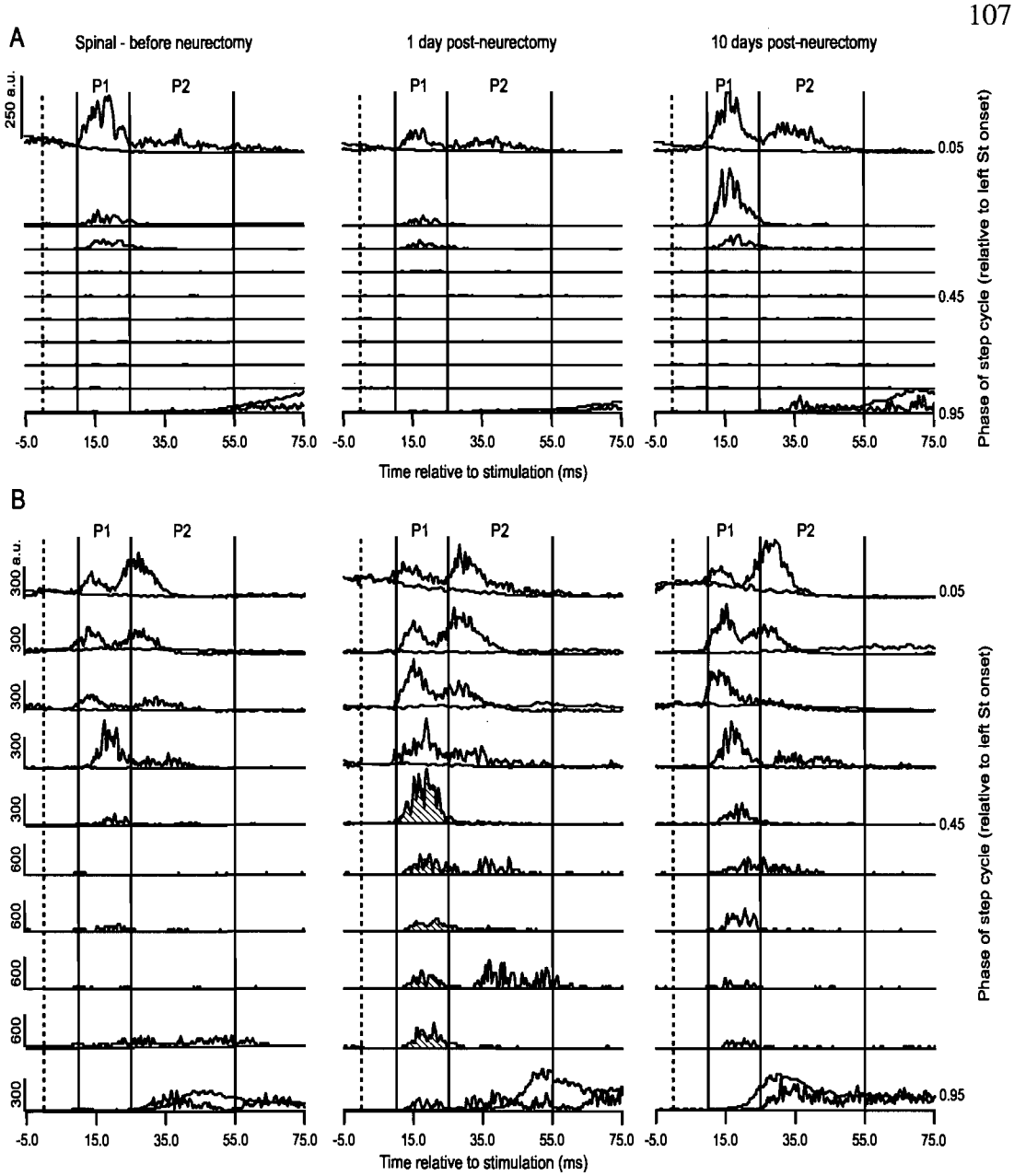

Figure 5. Averaged reflex responses before and after the LGS neurectomy for the left St of cats SD1 (Fig., 5A) and SD3 (Fig., 5B) before (left panels) 1 day (middle panel) and 10 days (right panel) after the neurectomy. Values are at the same scale in arbitrary units (a.u.) for all phases in cat SD1 whereas in cat SD2 phases 6-9 differ from those of phase 1-5 and 10 to highlight changes in P1 responses at 2 days post-denervation during stance (hatched areas). Windows used to determine P1 and P2 responses are indicated. The first horizontal trace for a given day is phase 0.0-0.01 of the step cycle synchronized to left St burst onset.

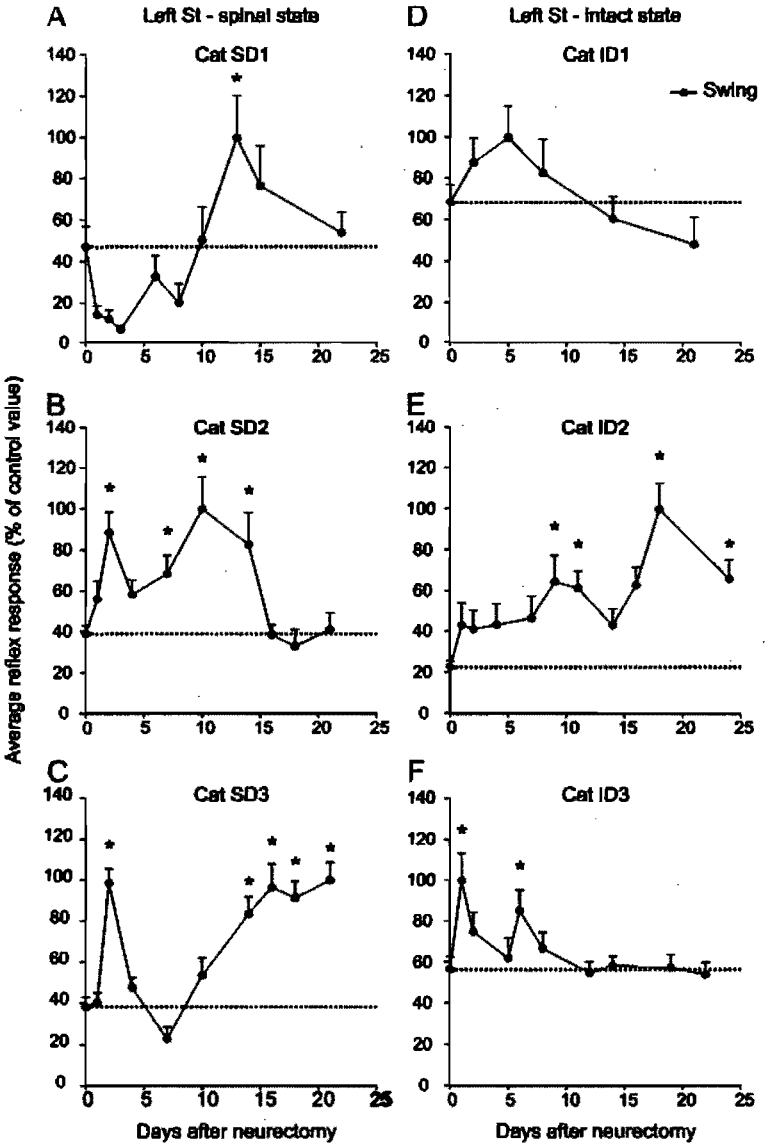

Figure 6. Averaged reflex responses of the left St during swing in cats SD1-3 (left panels) and ID1-3 (right panels) before (day 0) and for several days after denervating the left LGS, expressed as the maximal value before or after denervation. Asterisks indicate significant differences from the control value (i.e. before denervation) ($p < 0.05$). Each data point is the mean ± SEM of approximately 30-150 responses.

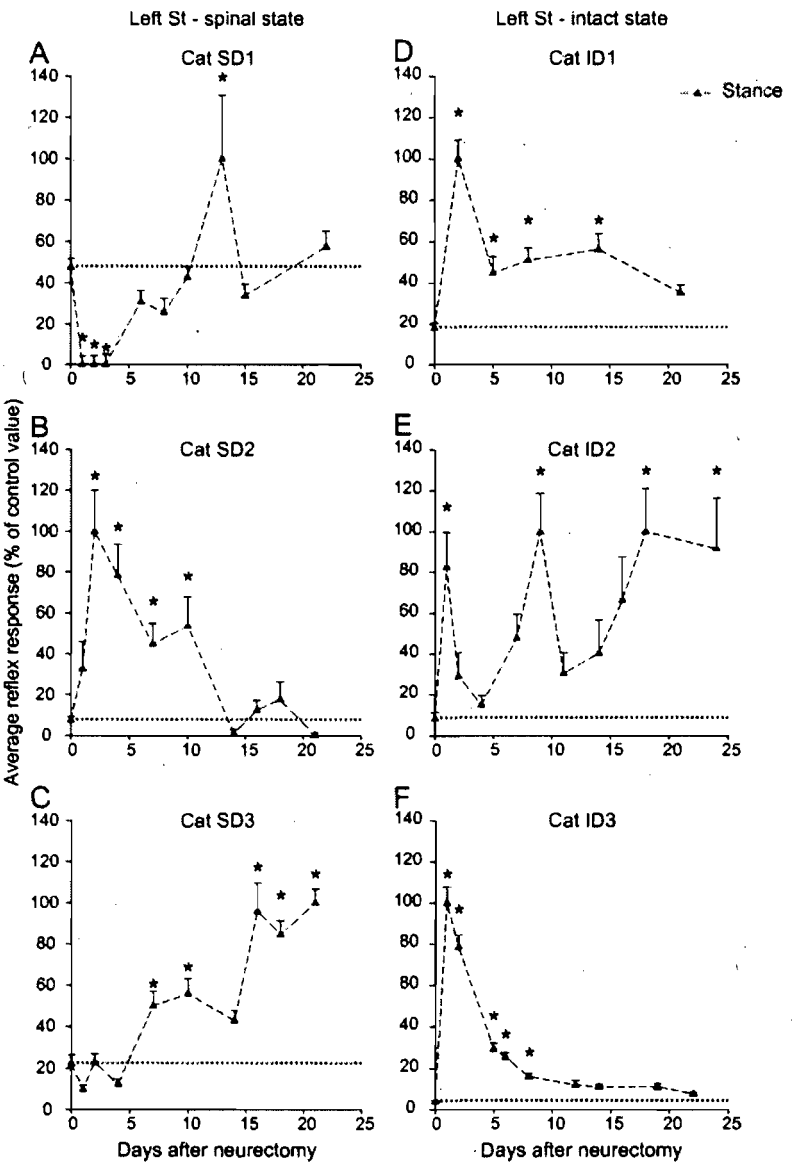

Figure 7. Averaged reflex responses of the left St during stance in cats SD1-3 (left panels) and ID1-3 (right panels) before (day 0) and for several days after denervating the left LGS, expressed as the maximal value before or after denervation. Asterisks indicate significant differences from the control value (i.e. before denervation) ($p < 0.05$). Each data point is the mean ± SEM of approximately 30-150 responses.

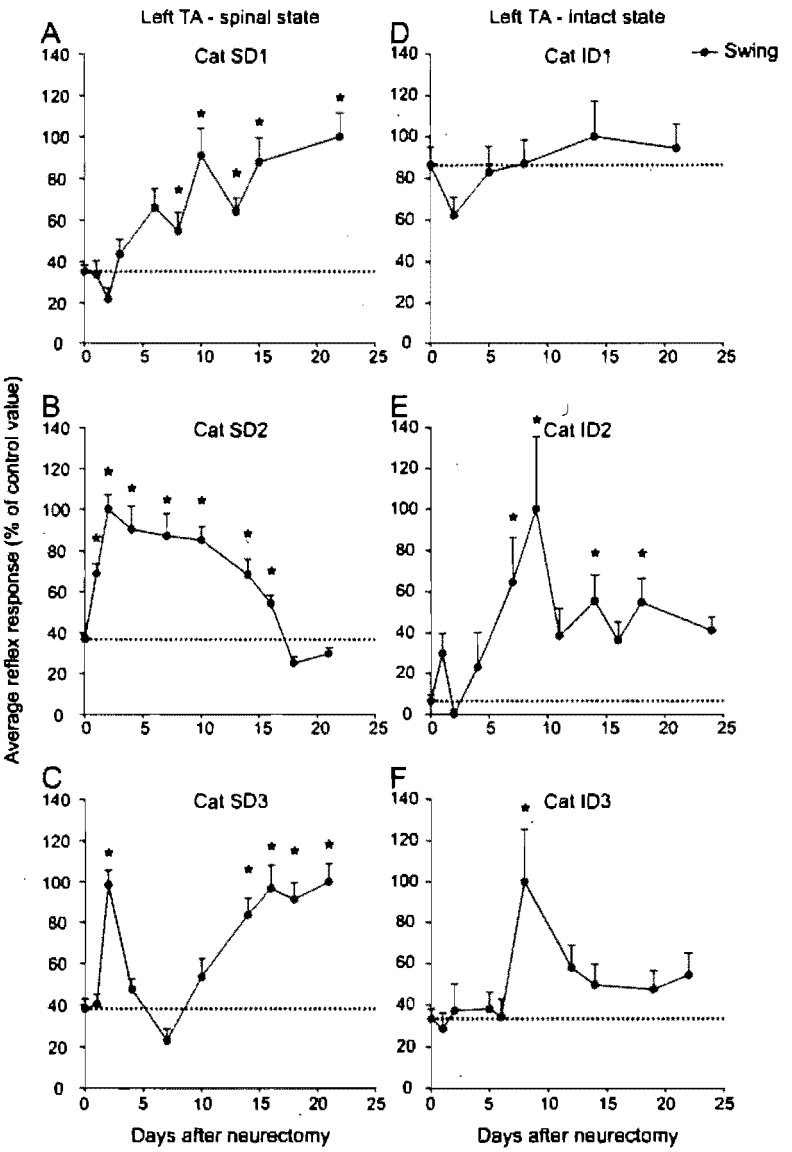

Figure 8. Averaged reflex responses of the left TA during swing in cats SD1-3 (left panels) and ID1-3 (right panels) before (day 0) and for several days after denervating the left LGS, expressed as the maximal value before or after denervation. Asterisks indicate significant differences from the control value (i.e. before denervation) ($p < 0.05$). Each data point is the mean ± SEM of approximately 30-150 responses.

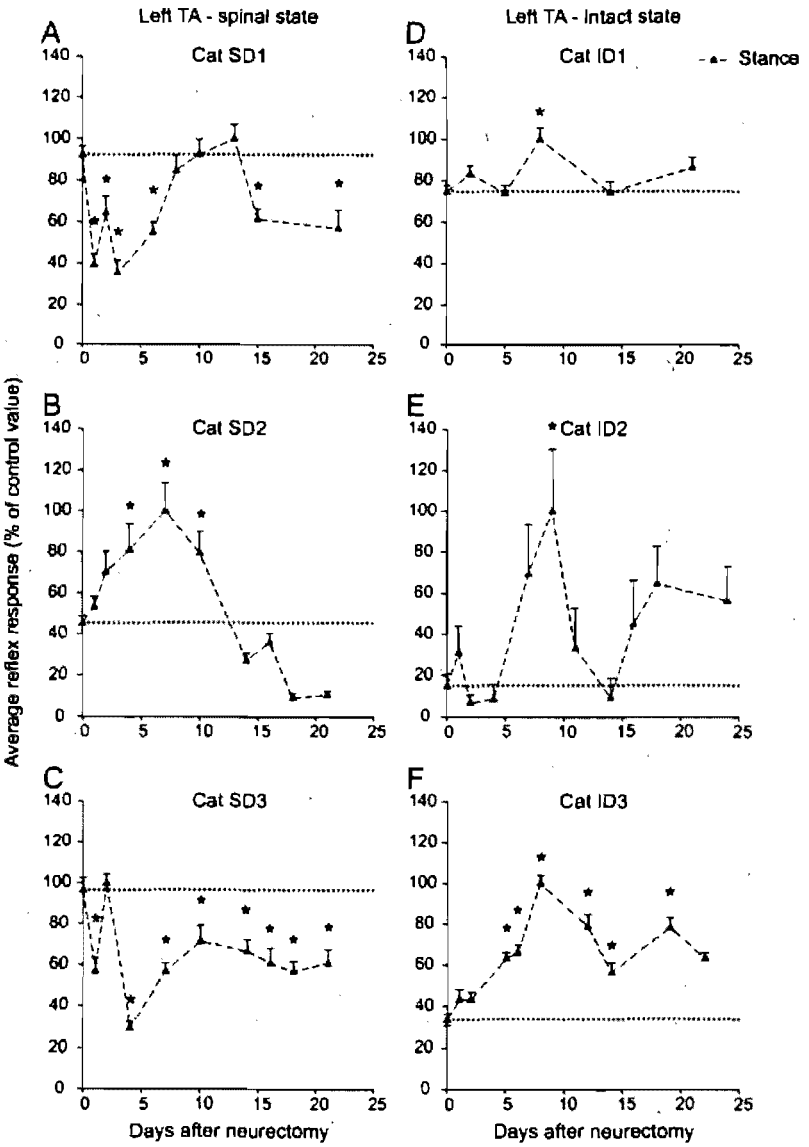

Figure 9. Averaged reflex responses of the left TA during stance in cats SD1-3 (left panels) and ID1-3 (right panels) before (day 0) and for several days after denervating the left LGS, expressed as the maximal value before or after denervation. Asterisks indicate significant differences ($p < 0.05$) from the control value (i.e. before denervation). Each data point is the mean ± SEM of approximately 30-150 responses.

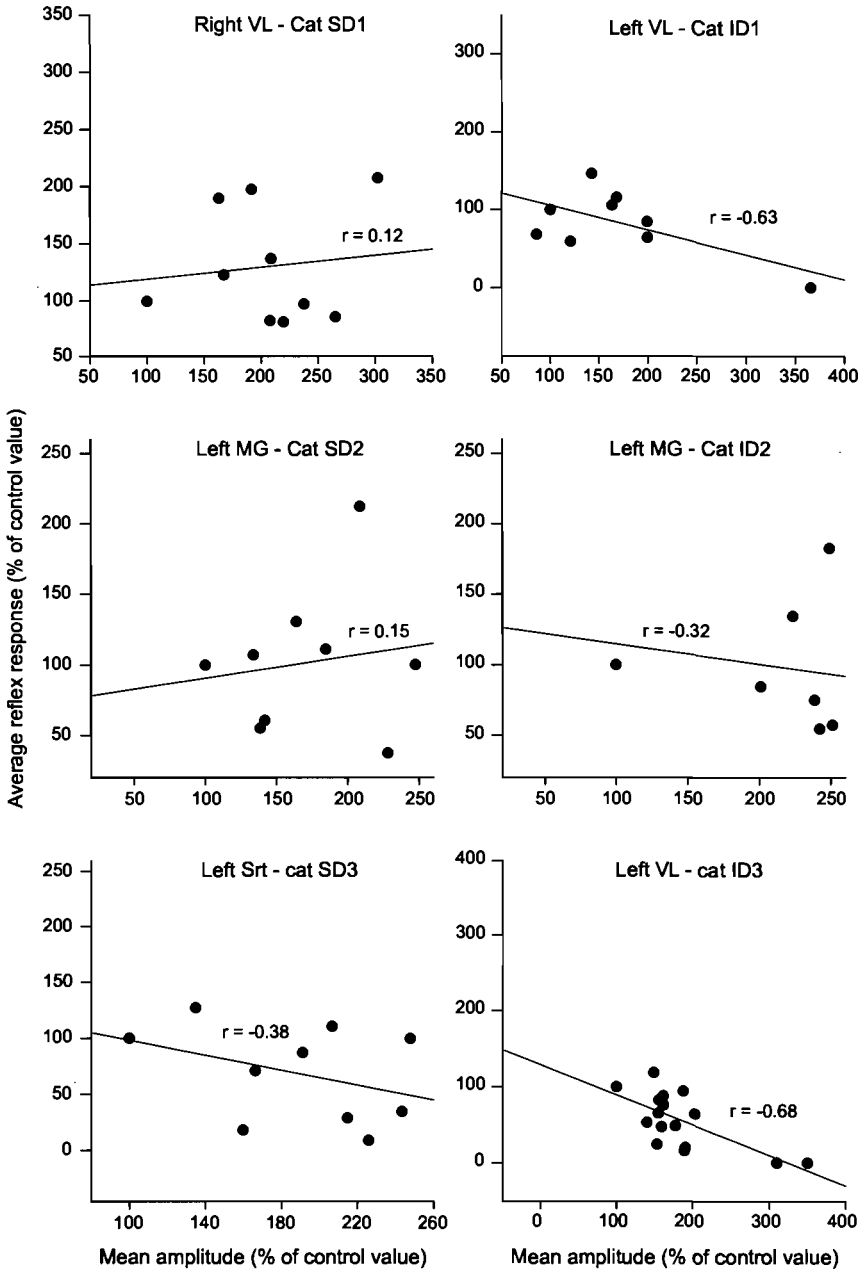

Figure 10. Regression analyses between the average reflex response and mean locomotor burst amplitude of selected muscles in cats SD1-3 (left panels) and ID1-3 (right panels) expressed as a percentage of the control value. Each data point is the average of approximately 30-150 reflex responses and 20 locomotor bursts. The correlation coefficient (r) is plotted next to the regression line.

Burst onset normalized to start of left St burst (% of step cycle)

SD1

Days	Left Srt*	Left VL*	Left TA*	Right Srt*	Right VL*	Right LG*
Control	-1 ± 1	31 ± 4	-8 ± 4	61 ± 4	-14 ± 2	-17 ± 3
1	-1 ± 2	31 ± 3	-2 ± 2*	37 ± 3*	-23 ± 4*	-30 ± 3*
2	2 ± 3	36 ± 5*	8 ± 4*	40 ± 4*	-29 ± 4*	-31 ± 5*
3	-2 ± 3	25 ± 3*	0 ± 6*	38 ± 4*	-27 ± 3*	-32 ± 4*
6	-5 ± 3*	24 ± 3*	-3 ± 3*	38 ± 6*	-16 ± 4	-29 ± 5*
8	7 ± 8*	32 ± 3	5 ± 7*	44 ± 6*	-25 ± 3*	-28 ± 4*
10	-3 ± 6	27 ± 5*	0 ± 6*	47 ± 5*	-17 ± 4	-25 ± 6*
13	1 ± 3	31 ± 4	1 ± 38*	40 ± 3*	-22 ± 5*	-36 ± 7*
15	1 ± 2	37 ± 3*	5 ± 4*	41 ± 3*	-18 ± 4*	-29 ± 6*
22	10 ± 8	30 ± 5	3 ± 4*	44 ± 5*	-23 ± 4*	-25 ± 4*

SD2

Days	Left Srt*	Left VL*	Right Srt	Right VL*	Left MG	Right LG
Control	-3 ± 6	34 ± 5	49 ± 13	-14 ± 7	30 ± 7	-17 ± 6
1	-4 ± 3	34 ± 5	47 ± 6	-15 ± 7	30 ± 6	-19 ± 6
2	0 ± 2*	40 ± 7*	49 ± 9	-10 ± 4*	35 ± 5	-15 ± 8
7	-5 ± 4	36 ± 5	48 ± 4	-10 ± 5	26 ± 5	-19 ± 6
10	-2 ± 3	35 ± 5	46 ± 3	-13 ± 6	31 ± 6	-16 ± 6
14	-4 ± 2	33 ± 3	47 ± 4	-13 ± 5	27 ± 4	-18 ±6
16	-2 ± 2	35 ± 4	50 ± 5	-10 ± 5	33 ± 5	-14 ± 5
18	2 ± 4*	36 ± 5	51 ± 6	-12 ± 8	33 ± 5	-18 ± 5
21	-1 ± 3	34 ± 3	44 ± 6	-11 ± 4	31 ± 3	-17 ± 5

SD3

Days	Left Srt*	Left VL*	Right VL*	Right TA*	Right MG*
Control	1 ± 8	30 ± 8	-21 ± 9	44 ± 9	-27 ± 10
1	-4 ± 3	32 ± 9	-18 ± 5	52 ± 7*	-25 ± 8
2	-8 ± 6*	22 ± 6*	-20 ± 9	46 ± 8	-24 ± 8
4	-12 ± 8*	17 ± 9*	-31 ± 12*	38 ± 12*	-36 ± 12*
7	-12 ± 8*	21 ± 8*	-24 ± 10	40 ± 10	-29 ± 10
14	-2 ± 2	31 ± 2	-17 ± 3	50 ± 3*	-20 ± 3*
16	-7 ± 10*	26 ± 8	-21 ± 12	46 ± 7	-23 ± 12
18	-4 ± 2	31 ± 2	-16 ± 5	47 ± 4	-20 ± 6*
21	-2 ± 2	31 ± 4	-20 ± 4	47 ± 5	-24 ± 6

Table 1. Burst onset of available muscles expressed as a percentage of the step cycle beginning witih left St burst onset before and after the neurectomy. Asterisks indicate significant differences ($p < 0.05$) from the control value (i.e. before denervation). Each value is the mean ± standard deviation of approximately 20 locomotor bursts.

Chapter 4 - Article 3. Adaptive changes of the locomotor pattern and cutaneous reflexes during locomotion studied in the same cats before and after spinalization

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Abstract

Descending supraspinal inputs exert powerful influences on spinal reflex pathways in the legs. Removing these inputs by completely transecting the spinal cord changes the state (i.e. the configuration of the spinal circuitry) of the locomotor network and undoubtedly generates a reorganization of reflex pathways. To study changes in reflex pathways after a complete spinalization, we recorded spinal reflexes during locomotion before and after a complete transection of the spinal cord at the 13th thoracic segment in cats. We chronically implanted electrodes in 3 cats, to record electromyography (EMG) in several hindlimb muscles and around the left tibial (Tib) nerve at the ankle to elicit reflexes during locomotion before and after spinalization in the same cat. Control values of kinematics, EMGs and reflexes were obtained during intact locomotion for 33–60 days before spinalization. After spinalization, cats were trained 3-5 times a week on a motorized treadmill. Recordings resumed once a stable spinal locomotion was achieved (26-43 days), with consistent plantar foot placement and full hindquarter weight support without perineal stimulation. Changes in Tib nerve reflex responses after spinalization in the same cat during locomotion were found in all muscles studied and were often confined to specific phases of the step cycle. The most remarkable change was the appearance of short-latency excitatory responses in some ipsilateral ankle extensors during stance. Short-latency excitatory responses in the ipsilateral tibialis anterior were increased during stance, whereas in other flexors such as semitendinosus and sartorius, increases were mostly confined to swing. Longer-latency excitatory responses in ipsilateral flexors were absent or reduced. Responses evoked in limb muscles contralateral to stimulation were generally increased throughout the step cycle. These reflex changes after spinalization provide important clues regarding the functional reorganization of reflex pathways during spinal locomotion.

Keywords: Reflex, locomotion, spinal cord injury

Introduction

Stimulation of the skin or cutaneous nerves of the foot in anesthetised preparations at rest and during fictive locomotion produces a mixture of inhibitory and excitatory post-synaptic potentials in several lumbosacral motoneurons, which indicates that parallel excitatory and inhibitory pathways converge on a given motor pool (Burke, 1999; McCrea, 2001). As a result, during normal locomotion stimulating cutaneous nerves evokes a complex pattern of response in multiple hindlimb muscles, which can include inhibitory and excitatory responses within the same muscle (Duysens & Loeb 1980; Abraham *et al.* 1985; Loeb, 1993). For example, during swing, short- (P1) and longer- (P2) latency excitatory responses are evoked in ipsilateral flexors at ~8-10 ms and ~25-30 ms, respectively. P1 and P2 responses can be modulated independently during the step cycle with large P1 responses being present during early- to mid-swing whereas P2 responses are generally more prominent in late stance (Rossignol et al. 2006). In ipsilateral extensors, P1 responses can also be evoked during the swing phase but during stance short-latency inhibitory (N1) and longer-latency excitatory (P2-P3) responses are evoked at ~8-10 ms and ~30-45 ms, respectively. In flexors and extensors contralateral to the stimulation P2 responses are usually observed at ~20-25 ms (Duysens & Loeb 1980).

The presence of excitatory and inhibitory responses in ipsilateral extensors suggests that both excitatory and inhibitory last order interneurons converge on motoneurons but that they are dynamically modulated according to the phase of the step cycle. Different structures could be involved in this dynamic modulation. For instance, interneurons interposed in cutaneous reflexes from the foot receive inputs from 1) other peripheral afferents, 2) the spinal central pattern generator (CPG) and 3) from supraspinal and propriospinal inputs (Rossignol *et al.* 2006). Removing any of these inputs creates a new "state", defined as the configuration and excitability of locomotor circuitry, in the spinal CPG and potentially impacts the modulation of inhibitory and excitatory reflex responses during locomotion. Lesions at various levels of the nervous system can modify the state of the system and alter the phase-

dependent modulation of spinal reflexes therefore providing a tool to investigate the role of various structures in the organization and modulation of reflex pathways (Burke 1999). In a recent study we recorded reflex responses evoked by stimulating the tibial (Tib) nerve at the ankle during locomotion before and after lesioning the mixed nerve supplying the lateral gastrocnemius and soleus (LGS) in otherwise intact cats (Frigon & Rossignol 2007a). The increase in several reflex pathways following the LGS denervation, which could have resulted from a dis-inhibition of short- and longer-latency excitatory pathways, suggests that afferent inputs from the LGS normally project to interneurons interposed in reflex pathways from the Tib nerve.

Although the pattern of reflex responses has been described during locomotion in intact and spinal cats the functional reorganization of reflexes from an intact to a spinal state in the same cat is unknown. In the present study we recorded reflex responses evoked by stimulating the Tib nerve in the same cat before and after a complete transection of the spinal cord at the 13th thoracic segment (T13). Numerous studies have investigated changes in spinal reflex pathways following spinal cord injury [for a review see (Frigon & Rossignol 2006)] but most of these studies have compared reflexes between groups of intact and groups of spinal cord-injured animals. One of the major limitations of such studies is that reflex pathways differ between individuals (Loeb, 1993; Zehr *et al.* 1997) making the functional reconfiguration of reflex pathways difficult to assess by comparing intact and spinal cord-injured groups. A few studies have used chronic recordings to examine reflex changes after spinal injury in the same animals (Bennett *et al.* 1999; Bennett *et al.* 2004; Lavrov *et al.* 2006) but not in a locomotor condition. Descending commands from the brain and brainstem are known to exert powerful influences on spinal reflex pathways (Holmqvist & Lundberg 1959) and therefore studying the reorganization of reflex pathways during locomotion after complete spinalization could provide important clues as to their intrinsic organization.

Methods

Animals and general procedures

Three adult cats (2 males, 1 female) weighing between 3.0 and 4.5 kg were selected based on their ability to walk for prolonged periods on a treadmill and trained for a few weeks at their preferred speed (0.4-0.5 m/s). Cats were then chronically implanted with electrodes for EMG recordings and nerve stimulation, allowed to recover from the implantation, and then values of EMGs and reflexes were recorded. After stable control data were obtained (33 – 60 days), a complete spinalization at T13 was performed. Recordings resumed following a few weeks of locomotor training once a stable full weight-bearing spinal locomotion was achieved (26 – 43 days). Stable locomotion in the intact and spinal states corresponded to periods where stepping was visibly the same from one session to another with similar limb movements, locomotor bursts, and reflexes. It should be emphasized that the modulation of phase-dependent reflexes cannot be rigorously studied unless the animal is capable of achieving long periods of several hundred consecutive steps. Therefore, the gradual evolution of reflexes in the weeks following spinalization before attaining stability of walking could not be studied. Cats were studied for several days after the expression of a stable spinal locomotion (25 – 37 days). At the termination of experiments, cats were sacrificed with an overdose of sodium pentobarbital.

The experimental protocol was in accordance with the guidelines of the animal Ethics Committee of the Université de Montréal. All surgical procedures were performed under general anesthesia and aseptic conditions. Prior to surgery, cats were injected with an analgesic (Anafen 2 mg/kg; subcutaneously) and pre-medicated (Atravet 0.1 mg/kg, Glycopyrrolate 0.01 mg/kg, Ketamine 0.01 mg/kg; intramuscularly). Cats were then intubated and maintained under gaseous anesthesia (isoflurane 2%) while heart rate and respiration were monitored. After surgery, an analgesic (Buprenorphine 0.01 mg/kg) was administered subcutaneously. An oral antibiotic (cephatab or apo-cephalex, 100 mg/day) was given for 10 days following surgery.

Implantation of electromyographic (EMG) electrodes

Chronic electromyographic (EMG) electrodes were implanted bilaterally in the following hindlimb muscles: semitendinosus (St: knee flexor/hip extensor), anterior part of sartorius (Srt: hip flexor/knee extensor), vastus lateralis (VL: knee extensor), lateral gastrocnemius (LG: ankle extensor/knee flexor), medial gastrocnemius (MG: ankle extensor/knee flexor), and tibialis anterior (TA: ankle flexor). A pair of Teflon-insulated multistrain fine wires (AS633; Cooner wire, Chatsworth, CA) was directed subcutaneously from head-mounted fifteen pin connectors (Cinch Connectors; TTI Inc., Pointe-Claire, Canada) and sown into the belly of each muscle for bipolar EMG recordings. Over the course of the study some EMG recordings were lost in muscles of the left (i.e. ipsilateral to stimulation) and right (contralateral to stimulation) hindlimbs. For instance, the ipsilateral Srt and TA of cat 1, the ipsilateral MG of cat 2 and the contralateral St and MG of cat 3 were lost, as determined by the disappearance of EMG signals. Moreover, some muscles discharged irregularly and the burst could not be delineated although reflex responses could be recorded in these muscles. This is the case for the contralateral Srt of cat 1 and both TAs of cat 3. EMG recordings were bandpass filtered (100-3000 Hz) and amplified (gains of 200-5000) using two Lynx-8 amplifiers (Neuralynx, Tucson, Arizona). EMG data were digitized (5000 Hz) using custom-made acquisition software. The duration, mean amplitude, and timing of EMG bursts during locomotion relative to ipsilateral St burst onset were calculated using custom software. Onsets and offsets of EMG bursts were determined visually. Mean amplitude was defined as the area under the rectified EMG burst divided by its duration and expressed as a percentage of the averaged intact value to evaluate locomotor EMG burst changes after spinalization.

Kinematics

Kinematic data of the left hindlimb were captured before and after spinalization using a Panasonic digital 5100 camera (1/1000 s shutter speed, 30 frames/sec = 60 fields = time resolution of 16,7ms) and a Sony RDR-GX315 DVD recorder during treadmill locomotion. Small reflective markers were placed over prominent bony landmarks at

each joint of the hindlimbs including the iliac crest, greater trochanter, lateral epicondyle, lateral malleolus, metatarso-phalangeal (MTP) joint, and the tip of the fourth toe. Joint angles (hip, knee, ankle, MTP) were reconstructed off-line using custom-made software with a resolution of 60 fields/sec from ≥ 20 step cycles. Step cycle duration was measured as the time between two successive left foot contacts. The duration of stance was measured as the time between contact and lift of the left foot whereas swing duration was calculated as the difference between step cycle and stance durations.

Spinalization and locomotor training

After establishing stable control (EMG and reflexes) recordings a laminectomy was performed at T13. The dura was removed and, after local lidocaine application (Xylocain, 2%), the spinal cord was completely transected with surgical scissors. Hemostatic material (Surgicel) was inserted within the gap and muscles and skin were sown back to close the opening. After a few days, cats were trained 3-5 times a week to walk on the treadmill. In the early days following spinalization training consisted of having two experimenters move the hindlimbs over the motorized treadmill to simulate locomotion while the forelimbs were positioned on a fixed platform located ~3 cm above the belt. The skin of the perineal region was stimulated to evoke stepping movements. A Plexiglas separator was placed between the limbs to prevent them from impeding each other because of increased adduction. Initially, the experimenter supported the hindquarters by lifting the tail. No pharmacological agents were used during training. Recording sessions resumed once the animals attained a stable locomotor pattern without perineal stimulation, with full hindquarter weight support and consistent plantar foot placement. The experimenter provided equilibrium by holding the tail (Barbeau & Rossignol 1987; Bélanger *et al.* 1996). To effectively compare reflex changes before and after spinalization all bouts of irregular stepping during spinal locomotion were precluded from analysis.

Tibial nerve stimulation and reflexes

A chronic bipolar stimulating electrode composed of wires (AS633; Cooner wire, Chatsworth, CA) embedded in a polymer (Denstply International) cuff (Julien & Rossignol 1982) was placed around the left Tib nerve at the ankle adjacent to the Achilles' tendon. The Tib nerve was stimulated with a Grass S88 stimulator connected in series with a constant current isolation unit (Grass PSIU6) and a custom-made current measurement unit that monitors the actual current delivered. Initially, stimulation was delivered at different intensities during locomotion with single 1 ms pulses 100 ms after ipsilateral St burst onset to determine the reflex threshold for obtaining small yet consistent short-latency (~10 ms) responses in the ipsilateral TA. Stimulation current was then set at 1.2 times this threshold to evoke reflexes. The cats did not seem to notice the stimulation during locomotion and as a result long sequences (> 10 minutes) of stimulation could be obtained. This intensity is slightly above the one required to record the threshold of an afferent volley in the sciatic nerve and reflex responses are thought to be mediated by the largest diameter Aβ afferents (Loeb, 1993). However, influences from group I or II afferents cannot be eliminated since the Tib nerve also supplies intrinsic foot muscles. The stimulation did not visibly alter limb trajectory during intact or spinal locomotion, which is important because perturbations of the limb could introduce responses linked to the movement, such as proprioceptive reflexes. The pattern of responses, as opposed to amplitude, is typically invariant unless very high stimulus intensities are used (Abraham *et al.* 1985; Loeb, 1993). Therefore, our responses were probably mediated by low-threshold cutaneous afferents.

A computer-generated pseudo-random sequence delivered 10 stimuli in the different 10 sub-phases of the cycle for a total of approximately 120-200 stimuli, each stimulus being given once every three cycles. Once reflexes were qualitatively and quantitatively reproducible from one session to another for a few weeks (33-60 days) the same stimulation current was used for the remainder of the study before and after spinalization. The current required to reach reflex threshold was assessed post-spinalization and did not change by more than a few μA.

Reflexes were measured as detailed previously (Frigon & Rossignol 2007a; Frigon & Rossignol 2007b). The EMGs were grouped into stimulated or control (non-stimulated) trials. The step cycle was divided into 10 phases by beginning the cycle from the ipsilateral St burst onset. Averaged EMG responses with stimulation were separated into these 10 bins according to the time in the cycle they were evoked. An average of at least 50 control cycles provided a template of baseline locomotor EMG (blEMG) during the step cycle. The latency of responses, denoted as prominent negative or positive deflections away from the blEMG, were determined manually using pre-defined latencies as guidelines (Duysens & Stein 1978; Drew & Rossignol 1985; Abraham *et al.* 1985; Pratt *et al.* 1991; Loeb, 1993) such as P1 or N1 (~8-10 ms) and P2 (≥ 25 ms), where "P" and "N" are positive (excitatory) and negative (inhibitory) responses, respectively. The latencies of responses are given in Table 2. Note that P2 latencies for St and TA are not provided because in most instances there was not clear distinction between P1 and P2 responses.

To quantify reflex responses windows were set before and after spinalization in muscles of the ipsilateral and contralateral hindlimbs. For ipsilateral flexors (see Fig. 5), time windows were the same before and after spinalization: St: P1 = 10-25 ms, P2 = 25-55 ms; TA: P1 = 10-30 ms, P2 = 30-55 ms. A time window of 15-50 ms was used for the ipsilateral Srt before and after spinalization because this muscle often shows a prolonged reflex response during that time period and not two distinct responses. For contralateral muscles (Srt, TA, VL) a time window of 15-40 ms was used before and after spinalization. For ipsilateral extensors (see Fig. 3), onsets and offsets of responses were manually determined pre-and post-spinalization if an inhibition was present because P2 latencies change somewhat throughout the step cycle as the duration of N1 varies from one phase to another. However, if P1 responses appeared during stance in ankle extensors after spinalization time windows of 10-25 ms and 25-55 ms were respectively used for P1 and P2.

To measure reflexes, the stimulated and non-stimulated EMG within the determined window was integrated. The non-stimulated integrated EMG within the same window was then subtracted from the integrated stimulated value. Because

reflex amplitude is known to scale (i.e. automatic gain control) with the level of EMG activity (Matthews 1986), the subtracted value was then divided by a fixed 15 ms block of blEMG in the same phase [see Fig. 1 of (Frigon & Rossignol 2007b) for an example], thus giving a reflex amplitude normalized to the level of baseline locomotor activity. Inhibitory responses, in our context, can only be quantified when there is a baseline level of EMG. Often the inhibitory response scales with the level of blEMG but the percent inhibition relative to blEMG remains the same. In order to use a single method to compare inhibitory and excitatory responses in two conditions with different levels of blEMG we divided the subtracted responses by the level of blEMG. Therefore, reflex responses are expressed as a percentage of the level of baseline locomotor activity in the intact and spinal states. If normalized reflex values in the spinal state were different from those in the intact state then reflex responses did not simply scale with changes in blEMG that occurred as a result of spinalization, which indicates that there was a change in the strength of the reflex pathway.

Statistics

For statistical analyses data from 3 different days in the intact state were pooled and compared with pooled data from 3 different days after spinalization. Planned comparisons were used to determine statistical differences between intact and spinal locomotion for locomotor EMG bursts (mean amplitude, onsets and offsets), durations of the step cycle, swing and stance, and reflex latencies. A Bonferroni correction for multiple comparisons was made [p is significant when $\leq$ 0.0071 (0.05/7)]. For reflex responses, a repeated measures 2 factor ANOVA (2 states x 6-10 phases) was performed to determine significant main effects and interactions between factors. When a significant interaction between state and phase was found paired t-tests were performed comparing reflex responses evoked in one phase of the step cycle in the intact state with the same phase in the spinal state. For example, P1 responses evoked in the first bin of the step cycle in the ipsilateral St in the intact state were compared against P1 responses evoked in the first bin of the step cycle in the ipsilateral St after spinalization. Significant interactions between state and phase

were found for all reflex responses except P2 responses of the ipsilateral MG. All values, unless otherwise stated, are means ± standard error of measurements (SEM).

Results

In the present study EMG bursts and Tib nerve reflexes were quantified during locomotion in the same cat before and after spinalization. All of the studied animals expressed a stable hindlimb locomotion following spinalization at the same treadmill speed as in the intact state, which could be maintained uninterrupted for prolonged periods (≥ 10 minutes). Cats 1, 2, and 3 respectively expressed a stable spinal locomotion at 41, 26, and 43 days post-spinalization without perineal stimulation. At this point EMG bursts and Tib nerve reflexes were consistent from one day to another, a prerequisite for quantitative analysis. During spinal locomotion, EMG bursts and the normalized amplitude of Tib nerve reflexes were altered in multiple muscles compared to the intact state.

Changes in the timing and magnitude of locomotor EMG bursts

The EMG activity of several muscles was recorded bilaterally before and after spinalization to quantify changes in the mean amplitude and timing of locomotor bursts after the complete spinal transection. Figure 1 shows locomotor EMG activity for selected muscles in cat 2 during intact and spinal locomotion. In both instances, the step cycle starts at left foot contact and EMGs are represented on the same ordinate scale for comparison. Some changes in the magnitude and timing of several bursts were observed following spinalization. For instance, the burst amplitude was increased in the ipsilateral St and in both VLs whereas that of LG decreased bilaterally. Srt and LG bilaterally started earlier within the normalized step cycle during spinal locomotion.

Table 1 details changes in the mean amplitude of locomotor EMG bursts after spinalization expressed as a percent difference from the intact state. The EMG burst increased in TA bilaterally in cat 2 and in the right TA of cat 1. Post-spinalization,

mean amplitude was increased in the left St of all cats and in the right St of cat 2. Thus, in flexors (St and TA) of the left hindlimb there was an increase in 7/8 recorded muscles. The mean amplitude of Srt, a hip flexor/knee extensor, was unchanged bilaterally in cat 2 and decreased bilaterally in cat 3. In cat 1, activity of the contralateral Srt became clonic after spinalization and the burst could not be quantified (see methods). In extensors (VL, LG and MG) there was a significant decrease of the mean amplitude in 11/16 muscles. In cat 1, all extensors (VL, LG, and MG) were decreased bilaterally. In cat 2, VL and LG activity was respectively increased and decreased bilaterally whereas in cat 3 the opposite occurred with decreased and increased activity in VL and LG, respectively. Therefore the mean amplitude of locomotor bursts in flexors was increased in the majority of cases while the mean amplitude of extensors was primarily decreased. Generally, if amplitude increased or decreased in a given muscle it changed in the same direction, albeit not the same magnitude, in the homologous muscle of the contralateral limb.

Table 2 provides onsets and offsets of EMG bursts during intact and spinal locomotion, expressed as % of the step cycle. In intact and spinal locomotion left foot contact indicated the beginning of the step cycle. In St bilaterally, burst onset occurred earlier in 1/5 muscles. Offset was significantly different in 5/5 St muscles with an earlier offset in 4/5. In TA bilaterally, onset and offset occurred earlier in 3/3 muscles. In Srt bilaterally, burst onset and offset occurred earlier in 4/4 muscles. In extensors bilaterally (VL, LG, MG) burst onset occurred earlier in 7/16 muscles with 6/7 of these being gastrocnemii. In these same extensors burst offset occurred earlier in 12/16 muscles. Therefore, in St and VL burst onset was primarily unchanged whereas that of Srt, TA and gastrocnemii occurred earlier. In most muscles the bursts terminated earlier within the normalized step cycle.

Table 3 gives the durations (in ms) of the step cycle and of swing and stance phases in the intact and spinal states for each cat at the same treadmill speed. In all cats there was a significant decrease ($p \leq 0.05$) in the duration of the step cycle and of swing and stance phases after spinalization. To maintain the same treadmill speed as before spinalization cats walked at a faster rate (steps per unit of time). Expressing

swing and stance durations as a function of step cycle duration in the intact and spinal states revealed that stance and swing occupied an identical percentage of the step cycle after spinalization in cat 1 whereas in cats 2 and 3 the percentage of swing increased slightly while that of stance decreased. Therefore, the structure of the step cycle is relatively unchanged after spinalization.

Changes in Tib nerve reflexes

The left Tib nerve was stimulated before and after spinalization in the same cats during locomotion to evaluate reflex changes taking place following a complete spinal transection. For comparison, reflex responses for a given muscle of the ipsilateral hindlimb in the 10 bins of the step cycle are separated into 4 events (shown in Figs. 3 and 4), including swing (first 3 phases), the swing-to-stance transition (phase 4), stance (phases 5-9), and the stance-to-swing transition (phase 10).

The most remarkable change in reflexes after spinalization was the appearance of a short-latency excitation in some ankle extensors during the stance phase instead of the more common short latency inhibition. Figure 2 shows reflex responses evoked by stimulating the left Tib nerve during different phases of the step cycle in cat 1 before (left panel) and after (right panel) spinalization in the ipsilateral LG. Short-latency inhibitory (N1) responses during stance (i.e. phases 5-9 in the intact state) disappeared and excitatory (P1) responses appeared in the spinal state. The P1 responses evoked during swing (i.e. the first 3 phases) were largely unaffected after spinalization. Thus, excitatory responses in ipsilateral extensors can appear in some phases of the step cycle even though bursting activity was similar, albeit decreased, during spinal locomotion (see scale and EMGs on the right side). When present, the appearance of short-latency excitatory responses during stance persisted for the duration of the study in several testing sessions.

Figure 3 illustrates responses evoked by Tib nerve stimulation in the ipsilateral knee flexor St for stimuli applied in different phases of the step cycle in cat 1 (top panel) and cat 2 (bottom panel) before (grey line) and after (black line) spinalization. After spinalization, P1 responses in cats 1 and 2 were increased while

P2 responses were decreased throughout the step cycle even though locomotor burst activity was similar between intact and spinal locomotion. Reflex changes in the ipsilateral St of cat 3 were similar to cat 2 and are not illustrated.

Table 4 provides the latency of responses before and after spinalization in each cat for selected muscles. The latency of P1 responses was significantly reduced in 4/7 ipsilateral flexors (St, Srt, TA) but never by more than 1-2 ms. In ipsilateral extensors (VL, LG, MG) the latency of N1 or P1 responses was unchanged in 8/8 muscles. After spinalization, the latency of P2 responses was significantly reduced in 5/8 ipsilateral extensors (i.e.. VL, LG, and MG). The latency of responses in muscles of the contralateral leg (Srt, VL, TA), contralateral to the stimulation, was reduced in 6/6 muscles.

Summary of reflex changes

Figures 4-7 provide a more detailed account of modifications of the normalized amplitude of reflex pathways from the left Tib nerve in muscles of the ipsilateral (Figs. 4-6) and contralateral (Fig. 7) legs. Responses are grouped into 10 phases relative to ipsilateral St onset and expressed as a function of the maximal intact value. Responses in each phase are divided by the blEMG occurring in the same phase to provide a measure of reflex responses. Therefore, by measuring the normalized amplitude of reflexes before and after spinalization changes in reflexes are independent of changes in the background level of muscle activity, which can influence reflex amplitude (Matthews, 1986). As can be seen from these figures, reflexes in most muscles were significantly altered in each cat during spinal locomotion.

Ipsilateral LG and MG

Figure 4 quantifies short-latency reflex responses in the ipsilateral LG and MG of cats 1-3 before and after spinalization. For the group, during stance (i.e. data points at 0.45 to 0.85), short-latency responses were significantly modified in 10/10 cases in the ipsilateral LG and MG. After spinalization, short-latency excitatory

responses appeared during stance in some cases while in other cases the inhibition was primarily decreased, except for LG of cat 3 where it was increased in 2/5 bins. Therefore, in ipsilateral gastrocnemii muscles the short-latency inhibition during stance is either reduced or short-latency excitation becomes apparent. During stance, longer-latency responses of the ipsilateral LG were significantly modified in 3/5 bins (data points at 0.65-0.85) for the group whereas those of the ipsilateral MG were not significantly altered after spinalization. P2 responses in the ipsilateral LG were mostly increased during stance after spinalization. Increased longer-latency responses could be observed in muscles with decreased (e.g. ipsilateral LG of cat 1) or increased (e.g. ipsilateral LG of cat 3) mean amplitude of the locomotor burst.

Ipsilateral St and TA

In the ipsilateral St (Fig. 5, two leftmost panels), P1 responses were significantly modified in 10/10 bins after spinalization for the group. During swing, (i.e. responses at 0.05 to 0.25) P1 responses were primarily increased post-spinalization. In the early part of swing (data points at 0.05 and 0.15), during which the ipsilateral St is active, P1 responses increased in all cats after spinalization. P1 responses were increased during stance in cat 1 but unchanged in cats 2 and 3. At the transition from swing to stance (i.e. response at 0.35), P1 responses were increased in cat 1 but decreased in cat 2 after spinalization. At the transition from stance to swing (i.e. response at 0.95), P1 responses were increased in cat 1 and decreased in cat 3. Therefore, the most consistent change between cats was an increase in P1 responses during the swing phase. P2 responses of the ipsilateral St, during swing were significantly modified in 7/10 bins for the group. In cat 2, P2 responses were decreased in most bins of the step cycle whereas in cats 1 and 3. P2 responses were mostly unchanged or decreased after spinalization. Decreases in P1 and/or P2 responses could be found even though ipsilateral St burst increased in all cats.

In the ipsilateral TA (Fig. 5, two rightmost panels), for the group (cats 2 and 3), P1 responses were significantly modified in 9/10 bins. During swing and stance, P1 responses were primarily increased. Thus, as with the ipsilateral St, a knee flexor,

short-latency excitatory responses in the ipsilateral TA, an ankle flexor, are also increased during swing in the majority of cases. For the group, P2 responses in the ipsilateral TA were modified in 10/10 bins and were almost always reduced after spinalization. Thus, in ipsilateral flexors, such as St and TA, short-latency excitatory responses are consistently increased during swing and longer-latency excitatory responses are generally reduced after spinalization.

Ipsilateral Srt and VL

In the ipsilateral Srt (Fig. 6, leftmost panels), for the group (cats 2 and 3), P1 responses were significantly modified in 6/10 bins. Responses of the hip flexor Srt were generally increased during swing post-spinalization similar to other ipsilateral flexors such as St and TA. In both cats, responses in the ipsilateral Srt were increased at the swing-to-stance transition and decreased at the stance-to-swing transition. Thus responses could be increased in some phases, particularly during swing and the transition to stance, even though left Srt burst activity was unchanged (cat 2) or decreased (cat 3) during spinal locomotion.

In the ipsilateral VL of cats 1-3 (Fig. 6, two rightmost panels), short-latency inhibitory responses (N1) during stance were significantly modified in 5/5 bins for the group. Similar to other ipsilalateral extensors, such as gastrocnemii muscles (Fig. 4), short-latency inhibition was reduced throughout the stance phase in the ipsilateral VL. However, contrary to LG and MG there were no short-latency excitatory responses during stance that appeared after spinalization. For the group, P2 responses of the ipsilateral VL were significantly modified in 3/6 bins. The large decrease in P2 responses in phases 0.35 or 0.45 occurred irrespective of locomotor burst activity because ipsilateral VL activity was increased in cat 2.

Contralateral muscles

For the group, excitatory responses in muscles of the limb contralateral to the stimulation were increased after spinalization in most phases of the step cycle (Fig. 7). For example, responses in the contralateral Srt (leftmost panels) were increased in

9/10 bins. In the contralateral TA (middle panels) responses were increased in 4/10 bins. In the contralateral VL (rightmost panels), responses were increased in 7/10 bins. In cats 1 and 3 the burst of the contralateral VL decreased after spinalization even though reflex responses in this muscle were mostly increased. Thus, irrespective of whether a muscle contralateral to the stimulation is a flexor (TA), an extensor (VL), or bifunctional (Srt) reflex responses are generally increased post-spinalization.

In summary, reflex responses were altered in all muscles studied post-spinalization and changes were often confined to specific phases of the step cycle. For instance, in ipsilateral flexors, such as St, TA, and Srt, short-latency excitatory responses were consistently increased during the swing phase from one cat to another. During stance, in ipsilateral extensors, such as LG, MG, and VL, there was less inhibition and in some ankle extensors there was the appearance of short-latency excitatory responses. In muscles of the limb contralateral to the simulation reflex responses were generally increased.

Discussion

The present study showed for the first time how, in the same cat, reflexes evoked by stimulating the Tib nerve through a stable chronically implanted nerve cuff were modified following a complete spinal transection during locomotion. Changes in reflexes were observed in flexors and extensors in both limbs as well as in bifunctional muscles. The clues provided by recording reflexes in the same animal before and after spinalization as to the organization of reflex pathways during locomotion and the reorganization from the intact to the spinal states will be discussed.

Methodological considerations

Changes in the properties of the stimulating cuff and recording electrodes over time could have accounted for altered reflexes. However, stable responses were recorded for prolonged periods before spinalization during which time substantial scar tissue

formed around electrodes and stabilized them. In several cases, changes in reflex responses were confined to specific phases of the step cycle. For instance, P1 responses in the ipsilateral Srt were mostly increased during swing (Fig. 6) and could be decreased in later phases. A change in the effective stimulation intensity would have affected all phases similarly. Moreover, the appearance of short-latency excitatory responses during the stance phase in some ankle extensors (Figs. 2 and 4) cannot be due to changes in the effective stimulus because such changes would only have affected the size of reflex responses and not the phasic modulation of responses. Therefore, we conclude that reflex changes were not due to changes in the physical conditions at stimulating or recording electrodes.

Unlike previous studies (Forssberg *et al.* 1975; Forssberg *et al.* 1977; Forssberg, 1979) that investigated responses to perturbations in spinal cats we evaluated reflexes before and after spinalization in the same cat during locomotion to determine changes in reflex pathways from the Tib nerve. Variability in spinal reflexes from one animal or individual to another makes comparisons between intact and lesioned groups problematic and undoubtedly reflect differences in neural circuitry that are genetically pre-determined and shaped during development and by experience or training (Loeb, 1993). Our approach of recording reflexes in the same animal before and after a lesion allows us to highlight similarities and differences in reflex changes from one cat to another.

Locomotor activity

Modifications in EMG bursts during locomotion were evaluated to determine if changes in reflexes paralleled those in the magnitude and timing of locomotor bursts. As in previous studies, changes in muscle activity could vary somewhat from one animal to another after spinalization, although in general, the locomotor pattern was similar to intact stepping (Smith *et al.* 1982; Lovely *et al.* 1986; Barbeau & Rossignol 1987; Lovely *et al.* 1990; Bélanger *et al.* 1996). As described previously (Smith et al. 1982; Lovely et al. 1990; Bélanger et al. 1996) step cycle, swing and stance durations were reduced after spinalization (Table 3).

Since reflex amplitude is influenced by the level of pre-existing motoneuron activity, or automatic gain control (Matthews, 1986; Duysens *et al.* 1993; Gritsenko et al. 2001) changes in the level of activity of motoneurons after spinalization could simply have accounted for changes in reflexes. Indeed, the loss and recovery of persistent inward currents and plateau potentials after spinal cord injury influences motoneuron and interneuron excitability and hence spinal reflexes (Bennett *et al.* 2001a; Bennett *et al.* 2001b; Heckman *et al.* 2003; Brownstone, 2006). However, reflex modifications presented here could not be due solely to altered motoneuron activity because we measured reflexes by dividing reflex responses by the baseline locomotor EMG occurring in the same phase. Moreover, increased or decreased responses could occur in muscles where mean amplitude of locomotor bursts were respectively decreased or increased, showing a clear dissociation between changes in reflexes and locomotor EMG bursts. Therefore, pre-motoneuronal mechanisms must be involved in modifying reflex responses.

Shifts in onsets and offsets of EMG bursts were observed for some muscles (Table 2) and could have influenced reflex responses. However, these shifts would only result in the appearance (or disappearance) of responses in one or two phases of the step cycle, which was not consistently observed. Again, dividing reflex responses by the blEMG in the same phase eliminates the underlying muscle activity as a factor in reflex changes.

If changes in burst amplitude of a given muscle occurred after spinalization, then the homologous muscle of the contralateral limb changed in the same direction (Table 1) suggesting that after complete spinalization the spinal CPG increases or decreases the activity of homologous muscles in parallel, which could be important for bilateral adjustments during locomotion. Parallel changes in homologous muscles could also be due to reciprocal connections. For instance, changes in muscle strength in one muscle through resistance training will increase the strength of the contralateral homologous non-trained muscle (Carroll et al. 2006).

Reflex changes after spinalization

Our results show not only how reflex responses from the Tib nerve are modified following spinalization but also provide clues as to the organization and modulation of these reflex pathways in the intact state. For instance, in ipsilateral extensors, during intact locomotion, short-latency (8-10 ms) excitatory responses can be evoked in the swing phase whereas during stance, short-latency (8-10 ms) inhibitory responses are followed by longer-latency (30-50 ms) excitatory responses (see Fig. 2). Short-latency excitatory inputs to ankle extensors evoked by stimulating cutaneous nerves of the foot have been demonstrated in reduced preparations (Wilson, 1963; LaBella & McCrea 1990; Rybak *et al.* 2006) and in spinal cats (Forssberg et al. 1975) but a clear demonstration in the same cat before and after spinalization had never been shown. After spinalization short-latency excitatory responses during swing are unchanged, but during stance inhibitory responses are reduced or short-latency excitatory responses appear. At the same time longer-latency responses begin at an earlier latency. The most likely explanation for these changes after spinalization is that there was a shift in the excitatory/inhibitory balance within spinal sensorimotor circuits that impacted the "normal" phase-dependent modulation of reflex pathways during locomotion. Firstly, in the intact state the short-latency excitatory pathway to ipsilateral extensors is probably actively suppressed during stance by the short-latency inhibitory reflex pathways, by the extensor half of the spinal CPG and/or by supraspinal inputs. Spinalization altered this modulation and as a result short-latency excitatory responses appeared during stance or the inhibition was reduced. Secondly, the short-latency inhibitory pathway probably also gates or competes with the longer-latency excitatory pathway. Consequently, because inhibition is reduced following spinalization longer-latency excitatory responses begin earlier (Table 4). In addition, reflex changes could be due to altered transmission in group I and II pathways because the Tib nerve also innervates intrinsic foot muscles. The contribution of group I and II pathways in the Tib nerve during locomotion in the cat are unknown and require further investigation in the intact and spinal states.

In ipsilateral flexors (Fig. 5), stimulation of cutaneous nerves from the foot evokes short- and longer-latency excitatory responses during intact and spinal locomotion. After spinalization short-latency excitatory responses are increased during early swing (St) or throughout most of the stance phase (TA) whereas longer-latency excitatory responses are reduced. Increased P1 responses in St during swing could result from a dis-inhibition of the short-latency excitatory pathway by the flexor half of the spinal CPG whereas increases in P1 responses in the TA during stance could be due to a dis-inhibition by the extensor half. The presence of P2 responses in ipsilateral flexors and extensors was shown in chronic spinal cats during fictive locomotion (Andersson et al. 1978; LaBella et al. 1992) and confirms that they can be organized within the spinal cord. The reduction of P2 responses in ipsilateral flexors, however, suggests an important supraspinal contribution to the excitability of this pathway. Spino-bulbo-spinal reflex pathways project primarily to flexors (Shimamura et al. 1991), which would explain why P2 responses were considerably reduced in St and TA (Fig. 5) after spinalization while longer-latency excitatory responses in ipsilateral extensors were unchanged or even increased (Fig. 4). Decreased P2 responses could also be due to increased refractoriness of motoneurons activated by the short-latency excitatory pathway. However, this could only partly explain changes in P2 responses because there is no clear relationship between P1 and P2 responses after spinalization (Fig. 5).

In muscles contralateral to the stimulation, excitatory responses were increased (Fig. 7) and occurred at an earlier latency (Table 4). Again it is probable that spinalization caused a dis-inhibition and/or enhanced facilitation of excitatory crossed pathways. The earlier onset of responses could be due to the fact that larger responses are easier to delineate and/or that an inhibitory influence was removed. For instance, a short-latency inhibitory pathway projects from cutaneous afferents of the foot to contralateral extensors (Edgley & Aggelopoulos 2006; Frigon & Rossignol 2007b) and may project to several motor pools. In this scenario, spinalization reduced the efficacy of the crossed inhibitory pathways and consequently responses occurred earlier, although this requires further investigation.

Therefore, the present results revealed how spinal reflex pathways are functionally reorganized during locomotion after spinalization. Evidently, locomotor training was responsible for some of the reflex changes because transmission in cutaneous and group I reflex pathways are modified by treadmill training, as recently shown during fictive locomotion (Cote *et al.* 2003; Cote & Gossard 2004). In the present study, the effects of spinalization and locomotor training on changes in reflex pathways cannot be dissociated because non-trained cats do not express a sufficiently stable locomotor pattern to allow rigorous quantification, as was done here. Moreover, the quality of spinal locomotion did not differ substantially from one cat to another because cats that could step remarkably well after spinalization were required for the quantitative analyses. Locomotor training facilitates the expression of spinal locomotion and as suggested by others a 'normalization' of the excitability of reflex pathways following spinalization could be one of the underlying mechanisms (Cote *et al.* 2003; Cote & Gossard 2004; Frigon & Rossignol 2006). In humans, locomotor training can also modify reflex pathways after spinal cord injury (for a review see Frigon and Rossignol 2006). In two case studies, changes in reflexes with locomotor training correlated with reduced symptoms of spasticity (Kiser et al. 2005) and improved walking ability (Trimble et al. 1998). Therefore, in animal models and humans spinal cord injury increases reflex excitability, which can be normalized by locomotor training. Further investigation is required to determine whether changes in reflex pathways are causal factors in the recovery of locomotor and/or motor functions.

Spinalization and locomotor training undoubtedly alters the excitatory/inhibitory balance within the spinal cord, hence modifying the excitability of reflex pathways. Indeed, post-spinalization reflex changes could be due to modifications in inhibitory influences within the spinal cord, such as altered pre-synaptic and/or reciprocal inhibition (Holmqvist & Lundberg 1959; Nelson & Mendell 1979; Nelson *et al.* 1979; Calancie *et al.* 1993; Faist *et al.* 1994; Frigon & Rossignol 2006). There is also evidence that treadmill training can modify the balance between excitation and inhibition within the spinal cord by reducing glycine-

and GABA-mediated inhibition in adult spinal cats (de Leon et al. 1999; Tillakaratne et al. 2000). Cutaneous reflexes, such as those from the Tib nerve, are mediated through polysynaptic pathways and changes could reflect modified gating or transmission at several pre- and post-synaptic loci at interneuronal levels and at the motoneuron. Although we favour a shift in the excitatory/inhibitory balance to explain most of our reflex changes other mechanisms cannot be excluded, such as activation of latent connections, changes in synaptic strength via long-term potentiation or depression, and formation of new connections through axonal sprouting (Edgerton *et al.* 2004; Rossignol, 2006; Cai *et al.* 2006; Maier & Schwab 2006).

In a given cat, changes in reflexes could differ between close synergists such as LG and MG (see LG and MG of cat 3 in Fig. 6). The different changes in reflex responses between close synergist could be due to the fact that LG and MG can receive different inputs from a given cutaneous nerve (LaBella et al. 1989) and that these inputs can be modulated differently (Degtyarenko et al. 1996). As a result, after spinalization the different input connections to both gastrocnemius muscles can be differently impacted leading to dissimilar reflex changes.

Functional significance

The general control model of locomotion is tripartite comprising a spinal CPG, descending supraspinal and propriospinal inputs, and sensory afferents from the periphery (Rossignol et al. 2006). On an ongoing basis the spinal CPG probably optimizes available sources of inputs to generate the appropriate locomotor pattern. For example, during normal walking, cutaneous feedback from the foot soles can contribute to locomotor activity as the foot contacts the ground but during swimming proprioceptive feedback from the moving muscles undoubtedly becomes an increasingly more important source of input to the spinal CPG. If the spinal CPG is already configured to optimize available inputs in the intact state it is anticipated that it will maximize remaining sources of inputs after injury to generate the locomotor pattern.

Although the spinal CPG appears relatively unchanged after a complete spinal transection, because the locomotor pattern is similar in the intact and spinal states, its input connections are drastically different. For instance, all descending inputs from supraspinal and propriospinal sources rostral to the lesion have been abolished and consequently the voluntary control of locomotion and that of posture and equilibrium is lost. As shown in this study reflex pathways from the Tib nerve are also altered. Reflex pathways, if not integral components of the CPG, certainly have powerful projections to it (Rossignol et al. 2006; McCrea 2001; Schomburg 1990) and we suggest that the spinal CPG maximizes available inputs (i.e. reflex pathways) to compensate for the loss of supraspinal influences. This compensatory principle was shown with other lesions. For example, after a cutaneous denervation of the hindpaws in cats corticospinal efficacy is increased (Bretzner and Drew 2005b) as are reflexes from an intact cutaneous nerve (Bernard et al. 2007). In a similar vein, after partial denervation of ankle extensors in cats the excitability of cutaneous reflexes is increased (Frigon and Rossignol 2007a). Sensory feedback from cutaneous receptors in the hindpaws and ankle extensors normally provide important signals to the locomotor circuitry to guide phase transitions and/or control the magnitude of locomotor bursts (Rossignol et al. 2006). After removing these inputs the locomotor system can compensate for the loss indicating that the spinal CPG can use other available sources of inputs to generate the locomotor pattern.

As stated by Sherrington (Sherrington 1906) all parts of the nervous system are interconnected and consequently removing any input potentially influences the excitability of all others. Descending supraspinal inputs are known to influence the excitability in cutaneous reflex pathways in anesthetised cats (Fleshman et al. 1988) and in intact cats during locomotion (Bretzner and Drew 2005a; Bouyer and Rossignol 2003). After spinalization the excitability from supraspinal inputs is abolished and cutaneous reflex pathways are altered. It stands to reason that cutaneous feedback from the periphery assumes a greater role after spinalization to generate locomotion. In the present study, several reflex changes were observed in phases of the step cycle where they would be most functionally relevant. For

example, in the ipsilateral St and Srt, a knee flexor and hip flexor, respectively, increases in reflex responses were mostly recorded during the swing phase (Figs. 5 and 6). Greater sensory feedback leading to the activation of St and Srt could reinforce and/or assist the initiation of swing. Additionally, reduced inhibition and/or increased excitation to ankle extensors (Fig. 4) could reinforce extensor activity during stance, thus explaining the absence or reduction of ankle yield during spinal locomotion (Smith et al. 1982; Bélanger et al. 1996). Large increases in short-latency responses in the ipsilateral TA (Fig. 5), an ankle flexor, during the stance phase could increase the stiffness around the ankle joint by co-activating flexors and extensors of the ankle.

The present paper showed how sensory feedback from the Tib nerve is altered during spinal locomotion by evaluating reflex changes before and after spinalization in the same cats but could not ascertain whether these changes are required from expressing spinal locomotion. From one cat to another, there were many similarities in reflex changes after spinalization, which could indicate that specific modifications take place in reflex pathways as a result of spinalization and locomotor training. Although an open question, interanimal differences in reflex changes could indicate why some cats express a spinal locomotion earlier and why the quality of spinal locomotion differs between cats.

Making use of the complex sensorimotor circuitry within the spinal cord by facilitating reflex changes might be effective in restoring walking ability after spinal cord injury. The methodology presented here can be used to systematically evaluate changes in numerous reflex pathways after spinal lesions of varying extents, which could provide more clues regarding the functional reorganization of spinal sensorimotor pathways after spinal cord injury. The methodology can also be used to test interventions, such as electrical or pharmacological stimulation, that could promote changes in reflex pathways. From an applied perspective, rehabilitative approaches specifically aimed at normalizing or shaping reflex pathways could facilitate the recovery of locomotion following spinal cord injury and deserve further investigation.

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Figures and tables

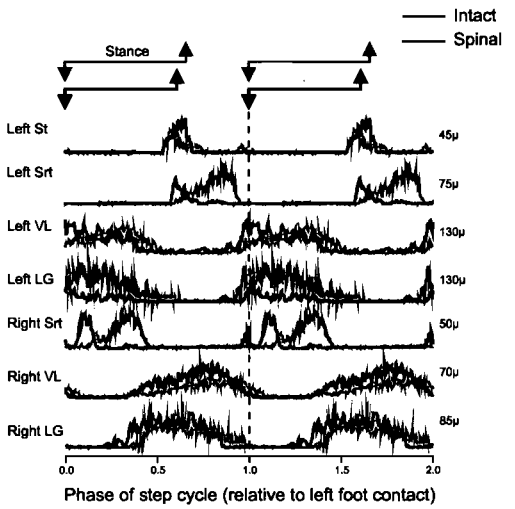

Figure 1. Rectified, averaged, single burst EMG traces for selected muscles during intact (grey) and spinal (black) locomotion in cat 2. In the intact and spinal states the step cycle begins and ends on left foot contact. Each EMG trace is the average of approximately 60 bursts.

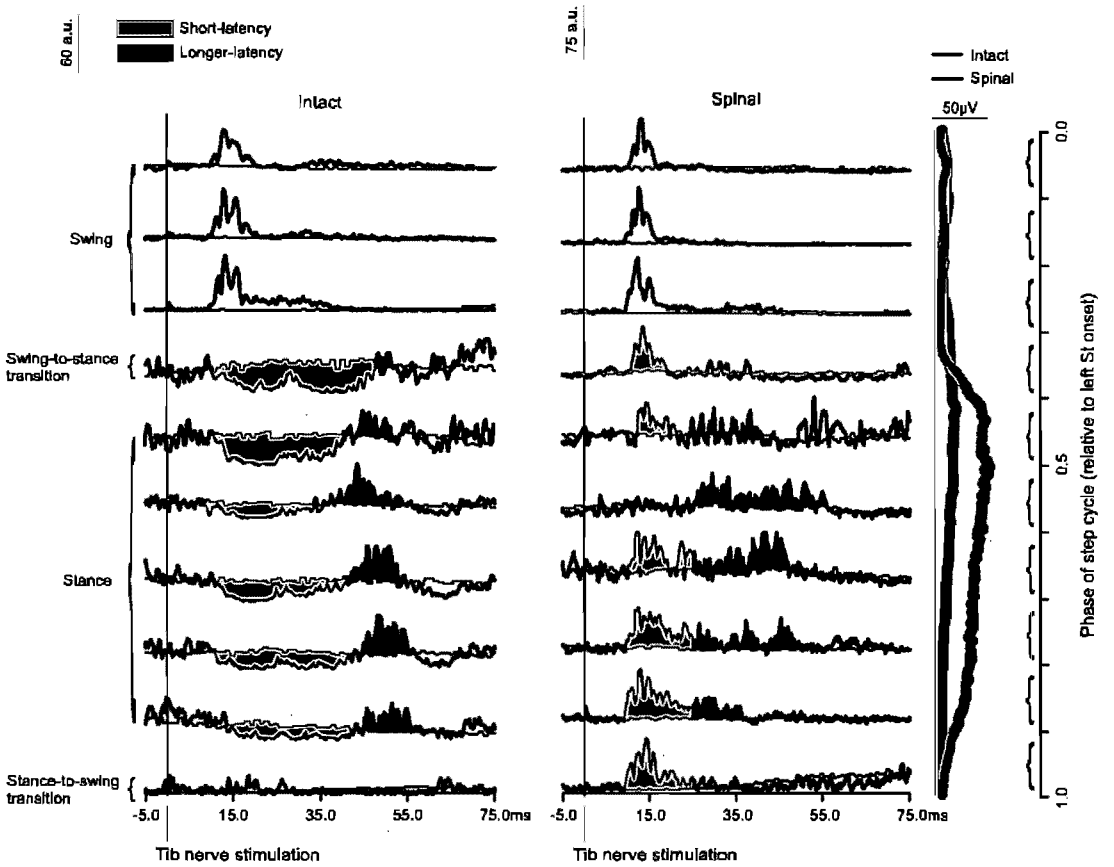

Figure 2. Averaged reflex responses of the left LG before (grey lines) and after (black lines) spinalization for cat 1. The thin lines in each phase indicate the blEMG during intact and spinal locomotion. Values in a single graph are at the same scale in arbitrary units (a.u.) but scales differ before and after spinalization. Windows for integrating N1 responses were manually determined in the intact state but after spinalization in this cat fixed windows were used, similarly to flexors, because there was a reversal from inhibition to excitation in most phases of the step cycle, making the delineation between responses difficult. The first horizontal trace for a given graph is phase 0.05 of the step cycle synchronized to the left St burst onset. Each trace is the average of approximately 10 stimuli. The rectified, averaged, single burst EMG traces of the left LG during intact and spinal locomotion are shown on the far right at the same scale.

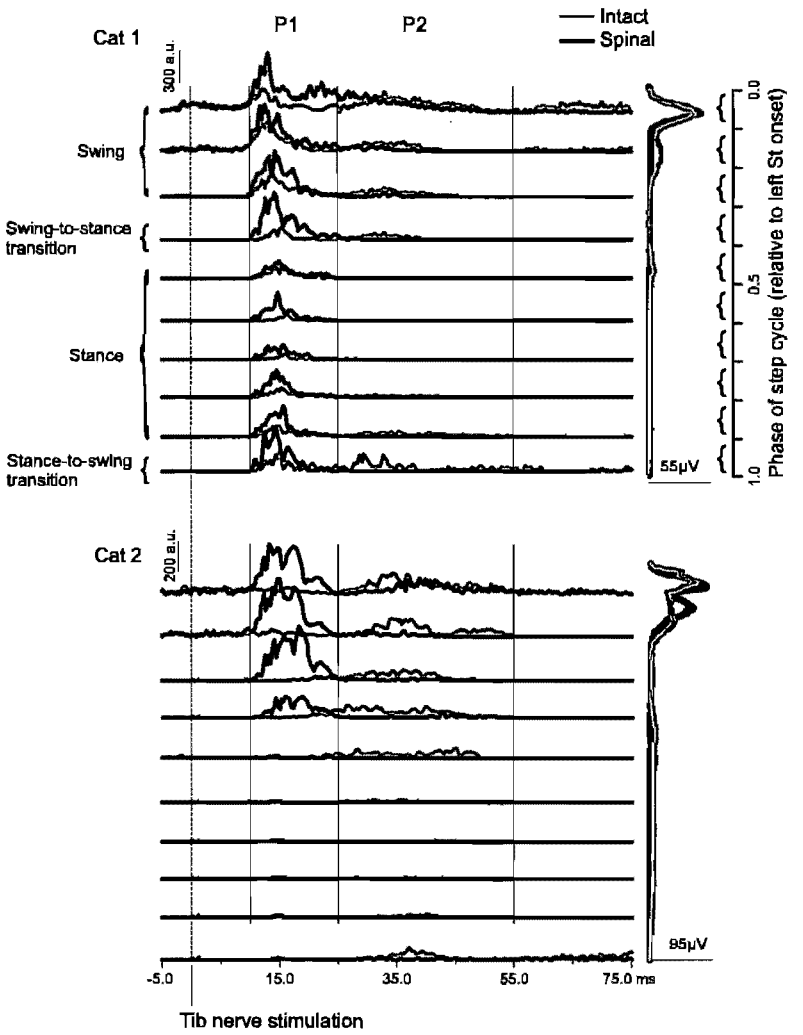

Figure 3. Averaged reflex responses of the left St before (grey lines) and after (black lines) spinalization for cat 1 (top panel) and cat 2 (bottom panel). Values in a single graph are at the same scale in arbitrary units (a.u.). Windows for integration in the left St of cats 1 and 2 were 10-25 ms and 25-55 ms for P1 and P2 responses, respectively. The first horizontal trace for a given graph is phase 0.05 of the step cycle synchronized to the left St burst onset. Each trace is the average of approximately 10 stimuli. The blEMG was removed for clarity. The rectified, averaged, single burst EMG traces of the left St during intact and spinal locomotion are shown on the far right at the same scale.

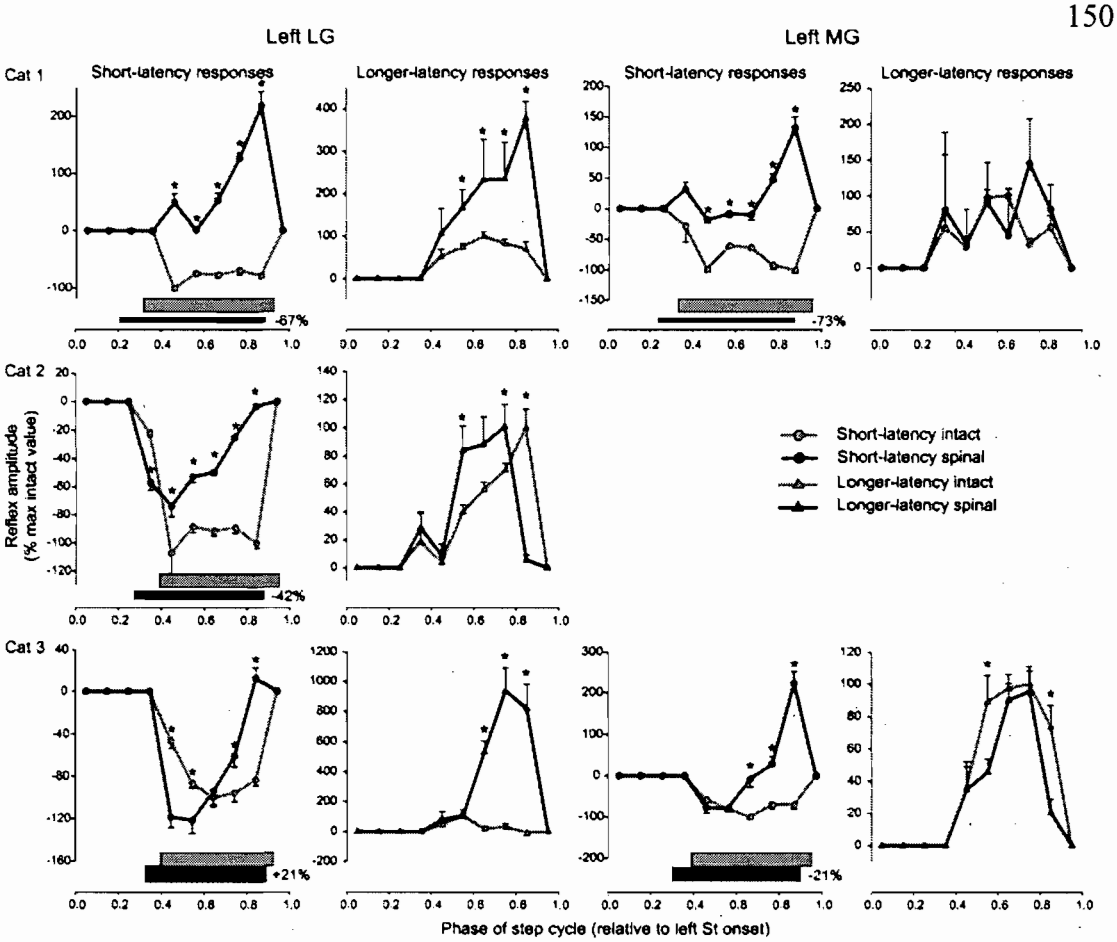

Figure 4. Phase plots summarizing changes in Tib nerve reflexes at 1.2T in ipsilateral ankle extensors (LG and MG, respectively) for cat 1 (top panels), cat 2 (middle panels) and cat 3 (bottom panels). Horizontal rectangles represent the phase of activity of each muscle during intact (grey) and spinal (black) locomotion. Significant differences ($p < 0.05$) from the intact value during a given phase after spinalization are indicated by an asterisk. Each data point is the mean ± SEM of approximately 30 responses.

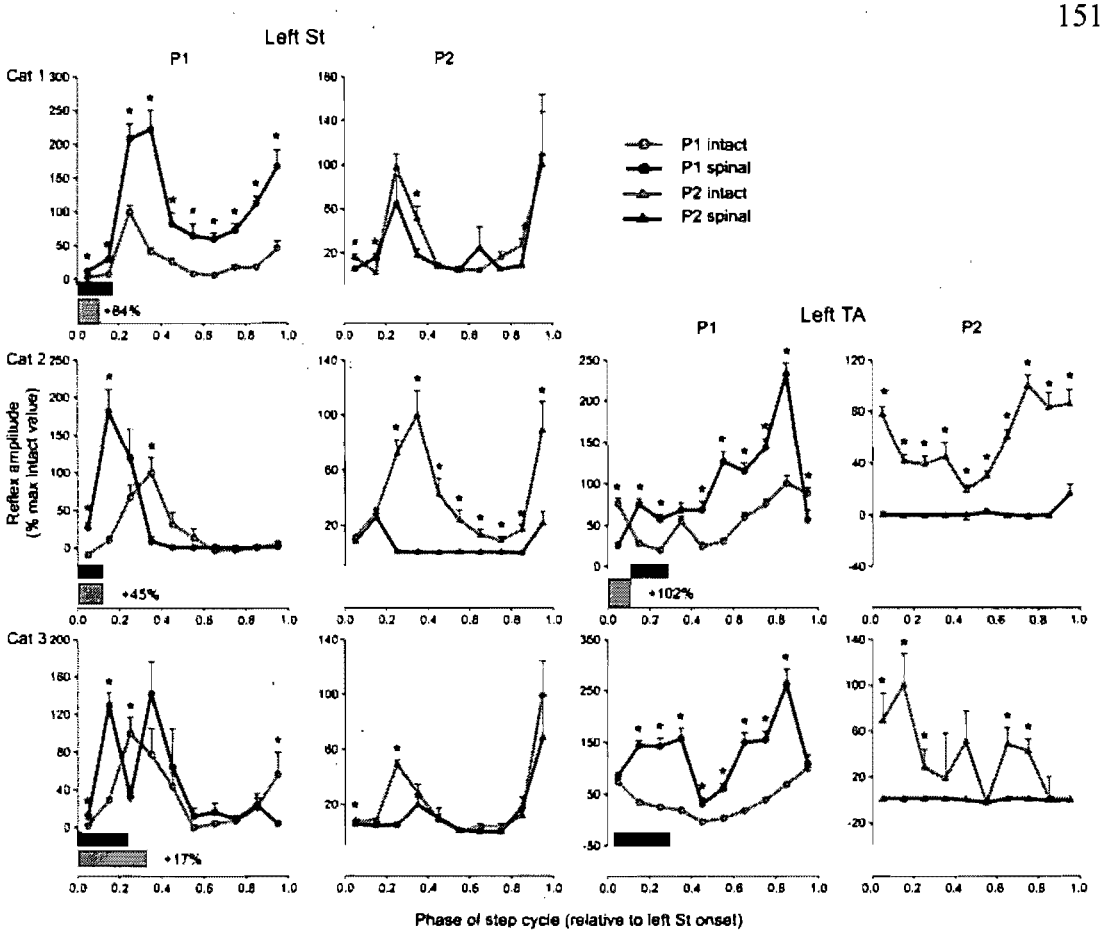

Figure 5. Phase plots summarizing changes in Tib nerve reflexes at 1.2T in ipsilateral flexors (St and TA, respectively) for cat 1 (top panels), cat 2 (middle panels) and cat 3 (bottom panels). Horizontal rectangles represent the phase of activity of each muscle during intact (grey) and spinal (black) locomotion normalized to left St burst onset. Significant differences ($p < 0.05$) from the intact value during a given phase after spinalization are indicated by an asterisk. Each data point is the mean ± SEM of approximately 30 responses.

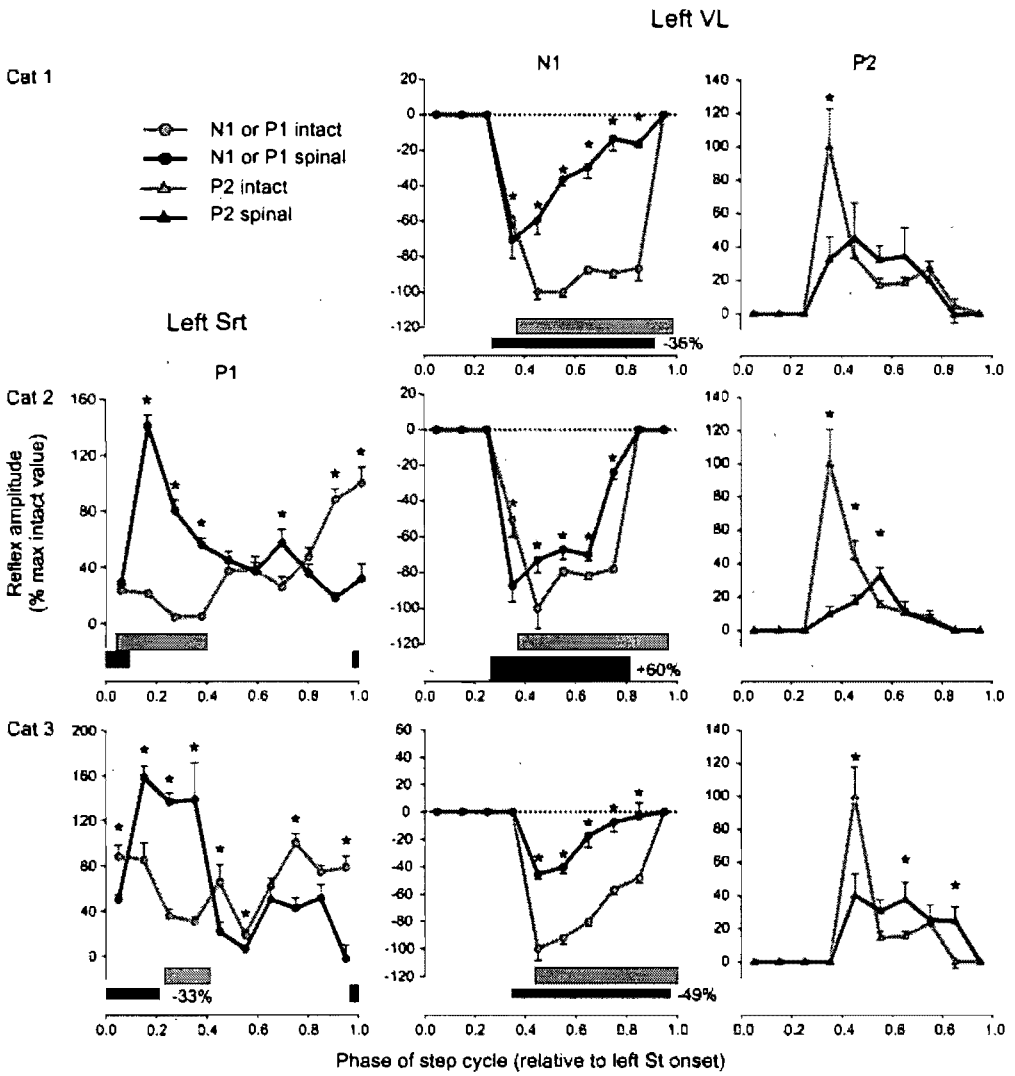

Figure 6. Phase plots summarizing changes in Tib nerve reflexes at 1.2T in the hip flexor/knee extensor Srt and the knee extensor VL for cat 1 (top panels), cat 2 (middle panels) and cat 3 (bottom panels). Horizontal rectangles represent the phase of activity of each muscle during intact (grey) and spinal (black) locomotion normalized to left St burst onset. Significant differences ($p < 0.05$) from the intact value during a given phase after spinalization are indicated by an asterisk. Each data point is the mean ± SEM of approximately 30 responses.

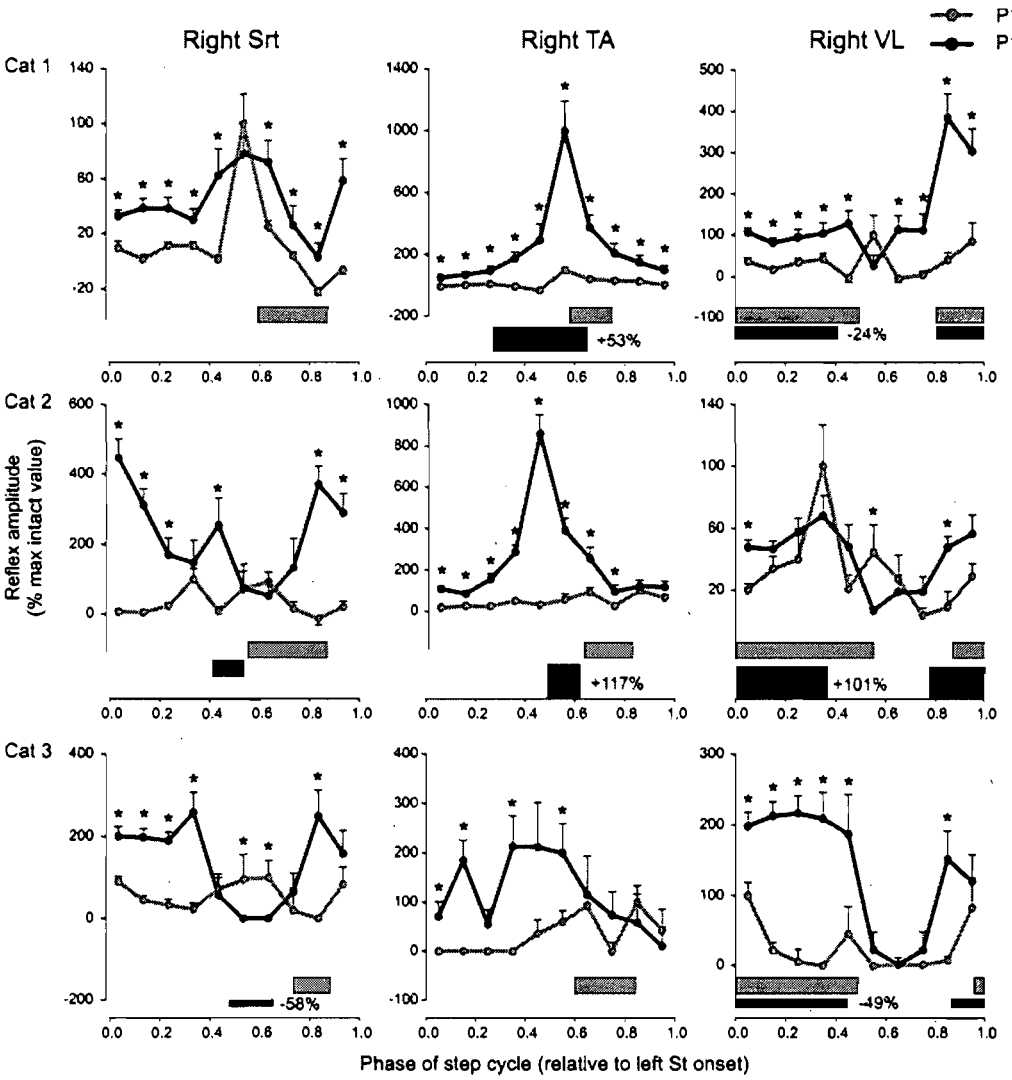

Figure 7. Phase plots summarizing changes in Tib nerve reflexes at 1.2T in contralateral muscles (Srt, TA and VL, respectively) for cat 1 (top panels), cat 2 (middle panels) and cat 3 (bottom panels). Horizontal rectangles represent the phase of activity of each muscle during intact (grey) and spinal (black) locomotion normalized to left St burst onset. Significant differences ($p < 0.05$) from the intact value during a given phase after spinalization are indicated by an asterisk. Each data point is the mean ± SEM of approximately 30 responses.

Difference in amplitude as a % of intact			
Muscle	Cat 1	Cat 2	Cat 3
LSt	+84%	+45%	+17%
LSrt	N/A	=	-33%
LVL	-36%	+60%	-49%
LLG	-67%	-42%	+21%
LMG	-73%	N/A	-21%
LTA	N/A	+102%	N/D
RSt	=	+33%	N/A
RSrt	N/D	=	-58%
RVL	-24%	+101%	-49%
RLG	-54%	-27%	+69%
RMG	-82%	+10%	N/A
RTA	+53%	+117%	N/D

Table 1. Changes in mean amplitude for all recorded muscles after spinalization expressed as the percent difference from the intact state. Some muscles were lost (N/A) over the course of the study and others could not be delineated (N/D). Each value is the average of 40-60 bursts.

	Cat 1		Cat 2		Cat 3	
Muscle	ON	OFF	ON	OFF	ON	OFF
LSt						
Intact	56 ± 3	74 ± 2	53 ± 3	77 ± 8	52 ± 3	73 ± 3
Spinal	58 ± 2	68 ± 2*	54 ± 3	69 ± 3*	54 ± 9	87 ± 7*
LSrt						
Intact	66 ± 2	93 ± 2	63 ± 4	94 ± 1	73 ± 2	92 ± 1
Spinal	N/A		52 ± 6*	67 ± 3*	51 ± 9*	78 ± 7*
LVL						
Intact	-10 ± 5	55 ± 3	-9 ± 4	52 ± 4	-7 ± 2	51 ± 3
Spinal	-15 ± 3*	30 ± 4*	-9 ± 3	41 ± 4*	-9 ± 5	49 ± 6
LLG						
Intact	-12 ± 2	50 ± 3	-7 ± 1	54 ± 7	-10 ± 1	45 ± 3
Spinal	-22 ± 7*	30 ± 7*	-9 ± 5	33 ± 5*	-12 ± 4	44 ± 5
LMG						
Intact	-11 ± 1	52 ± 1	N/A		-11 ± 2	47 ± 4
Spinal	-17 ± 4*	33 ± 5*	N/A		-12 ± 6	44 ± 6
LTA						
Intact	N/A		67 ± 2	83 ± 3	54 ± 4	79 ± 3
Spinal	N/A		60 ± 4*	71 ± 4*	N/D	
RSt						
Intact	95 ± 2	121 ± 3	108 ± 5	123 ± 6	N/A	
Spinal	96 ± 4	115 ± 4*	94 ± 7*	109 ± 8*	N/A	
RSrt						
Intact	114 ± 3	143 ± 6	115 ± 5	144 ± 6	124 ± 4	141 ± 4
Spinal	N/D		101 ± 7*	115 ± 5*	107 ± 2*	125 ± 2*
RVL						
Intact	35 ± 3	105 ± 2	34 ± 5	107 ± 4	46 ± 3	99 ± 2
Spinal	33 ± 4	95 ± 3*	34 ± 3	100 ± 4*	44 ± 3	97 ± 4
RLG						
Intact	38 ± 2	98 ± 2	40 ± 2	98 ± 6	42 ± 3	98 ± 4
Spinal	22 ± 4*	76 ± 9*	28 ± 5*	82 ± 4*	41 ± 8	93 ± 5*
RMG						
Intact	41 ± 3	93 ± 2	40 ± 3	94 ± 4	N/A	
Spinal	25 ± 4*	81 ± 8*	28 ± 6*	84 ± 8*		
RTA						
Intact	104 ± 2	131 ± 4	119 ± 3	141 ± 3	110 ± 3	135 ± 3
Spinal	74 ± 6*	111 ± 5*	99 ± 6*	116 ± 5*	N/D	

Table 2. Onsets and offsets of muscle bursts relative to left foot contact during intact and spinal locomotion expressed as a percentage of step cycle duration. Each value is the average of 15-40 bursts. * $p < 0.05$.

	Cat 1		Cat 2		Cat 3	
	ms	%	ms	%	ms	%
Step cycle duration						
Intact	1082 ± 9		1021 ± 15		983 ± 12	
Spinal	751 ± 6*		697 ± 6*		697 ± 14*	
Swing duration						
Intact	358 ± 4	33	345 ± 8	34	341 ± 8	35
Spinal	250 ± 4*	33	273 ± 5*	39	267 ± 12*	38
Stance duration						
Intact	725 ± 8	67	676 ± 12	66	641 ± 10	65
Spinal	501 ± 5*	67	424 ± 8*	61	430 ± 10*	62

Table 3. Absolute values of durations of the step cycle, flexion, and extension phases before and after spinalization in cats 1-3. * $p < 0.05$.

	Cat 1		Cat 2		Cat 3	
Muscle	N1 or P1	P2	N1 or P1	P2	N1 or P1	P2
Left St						
Intact	11 ± 1		11 ± 1		10 ± 2	
Spinal	10 ± 1*		10 ± 1*		9 ± 1	
Left Srt						
Intact			18 ± 3		16 ± 4	
Spinal			16 ± 4*		17 ± 3	
Left VL						
Intact	10 ± 1	45 ± 4	13 ± 1	48 ± 3	13 ± 2	45 ± 3
Spinal	10 ± 2	26 ± 8*	12 ± 2	47 ± 7	11 ± 2	32 ± 4*
Left LG						
Intact	11 ± 1	42 ± 4	11 ± 1	35 ± 4	11 ± 1	29 ± 6
Spinal	10 ± 1	24 ± 5*	11 ± 1	40 ± 5*	10 ± 1	29 ± 4
Left MG						
Intact	10 ± 1	40 ± 5			11 ± 2	33 ± 7
Spinal	10 ± 1	26 ± 3*			12 ± 1	31 ± 5
Left TA						
Intact			12 ± 1		10 ± 1	
Spinal			9 ± 1*		10 ± 1	
Right Srt						
Intact	21 ± 5		23 ± 5		18 ± 1	
Spinal	14 ± 4*		17 ± 6*		16 ± 2*	
Right VL						
Intact	17 ± 3		18 ± 3		21 ± 3	
Spinal	13 ± 2*		15 ± 3*		16 ± 3*	
Right TA						
Intact	18 ± 4		22 ± 4		22 ± 5	
Spinal	14 ± 2*		18 ± 3*		20 ± 4*	

Table 4. Latency of reflex responses for selected muscles in each before and after spinalization. Each value is the mean ± the standard deviation of ~ 20 responses. * $p < 0.05$.

Chapter 5 – Article 4. Partial denervation of ankle extensors before spinalization in cats impacts the expression of spinal locomotion and phasic modulation of reflexes

By Alain Frigon and Serge Rossignol

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Abstract

The expression of spinal locomotion is facilitated by locomotor treadmill training, which is thought to drive spinal plasticity by providing sensory feedback consistent with normal locomotion. Indeed, performing a peripheral denervation, which impacts sensory feedback, before spinalization impairs the proper expression of spinal locomotion. To study some of the underlying mechanisms we performed a denervation of the nerve supplying the left lateral gastrocnemius-soleus (LGS) muscles in 3 cats in the intact state (i.e. with an intact spinal cord) and following a period of recovery a complete spinalization at the 13th thoracic segment (T13) was made. After spinalization cats were trained on a treadmill to express a spinal locomotion. Reflexes evoked by stimulating the left Tib nerve at the ankle, the electromyography (EMG) of several hindlimb muscles, and kinematics were recorded during locomotion before and after the denervation, during the period of recovery, and following the complete spinalization. A denervation of the left LGS before spinalization influenced the expression of spinal locomotion in several ways. For instance, the ability to express a spinal locomotion differed from one cat to another ranging from a greater ankle yield in the denervated limb in one cat to an inability to recover locomotion after spinalization in another. Moreover, in the two cats that recovered locomotion after spinalization reflex changes were not the same as in 'normal' spinal cats, suggesting that the reorganization of spinal circuits is dissimilar to what normally takes place when a denervation is performed before spinalization. Peripheral sensory feedback appears critical for the normal 'development' of spinal locomotion.

Keywords: Plasticity, reflex, locomotion, denervation, spinalization

Introduction

Locomotion is a complex task requiring dynamic sensorimotor interactions at several levels of the nervous system. At the core is the spinal locomotor central pattern generator (CPG) that receives descending supraspinal and propriospinal inputs and sensory feedback from the periphery (Rossignol et al. 2006). Removing inputs from supraspinal structures and/or feedback from the moving limbs creates a new "state" within the spinal locomotor circuitry and consequently, the spinal CPG must optimize available inputs to maximize remnant locomotor capabilities (Rossignol 2006).

A few studies have shown that removing sensory inputs from the periphery by sectioning a nerve in the intact state (i.e. with an intact spinal cord) produces adaptive changes enabling locomotion to return to almost normal in adult cats (Pearson et al. 1999; Carrier et al. 1997; Bouyer and Rossignol 2003a; Frigon and Rossignol 2007a). Similarly, a denervation performed in the spinal state (i.e. after spinalization) also produces deficits but in most instances locomotion returns to pre-denervation values with time in adult cats (Carrier et al. 1997; Bouyer and Rossignol 2003b; Bouyer et al. 2001). However, performing a complete cutaneous denervation of the hindpaw in the spinal state abolished the ability to place the paw on the plantar surface during spinal locomotion (Bouyer and Rossignol 2003b). This is contrary to a complete cutaneous denervation performed in the intact state where plantigrade placement of the foot is only transiently impaired, suggesting that supraspinal structures can select other cues to adjust to the loss of cutaneous sensation (Bouyer and Rossignol 2003b; Bouyer and Rossignol 2003a). These studies showed that cutaneous inputs are required for proper paw positioning in the spinal state and that although the isolated spinal cord can compensate for the loss of sensory inputs it has a limited ability to do so.

Sensory feedback appears critical for the expression of spinal locomotion. For instance, a unilateral deafferentation by sectioning dorsal root ganglia at L3 to S1-S2 before spinalization prevented the expression of spinal locomotion (Giuliani and Smith 1987). More focalized lesions can also impair the "normal" expression of

spinal locomotion. For example, completely removing cutaneous innervation from the hindpaws in otherwise intact cats before spinalization prevented plantar foot placement and weight bearing during spinal locomotion (Bouyer and Rossignol 2003b). In another study, lesioning the nerves to some ankle flexor muscles in one hindlimb before spinalization produced an asymmetrical locomotion in the spinal state characterized by hyperflexion and absence of plantar foot contact on the denervated side (Carrier et al. 1997). Thus, the integrity of reflex pathways appears of critical importance for the "development" of spinal locomotion.

These results show that the effects of a neurectomy on spinal locomotion critically depend on whether the nerve is cut before or after spinalization. The spinal CPG appears unable to fully compensate for the loss of supraspinal commands if it was required to optimize remaining inputs after a peripheral nerve lesion in the intact state. The purpose of the present study was to investigate some of the mechanisms that could explain why a denervation before spinalization (i.e. in the intact state) negatively influences the expression of spinal locomotion. The left LGS nerve was denervated in the intact state in adult cats and following a period of recovery the animals were completely spinalized at the 13th thoracic (T13) segment. The LGS nerve was chosen because the effects of sectioning this nerve after spinalization have been investigated (Bouyer et al. 2001) whereas the effects of lesioning this nerve before spinalization have not. The tibial nerve (Tib) at the ankle, which innervates the majority of the plantar surface of the paw was stimulated before and after spinalization to determine if changes in these reflexes were the same as in our previous study of 'normal' spinal cats (Chapter 5). It could be the reorganization of reflex pathways differs after spinalization in cats where a peripheral denervation was performed before spinalization.

Methods

The general methodology is similar to what has been described before (Bélanger et al. 1996; Carrier et al. 1997; Bouyer and Rossignol 2003b; Bouyer and Rossignol 2003a; Bernard et al. 2007). The experimental protocol was in accordance

with the guidelines of the animal Ethics Committee of the Université de Montréal. All surgical procedures were performed under general anesthesia and aseptic conditions. Prior to surgery cats were injected with an analgesic (Anafen 100mg; subcutaneously) and pre-medicated (Atravet 0.01 ml/kg, Glycopyrrolate 0.05 ml/kg, Ketamine 0.1 ml/kg; intramuscularly). Cats were then intubated and maintained under gaseous anesthesia (isoflurane 2%) while heart rate and respiration were monitored. After surgery, an analgesic (Buprenorphine 0.05 ml/kg) was administered subcutaneously. An oral antibiotic (cephatab or apo-cephalex, 100 mg/day) was given for 10 days following surgery.

Animals and general procedures

Three adult cats of either sex (weight 3.0 - 7.0 kg) were first selected based on their ability to walk for prolonged periods on a treadmill and trained for a few weeks at their preferred speed (0.4-0.5 m/s). These are the same cats that were used to describe changes in Tib nerve reflexes after a LGS denervation in the intact state (Frigon and Rossignol 2007a). Figure 1 summarizes the sequence of events for each "denervated-spinal" (DS) cat starting with the neurectomy and expressed as a function of the time of spinalization. Cats in the present study are numbered in the same way as in our previous work. For instance cat DS1 in the present study is the same as cat 1 described in Frigon and Rossignol 2007a and so on. Cats were initially implanted with electrodes for chronic EMG recordings and nerve stimulation, allowed to recover from the implantation, and then values of EMGs, reflexes, and kinematics were recorded in the post-denervation state. Following a recovery period, a spinalization was made at T13. Cats DS1, DS2 and DS3 were spinalized 49, 122 and 54 days after the neurectomy, respectively. Following spinalization cats were trained 3-5 times a week on a motorized treadmill to express spinal locomotion. In 2 cats (DS1 and DS3), clonidine (150-200 ug/kg) was given intraperitoneally on a few occasions to initiate walking movements (Barbeau et al. 1987; Chau et al. 1998). Despite weeks of training and repeated clonidine injections cat DS3 never expressed a spinal locomotion. Cats DS1 and DS2 respectively expressed a spinal locomotion

at 116 and 39 days post-spinalization. Clonidine injections could trigger locomotion in cat DS1 before 116 days post-spinalisation and constant perineal stimulation and weight support had to be provided by the experimenter to elicit spinal locomotion in this cat. Cats were kept for 5-8 months and were tested periodically before and after spinalization. Each testing session lasted approximately 1 hour.

Kinematics

Kinematic data of the left and right hindlimbs were captured post-spinalization using a Panasonic digital 5100 camera (1/1000 s shutter speed, 30 frames/sec = 60 fields = time resolution of 16,7ms) and a Sony RDR-GX315 DVD recorder during treadmill locomotion. Small reflective markers were placed over prominent bony landmarks at each joint of the hindlimbs including the iliac crest, greater trochanter, lateral epicondyle, lateral malleolus, metatarso-phalangeal (MTP) joint, and the tip of the fourth toe. Joint angles (hip, knee, ankle, MTP) were reconstructed off-line using custom-made software with a resolution of 60 fields/sec from ≥ 20 step cycles. Step cycle duration was measured as the time between two successive left foot contacts. The duration of stance was measured as the time between contact and lift of the left foot whereas swing duration was calculated as the difference between step cycle and stance durations. Step length was measured as the horizontal distance covered by the left foot between two successive foot contacts. Stance length was measured as the horizontal distance covered by the left foot between contact and lift whereas swing length was calculated as the difference between step and stance lengths.

Implantation of electromyographic (EMG) electrodes, recording and processing

Chronic electromyographic (EMG) electrodes were implanted bilaterally in the following hindlimb muscles: semitendinosus (St: knee flexor/hip extensor), anterior part of sartorius (Srt: hip flexor/knee extensor), vastus lateralis (VL: knee extensor), lateral gastrocnemius (LG: ankle extensor/knee flexor), medial gastrocnemius (MG: ankle extensor/knee flexor), soleus (ankle extensor) and tibialis

anterior (TA: ankle flexor). A pair of Teflon-insulated multistrain fine wires (AS633; Cooner wire, Chatsworth, CA) was directed subcutaneously from head-mounted fifteen pin connectors (Cinch Connectors; TTI Inc., Pointe-Claire, Canada) and sown into the belly of each muscle for bipolar EMG recordings. EMG recordings were bandpass filtered (100-3000 Hz) and amplified (gains of 0.5-50K) using two Lynx-8 amplifiers (Neuralynx, Tucson, Arizona). EMG data were digitized (5000 Hz) using custom-made acquisition software. The duration, mean amplitude, and timing of the EMG bursts during locomotion relative to left St burst onset were calculated using custom software. Over the course of the study some EMG recordings were lost in each cat, as determined by the disappearance of EMG signals and in some cases the locomotor bursts could not be delineated. Mean amplitude was defined as the area under the rectified EMG burst divided by its duration and expressed as a percentage of the averaged control (i.e. before spinalization) value. Step cycle duration was measured as the time between two successive St bursts. Kinematic and EMG data were synchronized using an SMPTE time code generator (Evertz, time code master 5010).

Tibial nerve stimulation and reflexes

The stimulation and analysis procedures are the same as previously described (Frigon and Rossignol 2007a; Frigon and Rossignol 2007b). A chronic stimulating electrode composed of bipolar wires (AS633; Cooner wire, Chatsworth, CA) embedded in a polymer (Denstply International) cuff (Julien and Rossignol 1982) was placed around the left Tib nerve at the ankle adjacent to the Achilles' tendon. The Tib nerve was stimulated (Grass S88 stimulator) at different intensities during intact locomotion (i.e. before the neurectomy and spinalization) with a single 1 ms pulse at a constant time (100 ms after onset of St burst) to determine the threshold for obtaining small yet consistent short-latency (~ 10 ms) responses in the left TA. Stimulation current was then set at 1.2 times this threshold to evoke non-noxious reflexes and, once reflexes were qualitatively and quantitatively reproducible from one session to another for a few weeks ($\geq$ 22 days), the same current was used before

and after denervation and spinalization. At this intensity it has been shown that responses evoked in TA are only slightly higher in stimulation intensity than the threshold for recording an afferent volley in the sciatic nerve and are thought to be generated by the largest diameter Aβ afferents (Loeb 1993). However, influences from group I or II afferents cannot be eliminated since the Tib nerve also supplies intrinsic foot muscles. The stimulation did not visibly alter limb trajectory during intact or spinal locomotion, which is important because perturbations of the limb could introduce responses linked to the movement, such as proprioceptive reflexes. The pattern of responses, as opposed to amplitude, is typically invariant unless very high stimulus intensities are used (Abraham et al. 1985; Loeb 1993). Therefore, our responses were probably mediated by low-threshold cutaneous afferents. In all testing sessions, a stimulus was given once every three cycles at pseudo-random times to elicit responses throughout the step cycle for approximately 120-200 stimulations. After the initial implantation substantive scar tissue forms around the stimulating electrode and stabilizes it and it is thus improbable that the afferent volley evoked at the stimulating electrode changed over time.

The EMGs were grouped into stimulated or control (non-stimulated) trials. The step cycle was divided into 10 phases by synchronizing the cycle to the onset of the left St. Averaged responses of EMGs with stimulation were separated into these 10 bins according to the time they were evoked in the cycle. An average of at least 50 control cycles provided a template of baseline locomotor EMG (blEMG) during the step cycle. Onsets and offsets of responses, denoted as prominent negative or positive deflections away from the blEMG, were determined manually using pre-defined latencies as guidelines (Duysens and Stein 1978; Abraham et al. 1985; Loeb 1993; Pratt et al. 1991) such as P1 or N1 (~8-10 ms) and P2 (≥ 25 ms), where "P" and "N" are positive (excitatory) and negative (inhibitory) responses, respectively. For ipsilateral flexors (see Fig. 4), time windows were the same before and after spinalization: St: P1 = 10-25 ms, P2 = 25-55 ms; TA: P1 = 10-30 ms, P2 = 30-55 ms. A time window of 15-50 ms was used for the ipsilateral Srt before and after spinalization because this muscle often shows a prolonged burst during that time

period and not two distinct responses. For contralateral muscles (Srt, TA, VL) a time window of 15-40 ms was used before and after spinalization. Responses in the contralateral St are weak and inconsistent and were not measured. For ipsilateral extensors (see Fig. 5), onsets and offsets of responses were manually determined pre- and post-spinalization if an inhibition was present because P2 latencies change somewhat throughout the step cycle as the duration of N1 varies from one phase to another. However, if N1 reversed to a P1 after spinalization time windows of 10-25 ms and 25-55 ms were respectively used for P1 and P2.

Neurectomy

The left LGS nerve was cut by carefully separating the nerve from its surrounding tissue in the popliteal fossa and the proximal end was capped with flexible vinyl polysiloxane (Reprosil; Dentsply International, Milford, DE) to prevent nerve regeneration (Frigon and Rossignol 2007). The anaesthesia and surgery for the neurectomy lasted under an hour and cats were tested the next day to allow sufficient recovery. Post-mortem analyses confirmed that the LGS nerve was cut and did not regenerate in each cat.

Spinalization and training

Spinalization procedures were identical to a previous study (Bélanger et al. 1996). In the early days after spinalization training consisted of having two experimenters move the hindlimbs over the motorized treadmill to simulate locomotion while the forelimbs were positioned on a fixed platform located ~ 3 cm above the belt. The skin of the perineal region was stimulated to facilitate stepping movements. A Plexiglas separator was placed between the limbs to prevent them from impeding each other because of increased adduction. Initially, the experimenter supported the hindquarters by lifting the tail. After spinalization, recording sessions resumed once a steady locomotor pattern was attained with the experimenter providing equilibrium by gently holding the tail and/or weight bearing when necessary (Barbeau and Rossignol 1987; Bélanger et al. 1996)

Statistics

To determine statistical differences for the mean amplitude of locomotor EMG bursts and durations and lengths of the step cycle, swing and stance phases, data from the 3 last trials recorded before spinalization (control) were averaged and compared with pooled data from 3 sessions after spinalization using a one-way ANOVA. Reflexes were analysed identically except that each response in each phase of the step cycle was treated independently. For example, P1 responses evoked in the first bin of the step cycle in the left St in the control state were compared against P1 responses evoked in the first bin of the step cycle in the left St post-spinalization.

Results

Denervating the left LGS before spinalization influenced the expression of spinal locomotion with varying effects from one cat to another. Cat DS2 expressed a stable spinal locomotion 39 days post-spinalization with proper paw placement and full weight support but there was an asymmetry between hindlimbs. Cat DS1 eventually expressed a spinal locomotion at 116 days post-spinalization but constant perineal stimulation and weight support had to be provided by the experimenter. Cat DS3 never expressed a spinal locomotion despite weeks of treadmill training and repeated clonidine injections. Changes in kinematics, step cycle duration and length, and Tib nerve reflexes after spinalization are described in the following sections for cats DS1 and DS2 and comparisons are made with data obtained in cats that were otherwise intact before spinalization (IS: intact-spinal) (Chapter 5).

Comparisons of left and right hindlimb kinematics during spinal locomotion

Kinematic data of both hindlimbs were recorded before and after spinalisation at the same treadmill speed. Figure 2 shows stick figures (top panels) and angular excursions of the hip, knee, ankle and MTP joints (bottom panels) of the left (left panels) and right (right panels) hindlimbs during locomotion 72 days post-spinalization (194 days post-neurectomy) in cat DS2 without (black lines) and with

(red lines) perineal stimulation. Cat DS2 had full hindquarter support and the alternation between hindlimbs was symmetrical. The major difference between both legs was a large ankle yield of the left leg (i.e. the denervated side) following foot contact (grey rectangle), which was not present in the right leg. Whereas perineal stimulation could reduce the magnitude of the ankle yield in the left leg (bottom left panel, shaded area) it did not affect other joints in the left or right hindlimb. In our previous study this cat [(cat 2 of (Frigon and Rossignol 2007a)] had very little deficit after denervating the left LGS in the intact state.

Figure 3 shows stick figures (top panels) and angular excursions of the hip, knee, ankle and MTP joints (bottom panels) of the left (left panels) and right (right panels) hindlimbs during locomotion in cat DS1 72 days post- spinalisation (121 days post-neurectomy). This sequence was taken more than 35 minutes following clonidine injection without (black lines) or with (red lines) perineal stimulation. Cat DS1 had no hindquarter support, which was provided by the experimenter. The stick diagrams and joint angles during the step cycle illustrate the asymmetrical locomotion between both legs. While the right leg had an organized locomotor pattern, the left hindlimb (i.e. the denervated side) made small strides with little excursion at each joint. Perineal stimulation improved locomotor movements of the left leg but had little effect on the right leg. With time, continued training and clonidine injections, cat DS1 attained a symmetrical gait at approximately 116 days post-spinalization but weight bearing never recovered and vigorous perineal stimulation had to be constantly provided to elicit spinal locomotion. In our previous study, cat DS1 [cat 1 of (Frigon and Rossignol 2007a)] had a large increase in ankle yield of the left leg after a LGS neurectomy in the intact state, which returned to intact values in approximately two weeks.

To make comparisons with cats that had no denervation before spinalisation (IS; intact-spinal) kinematic data from 3 spinal cats used in a previous study (Chapter 5) were used (Table 1, Figures 4 and 5). Table 1 shows durations of the step cycle and its sub-phases before and after spinalisation in DS and IS cats. In cats IS1-IS3 and in cat DS1 the duration of the step cycle, swing, and stance was reduced after

spinalisation. However, in cat DS2 step cycle duration increased, as did the duration of swing and stance. The length of the step cycle and of swing and stance was also reduced in cats IS1-IS3 and in cat DS1 whereas in cat DS2 swing length was slightly but significantly increased.

One of the changes observed after spinalization is a reduced ability to place the foot in front of the hip at ground contact during locomotion (Bélanger et al. 1996). Figure 4 shows the position of the toe relative to the hip at foot contact and foot lift in DS and IS cats. In cats DS1 and IS1-IS3 the distance from the toe to the hip at foot contact decreased after spinalisation but in cat DS2 it increased. Moreover, in cats IS1-IS3 there was a reduced distance from the toe to the hip at foot lift after spinalisation whereas in cats DS1 and DS2 there were no significant changes.

Spinal locomotion is also characterized by several changes in the peak-to-peak excursions at the hip, knee, ankle, and MTP joints (Bélanger et al. 1996). In the present study, angular excursions at the hip and knee joints were reduced in cats IS1-IS3 and in cat DS1 post-spinalization but in cat DS2 they were increased (Fig. 5A and 5B). At the MTP joint, angular excursion decreased post-spinalization in cats IS1-IS3 but increased in cats DS1 and DS2. Changes at the ankle joint post spinalization were more variable. In cats DS1 and IS1 ankle joint excursion increased whereas in cat IS3 it decreased. It was unchanged in cats DS2 and IS2. Thus, changes in angular excursions can differ in cats with a denervation performed spinalization.

Changes in locomotor EMG bursts after spinalization

The activity of several muscles was recorded before and after spinalization to quantify changes in locomotor bursts during spinal locomotion. Tables 2 and 3 provide an account of EMG bursts during locomotion before and after spinalization for cats DS1 and DS2. The step cycle begins at left foot contact before and after spinalization to determine if the mean amplitude (Table 2) and timing (Table 3) of locomotor EMG bursts was modified after spinalization. In cat DS1, the mean amplitude increased significantly in the left St but decreased in the left Srt, MG, and

VL and in the right Srt and Sol. In cat DS2, mean amplitude increased in the left St, TA and MG and in the right St and Srt but decreased in extensors of the right leg (VL, MG, Sol). Thus, in flexors (St and TA) the mean amplitude increased in 3/3 recorded muscles whereas mean amplitude decreased in 7/8 extensors. Burst onset of extensors (VL, MG, Sol) of both hindlimbs in cat DS1 occurred earlier in 3/4 cases and in 2/3 cases in cat DS2. Burst offset occurred earlier in 3/4 and 4/4 extensors bilaterally in cat DS1 and DS2, respectively. The burst onset of St bilaterally occurred later in 2/3 cases while TA burst started earlier and finished later in 1/1 case. Burst offset in St finished later in 1/3 case. The burst onset and offset of the left Srt was earlier in cat DS1 but later in cat DS2 for the right Srt.

Changes in Tib nerve reflexes after spinalization

To evaluate changes in reflexes post-spinalization, the Tib nerve was stimulated during locomotion before and after spinalization in the same cat. Figures 6-9 show Tib nerve reflexes for selected muscles in cats DS1 and DS2 before and after spinalization, expressed as a function of the maximal control value (i.e. before spinalization). Although all phase values are represented, for comparison between conditions, reflex responses for a given muscle of the left hindlimb in the 10 bins of the step cycle are separated into 4 events: swing (data points at 0.05-0.25), the swing-to-stance transition (data point at 0.35), stance (data points at 0.45-0.85), and the stance-to-swing transition (data point at 0.95).

In the left TA (Fig. 6, left panels), P1 responses were significantly reduced during stance in 8/10 cases after spinalization. P1 responses were also reduced at the swing-to-stance and stance-to-swing transitions in both cats. After spinalization, P2 responses (Fig. 6, right panels) were reduced during stance in 6/10 cases and in the transitions from swing-to-stance and stance-to-swing in both cats.

In the left St (Fig. 7, left panels) P1 responses were significantly modified during swing in 3/6 cases with a decrease in 2/3 cases and an increase in 1/3 cases after spinalization. At the transition from swing to stance P1 responses were increased (DS1) or decreased (DS2). At the swing-to-stance transition P1 responses

were unchanged (DS1) or increased (DS2). During stance, P1 responses were decreased in 4/10 cases post-spinalization. In cat DS1, P2 responses were decreased in 3/3 cases during swing, in 5/5 cases during stance, and at the stance-to-swing transition (Fig. 7, upper right panels). In cat DS2 P2 responses were increased in 1/5 case during stance and decreased at the stance-to-swing transition (Fig. 7, lower left panels).

In the left MG (Fig. 8, left panels), short-latency responses were significantly modified in 10/10 cases during stance post-spinalization. In cat DS1, short-latency inhibitory disappeared and short-latency excitatory responses appeared in 5/5 cases whereas in cat DS2 N1 responses were reduced in 5/5 cases. P2 responses (Fig. 8, right panels) were significantly modified in 10/10 phases. Post-spinalization, in cat DS1, P2 responses were decreased in 5/5 cases during stance while in cat DS2 they were increased in 5/5 cases.

Figure 9 shows responses in the right Srt (left panels) and right VL (right panels) before and after spinalization in cats DS1 (top panels) and DS2 (bottom panels). In both cats, responses increased in the right Srt in 15/20 cases and in the right VL in 15/20 cases.

The latencies of reflex responses in selected muscles are shown in Table 4 for cats DS1 and DS2 before and after spinalization. In the left St after spinalization, the latency of P1 responses was unchanged whereas in TA, latency decreased by 1-2 ms. The latency of N1 or P1 responses decreased in the left MG of cat DS1. In both cats, P2 responses of the left MG occurred earlier. In the right Srt and VL reflex responses occurred earlier post-spinalization.

Discussion

Lesioning the left LGS nerve before spinalization had numerous effects on the expression of spinal locomotion. In general a denervation before spinalization negatively influenced the expression of spinal locomotion with deficits ranging from a greater ankle yield on the denervated side to an inability to express spinal locomotion. Although there were similarities in some of the changes in reflexes

when compared to 'normal' spinal cats (Frigon and Rossignol, companion paper) there were also notable differences, which might explain why the expression of spinal locomotion was negatively influenced by denervating the left LGS before spinalization.

Hindlimb kinematics in DS cats during spinal locomotion

As stated in the introduction, a few studies have shown that lesioning a peripheral nerve (Bouyer and Rossignol 2003b; Carrier et al. 1997) before spinalization negatively influenced the expression of spinal locomotion in cats. The effects of denervating the left LGS are described in Frigon and Rossignol 2007a but it should be noted that all cats had reached a steady state in their locomotor pattern before the spinalization was performed. The present results show that a neurectomy of the left LGS before spinalization generated several kinematic deficits in the expression of locomotion after spinalization. This is in stark contrast to a LGS neurectomy performed after spinalization, where initial deficits, such as an increase in ankle yield, disappear within approximately 2 weeks (Bouyer et al. 2001). In the present study, in cat DS2, spinal locomotion was expressed within 40 days post-spinalization and despite a rhythmic alternation between legs and full hindquarter support there was a much greater ankle yield on the denervated side compared to the intact leg that persisted throughout the study (Fig. 2). In cat DS1, clonidine injections 72 days post-spinalization revealed an asymmetry between both hindlimbs with the denervated leg performing small, disorganized steps (Fig. 3). Spinal locomotion was eventually expressed at 116 days post-spinalization without clonidine, which is a relatively long time when compared to 'normal' spinal cats that express a spinal locomotion in 3-4 weeks (Barbeau and Rossignol 1987; Bélanger et al. 1996).. Moreover, in cat DS1, weight bearing never recovered and constant perineal stimulation had to be provided by the experimenter. Cat 3 never recovered spinal locomotion despite repeated clonidine injections and intensive training for 3 months. Differences in the quality of spinal locomotion between cats could be due to several factors. The allotted recovery time post-neurectomy does not appear to be the

primary factor influencing the expression of spinal locomotion because, although considerably more time (122 days) was allowed post-neurectomy in cat 2 before spinalization cat 3 was given more time (54 days) than cat 1 (49 days) between the neurectomy and spinalization. The magnitude of deficit (e.g. increased ankle yield) initially incurred following the denervation could be a factor [see (Frigon and Rossignol 2007a)]. Cat DS2 had very little deficit after the initial neurectomy whereas cat DS1 had an increased ankle yield that recovered within about 2 weeks. Cat DS3 on the other hand had a very large increase in ankle yield, which never fully recovered even 54 days post-neurectomy. Therefore, our working hypothesis is that, the extent of the deficit incurred after the neurectomy in the intact state and the amount of recovery attained at the time of spinalization are responsible for interanimal differences in the expression of spinal locomotion.

Previous studies also demonstrated that denervations followed by spinalization could produce deficits specific to the lesioned nerve during locomotion. For example, cutaneous inputs, known to be important for proper paw placement during locomotion (Rossignol et al. 2006; Zehr and Stein 1999; Frigon and Rossignol 2006) were lesioned in intact cats, which produced improper paw placement that recovered with time (Bouyer and Rossignol 2003b). However, after complete spinalization proper foot contact was again lost and this time it never recovered. Instead of plantar foot placement there was a persistent paw drag during spinal locomotion suggesting that in the intact state the recovery was maintained by supraspinal commands (Bouyer and Rossignol 2003b). Denervating the left LGS in the intact or spinal state produces an increased ankle yield, which returns to normal values within approximately 2 weeks in most cats (Pearson et al. 1999; Bouyer et al. 2001). The presence of an increased ankle yield after spinalization in cat DS2 (Fig. 2) on the denervated side suggests that supraspinal inputs were involved in the initial compensation and that removing these commands with spinalization removed the adaptation. Perineal stimulation could reduce but not abolish the deficit indicating that under normal circumstances sensory feedback from the LGS muscles provides

excitability to ankle extensors to control the magnitude of ankle yield during early stance.

After spinalization step cycle, swing, and stance durations are normally decreased [(Bélanger et al. 1996), Table 1] but in cat DS2 step cycle, swing, and stance durations were increased after spinalization (Table 1). Step length is also normally decreased after spinalization owing to a reduced ability to position the paw in front of the hip [(Bélanger et al. 1996); Table 1, Fig. 4]. However, in cat DS2 step length was unchanged after spinalization (Table 1) and the distance from the toe to the hip at foot contact was increased (Fig. 4). In both DS cats the position of the toe relative to the hip at foot lift was also unchanged whereas in IS cats it was decreased. In cat DS2 the peak-to-peak angular excursion of the hip, knee, and MTP joints were also increased after spinalization whereas in all 3 IS cats these joint excursions were decreased (Fig. 5). Therefore, the results showed that a denervation before spinalisation could produce changes in the duration and length of the step cycle and its sub-phases and in angular excursions that are not normally observed in "normal" spinal cats. Cat DS2 could be using an adaptive strategy to maximize available inputs from the periphery. For example, an increased distance of the toe at foot contact relative to the hip would increase the stretch imposed on ankle extensors. Increased group I transmission could reinforce the stance phase (Pearson 2008).

Changes in tibial nerve reflexes

The gain of reflexes evoked by stimulating the left Tib nerve was considerably modified following spinalization, as was also shown previous in normal spinal cats (i.e. in spinal cats not subjected to an LGS neurectomy in the intact state). Although there were many similarities in reflex changes between DS and IS cats there were also differences. The most notable difference between DS and IS cats was a decrease in P1 responses of the left TA during the stance phase after spinalization (Fig. 6). In IS cats P1 responses are generally increased during stance after spinalization (see Article 4). Furthermore, in IS cats P1 responses of the left St were increased in

phases 0.05-0.15, or early swing (see Article 4) but in DS cats P1 responses only increased in phase 0.05 in one cat.

Changes in other muscles were similar to those described previously. For instance, in the left MG, there was the appearance of short-latency excitatory responses (DS1) or a reduction in short-latency inhibition (DS2), which is observed in IS cats (Fig. 8). Reflex responses evoked in muscles contralateral to the stimulation were also generally increased throughout the step cycle in DS cats (Fig. 9), which is observed in IS cats. Therefore, a denervation before spinalization can generate changes in reflex responses that are different (left TA and St) and similar (left MG, contralateral muscles) to those observed in normal spinal cats. It is possible that the abnormal changes in reflex activity to ipsilateral flexors produced some of the deficits observed in spinal locomotion. At the very least reflex changes between DS and IS cats illustrate that spinal sensorimotor circuits mediating cutaneous reflex pathways are different in the two states. That some of the changes in reflex pathways (i.e. to ipsilateral extensors and contralateral muscles) were consistent between DS and IS cats suggest that some modifications in reflex pathways occur as a result of the complete spinalization, irrespective of whether or not a denervation is performed before spinalization.

Functional significance

Sensory feedback is undoubtedly critical in the spinal state to adapt to changes in the external environment but the integrity of reflex pathways before spinalization appears of paramount importance for the proper expression of locomotion. It is probable that in the intact state the spinal locomotor CPG optimizes all available inputs following a denervation, which included sensory feedback from the periphery and descending inputs from supraspinal and propriospinal sources. After spinalization, the spinal locomotor CPG appears unable to fully compensate for the loss of supraspinal inputs, which no doubt contributed to the compensation at a spinal level following the denervation in the intact state. Perineal stimulation could reduce the deficits but not make them disappear. In a previous study injecting clonidine in

spinal cats where a cutaneous denervation of the hindpaws was performed in the intact state increased spinal excitability (i.e. increased hip and knee flexor bursts) but could not restore proper paw placement (Bouyer and Rossignol 2003b). Thus the deficits incurred in cats denervated before spinalization do not simply result from inadequate excitability within spinal circuits. Proprioceptive and cutaneous inputs from different sources have specific roles to play in the "development" of spinal locomotion.

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Figures and tables

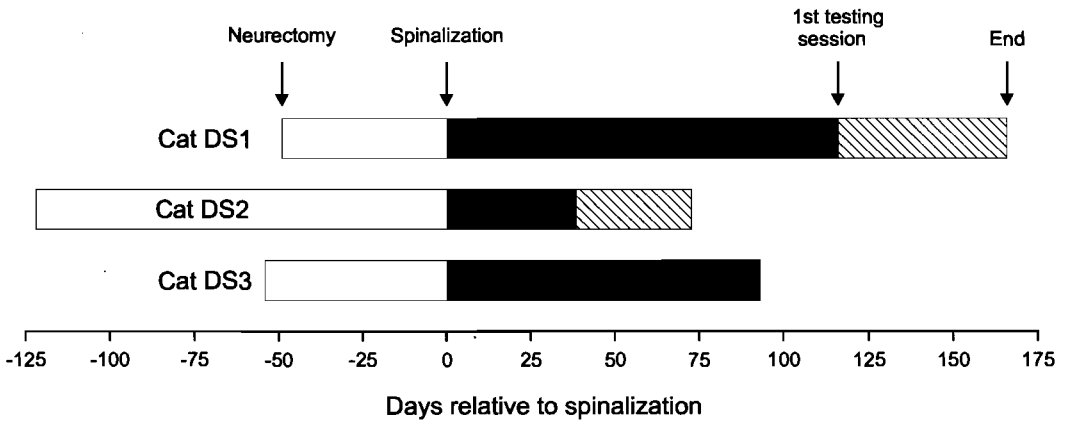

Figure 1. Sequence of events in each DS cat starting with the neurectomy of the left LGS and expressed relative to the time of spinalization.

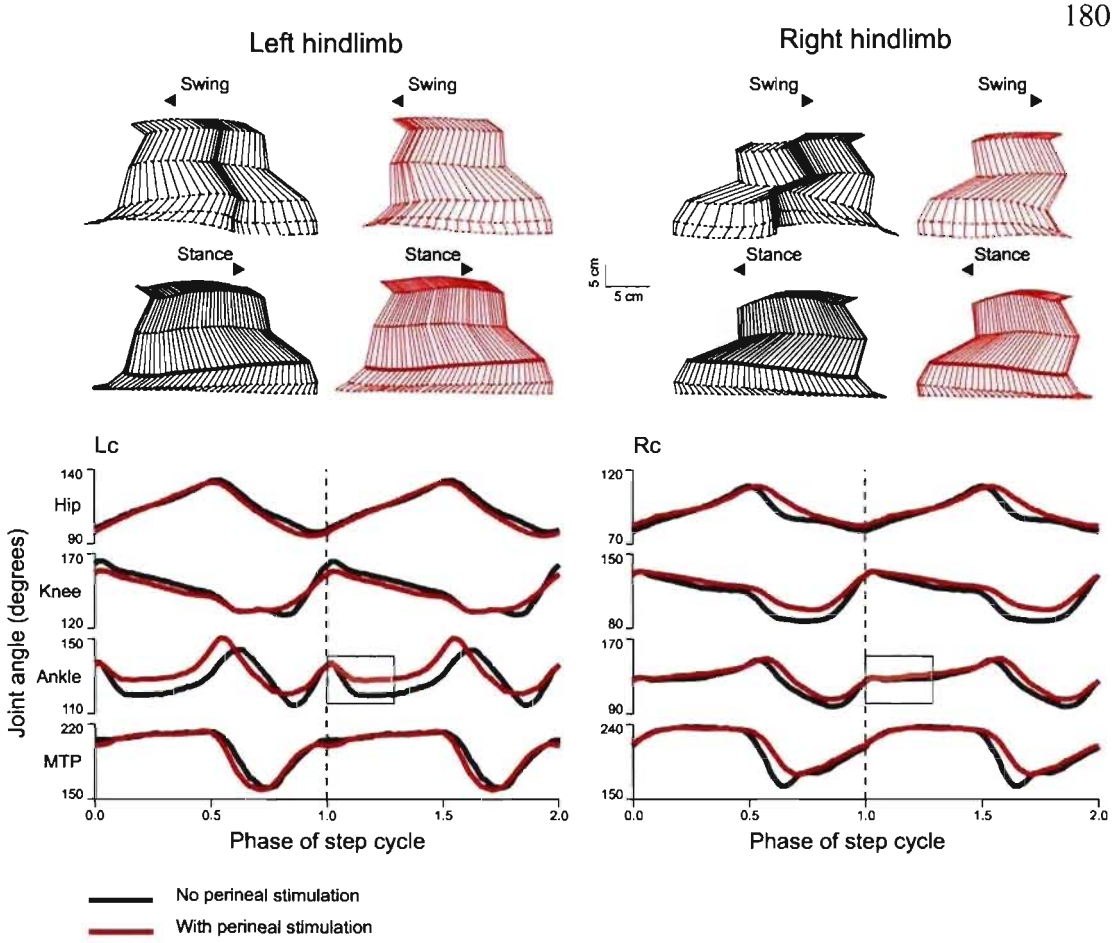

Figure 2. Stick figure reconstructions (top panels) and joint angles (bottom panels) of the left (left panels) and right (right panels) hindlimbs during locomotion without (black) and with (red) perineal stimulation 72 days post-spinalization in cat DS2. Angles of the hip, knee, ankle, and metatarsophalangeal (MTP) joints for each leg are normalized to foot contact during spinal locomotion. Each line is the average of 20-30 step cycles. Shaded areas in ankle joint plots highlight the period of ankle yield during early stance.

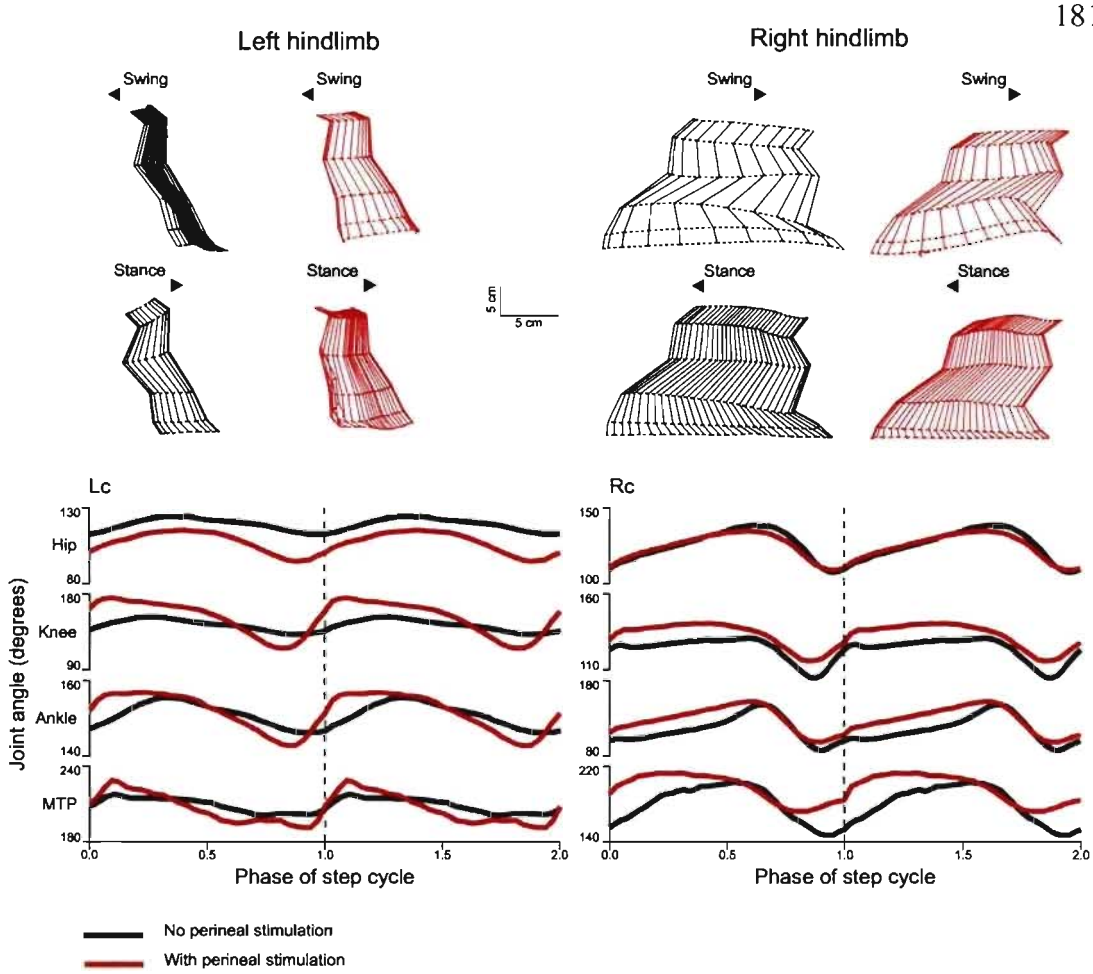

Figure 3. Stick figure reconstructions (top panels) and joint angles (bottom panels) of the left (left panels) and right (right panels) hindlimbs during locomotion without (black) and with (red) perineal stimulation 72 days post-spinalization in cat DS1 more than 35 minutes after clonidine injection. Angles of the hip, knee, ankle, and metatarsophalangeal (MTP) joints for each leg are normalized to foot contact during spinal locomotion. Each line is the average of 20-30 step cycles.

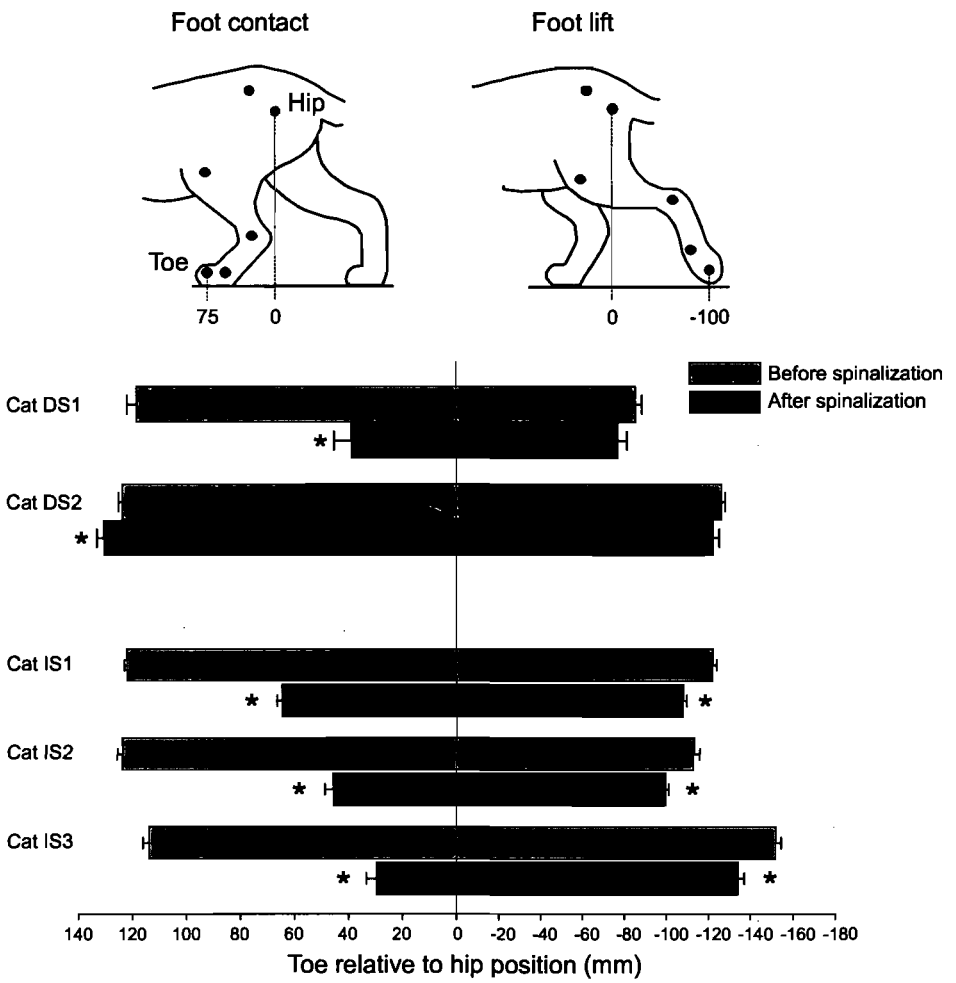

Figure 4. Position of the toe relative to the hip at foot contact and foot lift before and after spinalization in DS and IS cats. Each bar is the average of approximately 60 responses ± SEM.

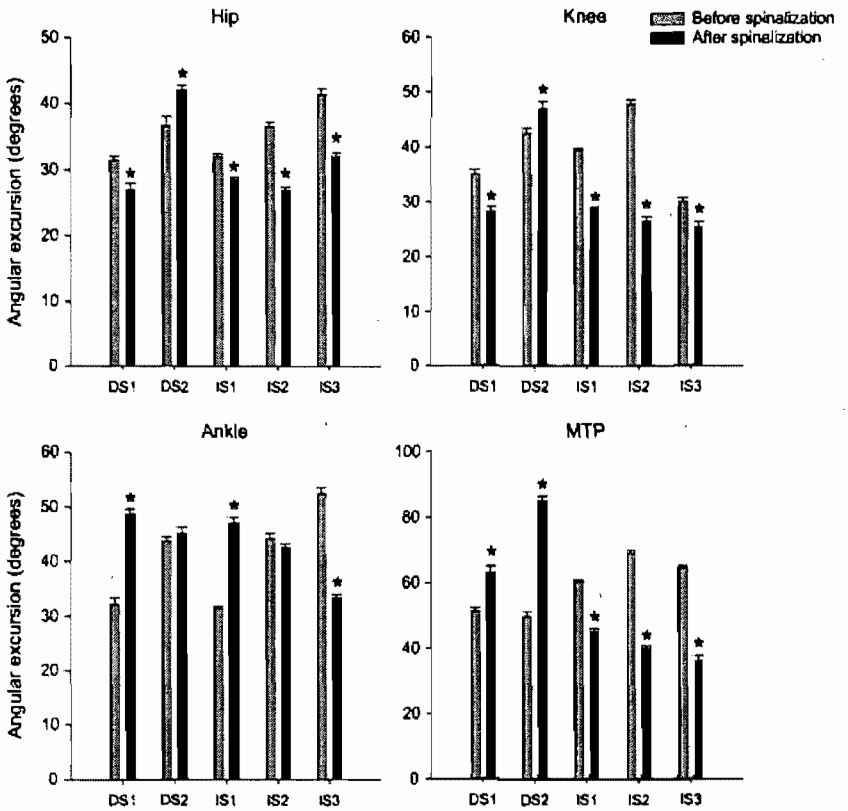

Figure 5. Peak-to-peak angular excursions of the hip, knee, ankle, and MTP joints in DS and IS cats before and after spinalization. Asterisks indicate significant differences ($p < 0.05$) from control values. Each bar is the average of approximately 60 responses ± SEM.

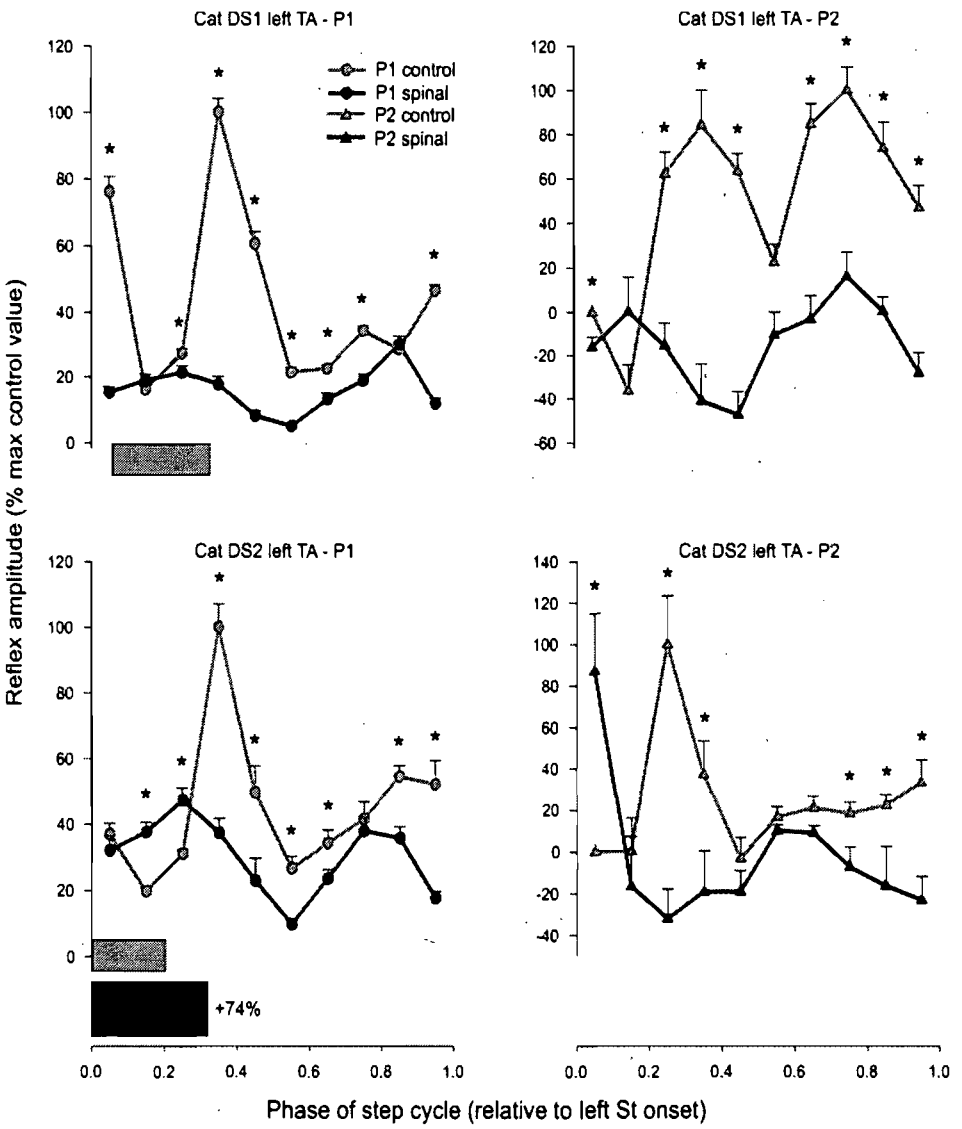

Figure 6. Phase plots summarizing changes in Tib nerve reflexes at 1.2T in the left TA in cats DS1 (top panels) and DS2 (bottom panels). The P1 (left panels) and P2 (right panels) responses are shown. Horizontal rectangles represent the phase of activity of each muscle before (grey) and after (black) spinalization. The number besides the black rectangle indicates the percent difference from control (i.e. before spinalization). In cat DS1 the locomotor burst could not be distinguished and is not illustrated although reflex responses were evoked. Asterisks indicate significant differences ($p < 0.05$) from control in a given phase. Each data point is the mean ± SEM of approximately 30 responses.

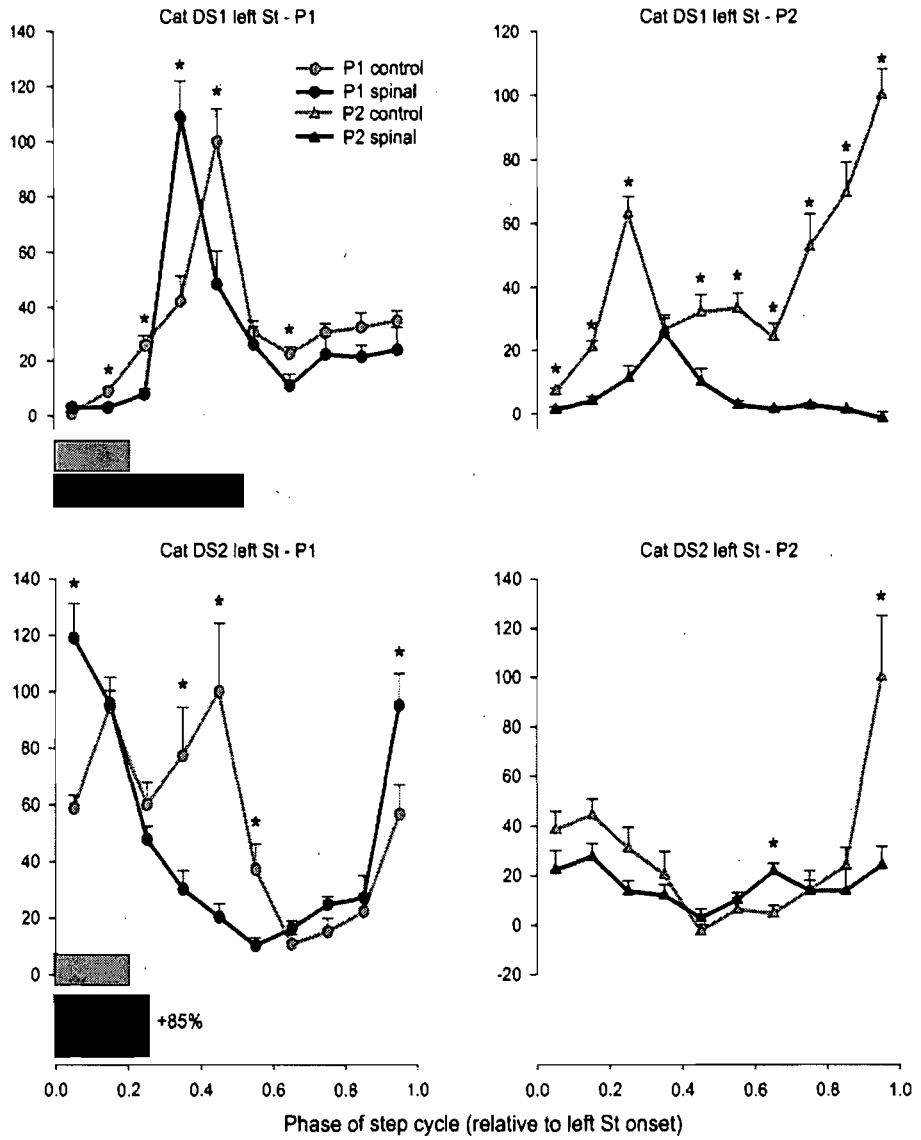

Figure 7. Phase plots summarizing changes in Tib nerve reflexes at 1.2T in the left St of cats DS1 (top panels) and DS2 (bottom panels). The P1 (left panels) and P2 (right panels) responses are shown. Horizontal rectangles represent the phase of activity of each muscle before (grey) and after (black) spinalization. The number besides the black rectangle indicates the percent difference from control (i.e. before spinalization). Asterisks indicate significant differences ($p < 0.05$) from control during a given phase. Each data point is the mean ± SEM of approximately 30 responses.

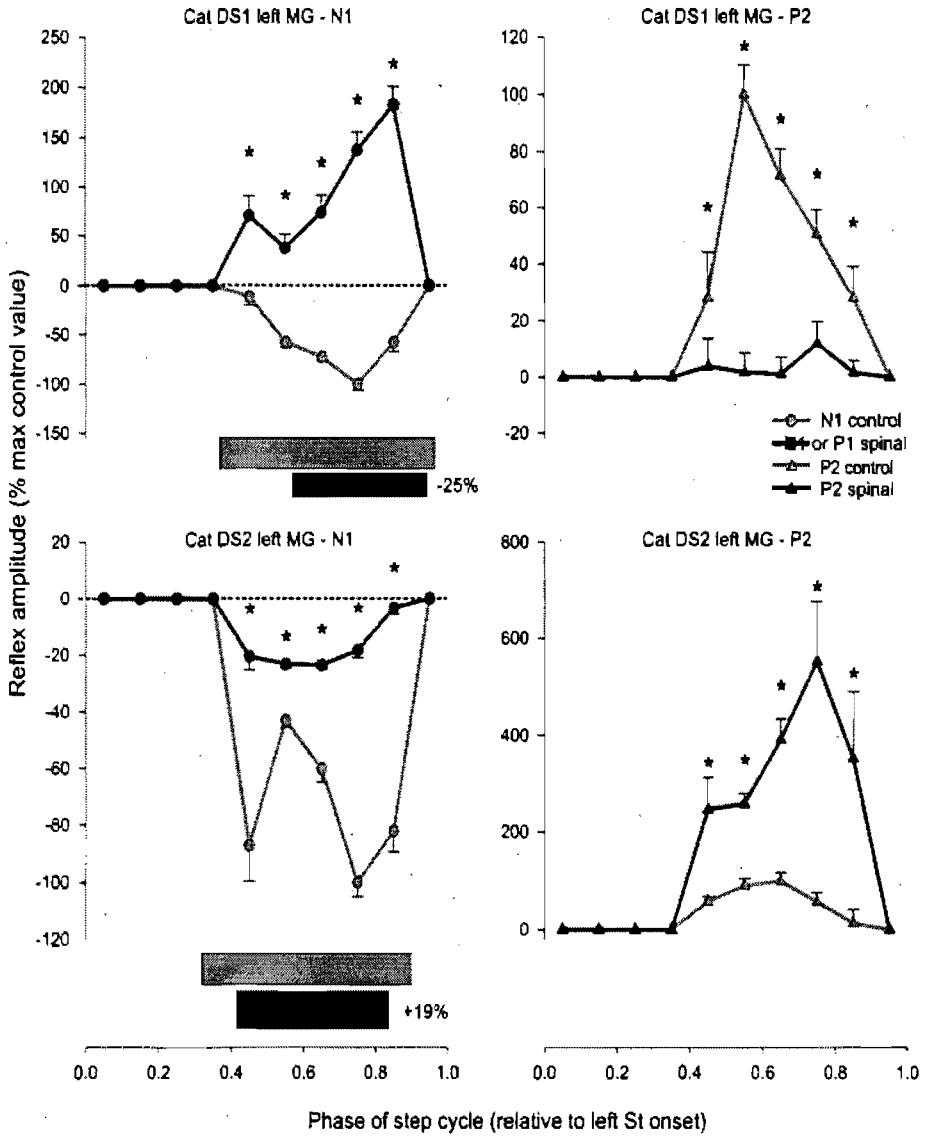

Figure 8. Phase plots summarizing changes in Tib nerve reflexes at 1.2T in the left MG of cats DS1 and DS2. Short- (left panels) and longer-latency (right panels) responses are shown before and after spinalization. Horizontal rectangles represent the phase of activity of each muscle before (grey) and after (black) spinalization. The number besides the black rectangle indicates the percent difference from control (e.g. before spinalization). Asterisks indicate significant differences ($p < 0.05$) from control during a given phase. Each data point is the mean ± SEM of approximately 30 responses.

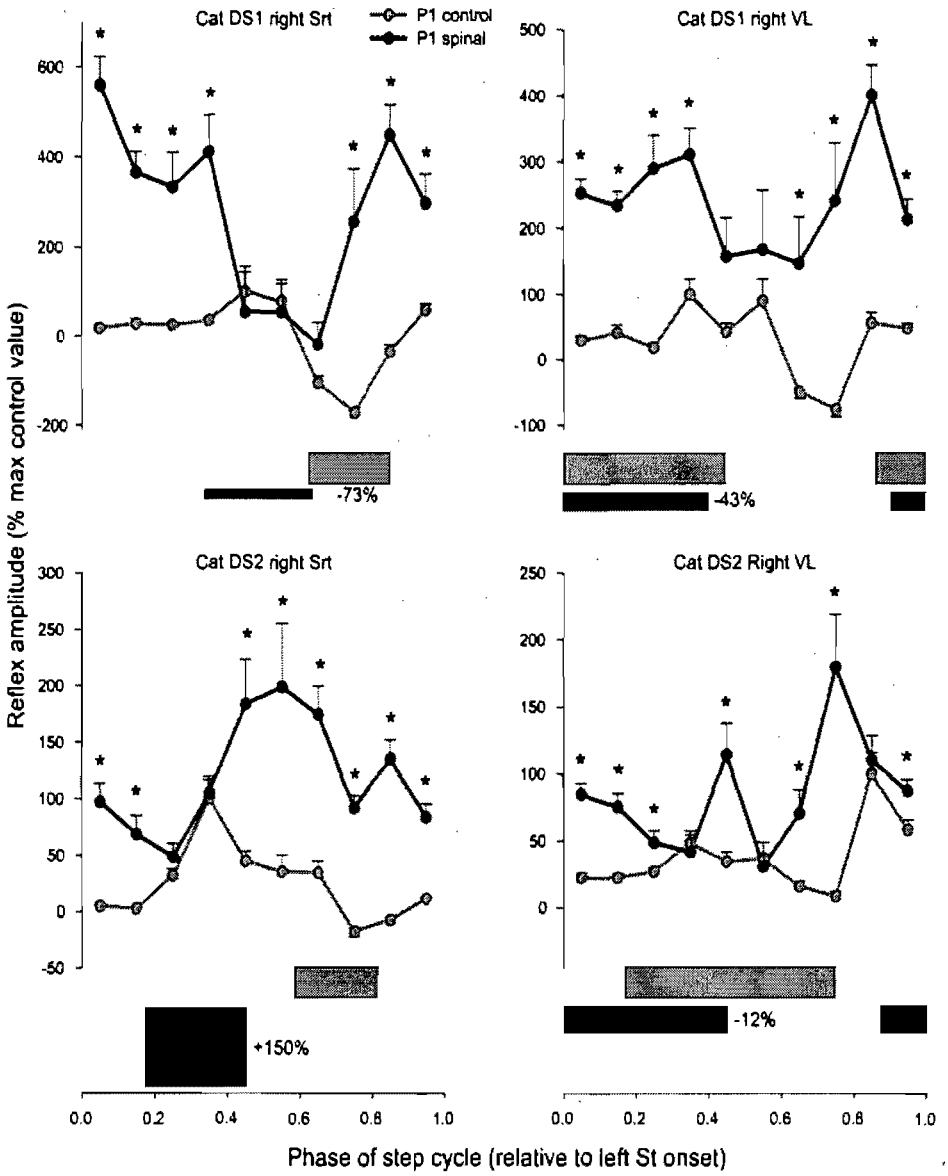

Figure 9. Phase plots summarizing Tib nerve reflexes at 1.2T in the right Srt (left panels) and right VL (right panels) in cats DS1 and DS2 before and after spinalization. Horizontal rectangles represent the phase of activity of each muscle before (grey) and after (black) spinalization. The number besides the black rectangle indicates the percent difference from the control state (e.g. before spinalization). Asterisks indicate significant differences ($p < 0.05$) from control during a given phase. Each data point is the mean ± SEM of approximately 30 responses.

Swing and stance durations and step lengths

	Cat DS1		Cat DS2		Cat IS1		Cat IS2		Cat IS3	
	ms	%	ms	%	ms	%	ms	%	ms	%
Step cycle duration										
Before spinalization	1038 ± 24		931 ± 12		1082 ± 9		1021 ± 15		983 ± 12	
After spinalization	683 ± 12*		1076 ± 16*		751 ± 6*		697 ± 6*		697 ± 14*	
Swing duration										
Before spinalization	332 ± 10	32	311 ± 6	33	358 ± 4	33	345 ± 8	34	341 ± 8	35
After spinalization	256 ± 10*	37	424 ± 16*	39	250 ± 4*	33	273 ± 5*	39	267 ± 12*	38
Stance duration										
Before spinalization	706 ± 21	68	619 ± 9	67	725 ± 8	67	676 ± 12	66	641 ± 10	65
After spinalization	427 ± 11*	63	652 ± 9*	61	501 ± 5*	67	424 ± 8*	61	430 ± 10*	62
	mm		mm		mm		mm		mm	
Step length										
Before spinalization	433 ± 6		517 ± 4		501 ± 4		493 ± 5		552 ± 5	
After spinalization	238 ± 6*		532 ± 6		358 ± 3*		303 ± 5*		343 ± 4*	
Swing length										
Before spinalization	213 ± 6		254 ± 4		250 ± 4		245 ± 5		277 ± 5	
After spinalization	120 ± 4*		265 ± 4*		180 ± 2*		152 ± 3*		170 ± 4*	
Stance length										
Before spinalization	219 ± 7		262 ± 5		251 ± 3		248 ± 4		275 ± 6	
After spinalization	118 ± 4*		266 ± 3		178 ± 2*		151 ± 3*		173 ± 3*	

Table 1. The top half of the table gives durations of the step cycle, swing, and stance phases before and after spinalization in denervated-spinal (DS) cats and intact-spinal (IS) cats. The duration of swing and stance are also expressed as a percentage of the step cycle before and after spinalization. The bottom half of the table gives the horizontal length of the step cycle, swing, and stance before and after spinalization in DS and IS cats. Asterisks indicate significant differences of values recorded before and after spinalization. All values are the mean of 20-50 values ± the standard error of measurement.

Muscle	Cat DS1	Cat DS2
LSt	+78%*	+85%*
LSrt	-15%*	N/A
LVL	-38%*	N/A
LLG	N/A	N/A
LMG	-25%*	+19%*
LSol	N/A	N/A
LTA	N/D	74%*
RSt	N/A	+156%*
RSrt	-73%*	+150%*
RVL	-43%*	-12%
RLG	N/A	N/A
RMG	N/A	-14%*
RSol	-79%*	-17%*
RTA	N/D	N/A

Table 2. Mean amplitude of locomotor bursts after spinalization expressed as the percentage difference from the control value. Asterisks indicate significant differences from control. Each value is the average of approximately 60 bursts.

Muscle		Cat DS1 ON	Cat DS1 OFF	Cat DS2 ON	Cat DS2 OFF
LSt					
	Intact	56 ± 4	77 ± 3	52 ± 4	69 ± 5
	Spinal	55 ± 4	80 ± 8	54 ± 3*	74 ± 4*
LSrt					
	Intact	64 ± 3	93 ± 2	N/A	
	Spinal	56 ± 5*	74 ± 5		
LVL					
	Intact	-2 ± 4	55 ± 3	N/A	
	Spinal	-9 ± 5*	56 ± 7		
LMG					
	Intact	-12 ± 3	51 ± 4	-9 ± 2	50 ± 4
	Spinal	-14 ± 6	43 ± 5*	-5 ± 2*	35 ± 7*
LTA					
	Intact	59 ± 4	87 ± 4	60 ± 3	80 ± 2
	Spinal	N/D		55 ± 7*	85 ± 7*
RSt					
	Intact	107 ± 4	129 ± 6	103 ± 2	122 ± 3
	Spinal	N/A		104 ± 3	124 ± 4
RSrt					
	Intact	111 ± 4	138 ± 6	118 ± 4	142 ± 5
	Spinal	110 ± 12	123 ± 12*	128 ± 6*	146 ± 8*
RVL					
	Intact	43 ± 4	106 ± 3	50 ± 3	103 ± 2
	Spinal	24 ± 6*	86 ± 10*	40 ± 4*	100 ± 2*
RLG					
	Intact	36 ± 3	103 ± 3	44 ± 3	97 ± 4
	Spinal	N/A		N/A	
RMG					
	Intact	N/A		40 ± 3	100 ± 3
	Spinal			42 ± 4*	85 ± 5*
RSol					
	Intact	36 ± 2	99 ± 4	39 ± 3	97 ± 3
	Spinal	23 ± 7*	86 ± 8*	41 ± 3	84 ± 7*
RTA					
	Intact	114 ± 3	139 ± 7	106 ± 3	133 ± 5
	Spinal	N/D		N/A	

Table 3. Onsets (ON) and offsets (OFF) of locomotor bursts after spinalization expressed as a percentage of the step cycle normalized to left foot contact. Asterisks indicate significant differences from control. Each value is the average of approximately 60 bursts.

Latency of reflex responses before and after spinalization				
	Cat DS1		Cat DS2	
Muscle	N1 or P1	P2	N1 or P1	P2
LSt				
Before spinalization	11 ± 2		9 ± 1	
After spinalization	10 ± 2		8 ± 1	
LMG				
Before spinalization	11 ± 2	35 ± 7	10 ± 1	42 ± 8
After spinalization	10 ± 1*	29 ± 4*	9 ± 1	22 ± 4*
LTA				
Before spinalization	11 ± 1		10 ± 1	
After spinalization	10 ± 1*		8 ± 1*	
RSrt				
Before spinalization	28 ± 10		24 ± 5	
After spinalization	22 ± 9*		14 ± 5*	
RVL				
Before spinalization	19 ± 1		18 ± 4	
After spinalization	15 ± 2*		13 ± 4*	

Table 4. Latency of reflex responses for selected muscles before and after spinalization. Asterisks indicate significant differences from control. Each value is the average of approximately 20-30 responses.

Chapter 6 - Article 5. Short-latency crossed inhibitory responses in extensor muscles during locomotion in the cat

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Abstract

During locomotion, contacting an obstacle generates a coordinated response involving flexion of the stimulated leg and activation of extensors contralaterally to ensure adequate support and forward progression. Activation of motoneurons innervating contralateral muscles (i.e. crossed extensor reflex) has always been described as an excitation but the present paper shows that excitatory responses during locomotion are almost always preceded by a short period of inhibition. Data from 7 cats chronically implanted with bipolar electrodes to record electromyography (EMG) of several hindlimb muscles bilaterally were used. A stimulating cuff electrode placed around the left tibial (Tib) and left superficial peroneal (SP) nerves at the level of the ankle in 5 and 2 cats, respectively, evoked cutaneous reflexes during locomotion. During locomotion, short-latency (~13 ms) inhibitory responses were frequently observed in extensors of the right leg (i.e. contralateral to the stimulation), such as gluteus medius and triceps surae muscles, which were followed by excitatory responses (~25 ms). Burst durations of the left Srt, a hip flexor, and ankle extensors of the right leg increased concomitantly in the mid- to late-flexion phases of locomotion with nerve stimulation. Moreover, the onset and offset of Srt and ankle extensor bursts bilaterally were altered in specific phases of the step cycle. Short-latency crossed inhibition in ankle extensors appears to be an integral component of cutaneous reflex pathways in intact cats during locomotion, which could be important in synchronizing EMG bursts in muscles of both legs.

Keywords: Spinal reflex, locomotion, crossed inhibition

Introduction

Bilateral postural adjustments mediated by ipsilateral and crossed spinal reflex pathways are important to adapt to the environment during walking (Zehr and Stein 1999; Burke 1999; McCrea 2001; Rossignol et al. 2006). For instance, mechanical stimulation of the foot dorsum during the swing phase of locomotion generates a coordinated reflex, the stumbling corrective reaction, in several leg muscles bilaterally allowing the perturbed limb to progress over the obstacle (Prochazka et al. 1978; Forssberg 1979; Wand et al. 1980; Buford and Smith 1993; Zehr and Stein 1999). In the stumbling corrective reaction the ipsilateral leg is lifted over the obstacle while the contralateral leg supports the stimulated limb (i.e. crossed extensor reflex). The locomotor program must be capable of adjusting both hindlimbs when one limb is perturbed so that progression and equilibrium is preserved (Saltiel and Rossignol 2004b) but pathways involved in this bilateral coupling are unclear. Recent work in cats has delineated interneuronal spinal pathways involved in stumbling corrective reactions in the ipsilateral limb during fictive locomotion (Quevedo et al. 2005a; Quevedo et al. 2005b) but pathways to the contralateral leg were not studied.

Electrically stimulating cutaneous afferents from the foot has been used as an alternative to mechanical stimulation to evaluate responses in several leg muscles (Duysens and Pearson 1976; Duysens 1977; Duysens and Loeb 1980; Abraham et al. 1985; Pratt et al. 1991; Buford and Smith 1993; Loeb 1993). During swing, electrical stimulation of cutaneous afferents from the paw evokes, in the ipsilateral limb, short- (P1) and longer- (P2) latency excitatory responses in flexors in the swing phase whereas during stance, flexors are typically silent, and responses in extensors are characterized by short-latency inhibition (N1) followed by longer-latency (P2-P3) excitation (Duysens and Loeb 1980; Abraham et al. 1985; Pratt et al. 1991; Loeb 1993). Similar responses are also evoked in the forelimbs during locomotion (Drew and Rossignol 1987; Zehr and Duysens 2004). In the contralateral hindlimb, excitatory responses (P2) are observed in extensors at a latency of 20-25 ms (Duysens and Loeb 1980) and it was proposed that crossed excitatory pathways coordinate

activity between limbs during locomotion (Sherrington 1910a; Lundberg 1979; Gauthier and Rossignol 1981; Lundberg et al. 1987; Rossignol et al. 2006).

However, by conditioning monosynaptic reflexes to examine motoneuron excitability it was shown that crossed inhibitory pathways also exist (Lloyd 1944; Curtis et al. 1958; Holmqvist and Lundberg 1959). In another study, inhibitory post-synaptic potentials mediated by a disynaptic pathway were recorded in motoneurons of the sacral cord following stimulation of low-threshold fibers of the contralateral dorsal root of the same segment (Curtis et al. 1958). Crossed disynaptic inhibition of sacral motoneurons was shown to be mediated by group Ia muscle spindle afferents (Jankowska et al. 1978). Stimulation of group II or cutaneous afferents in non-locomotor anesthetized cats also evokes short-latency inhibition in contralateral ankle extensor motoneurons (Arya et al. 1991; Aggelopoulos et al. 1996; Edgley and Aggelopoulos 2006). During locomotion, inhibition of contralateral extensors while the ipsilateral limb is perturbed during swing would tend to destabilize the animal. Reflex pathways during locomotion are often thought as adjusting phase onset and offset by terminating stance and initiating swing, for example, but rarely within the context of adjusting the coordination between limbs.

A few studies have suggested that probably several interlimb coordination mechanisms exist (Forssberg et al. 1980; Saltiel and Rossignol 2004a; Saltiel and Rossignol 2004b) and it is possible that crossed inhibition plays a critical role. Therefore, because crossed inhibitory responses are elicited by stimulating group II or cutaneous afferents in reduced non-locomotor preparations (Arya et al. 1991; Aggelopoulos et al. 1996; Edgley and Aggelopoulos 2006) we hypothesized that these responses could also be present in walking cats.

Methods

Animals and general procedures

Data were obtained from 7 adult cats (6 males, 1 female) weighing between 3.0 and 7.0 kg. Cats in the present study were used in other experiments (Frigon and Rossignol 2007) but specific observations have not been published previously. Cats

were first selected based on their ability to walk for prolonged periods on a treadmill and trained for approximately 2 weeks at their preferred speed (0.3-0.5 m/s). Cats were subsequently implanted with chronic electrodes for EMG recordings and nerve stimulation, allowed to recover from the implantation, and baseline values of EMGs and reflexes were recorded for 33-60 days.

The experimental protocol was in accordance with the guidelines of the animal Ethics Committee of the Université de Montréal. All surgical procedures were performed under general anesthesia and aseptic conditions. Prior to surgery, cats were injected with an analgesic (Anafen 2 mg/kg; subcutaneously) and pre-medicated (Atravet 0.1 mg/kg, Glycopyrrolate 0.01 mg/kg, Ketamine 0.01 mg/kg; intramuscularly). Cats were then intubated and maintained under gaseous anesthesia (isoflurane 2%) while heart rate and respiration were monitored. After surgery, an analgesic (Buprenorphine 0.01 mg/kg) was administered subcutaneously. An oral antibiotic (cephatab or apo-cephalex, 100 mg/day) was given for 10 days following surgery.

EMG

Chronic electromyographic (EMG) electrodes were implanted bilaterally in the following hindlimb muscles for all cats: semitendinosus (St: knee flexor/hip extensor), anterior part of sartorius (Srt: hip flexor/knee extensor), vastus lateralis (VL: knee extensor), lateral gastrocnemius (LG: ankle extensor/knee flexor) and tibialis anterior (TA: ankle flexor). The medial gastrocnemius (MG: ankle extensor/knee flexor), soleus (Sol: ankle extensor) and gluteus medius (GM: hip extensor) were also implanted in 5, 3, and 2 cats, respectively. A pair of Teflon-insulated multistrain fine wires (AS633; Cooner wire, Chatsworth, CA) was directed subcutaneously from head-mounted fifteen pin connectors (Cinch Connectors; TTI Inc., Pointe-Claire, Canada) and sewn into the belly of each muscle for bipolar EMG recordings. EMG recordings were bandpass filtered (100-3000 Hz) and amplified (gains of 0.5-50K) using two Lynx-8 amplifiers (Neuralynx, Tucson, Arizona). EMG data were digitized (5000 Hz) using custom-made acquisition software.

Step cycle duration was measured as the time between two successive Srt bursts. Burst duration was determined as the time from onset to offset. The effects of Tib nerve stimulation, evoked at different phases of the step cycle, on durations of the step cycle and selected EMG bursts (Srt and ankle extensors bilaterally) were assessed in 5 cats and expressed as the difference from the control (i.e. non-stimulated) values in ms. Stimulated cycles were grouped in one of 10 bins according to the time stimulation was delivered during the step cycle while non-stimulated cycles were averaged to provide control values. The mean control value was then subtracted from the mean stimulated values in each of the 10 bins providing a difference from control in ms in each phase. Correlation coefficients (r) were calculated (Sigmaplot 9.0) to determine the strength of the linear association between burst durations of the left Srt and right ankle extensors and between the right Srt and left ankle extensors with Tib nerve stimulation during different phases of the step cycle.

Nerve stimulation

A chronic stimulating electrode composed of bipolar wires (AS633; Cooner wire, Chatsworth, CA) embedded in a polymer (Denstply International) cuff (Julien and Rossignol 1982) was placed around the left tibial (Tib) nerve at the ankle adjacent to the Achilles' tendon in 5 cats and around the left superficial peroneal (SP) on the dorsum of the foot in 2 cats. Both nerves were stimulated (Grass S88 stimulator) at varying intensities during locomotion with a single 1 ms pulse at a constant time (100 ms after onset of left St burst) to determine the threshold for obtaining a small yet consistent short-latency (~10 ms) response in TA. Stimulation current was then set at 1.2-1.5 times this threshold. During testing sessions, stimuli were given once every three cycles. The time of the stimulus was varied pseudo-randomly to evoke responses at different times during the step cycle for a total of approximately 120-200 stimulations. In one session left SP nerve stimulation was triggered at 100 ms for ~100 stimulations following left St burst onset.

Reflexes were measured as detailed previously (Frigon and Rossignol 2007) and only responses evoked in extensors bilaterally will be described. Figure 1 provides a detailed description of the methodology used to quantify reflex responses. Briefly, the EMGs were grouped into stimulated or control (non-stimulated) trials. The step cycle was divided into 10 phases by synchronizing the cycle to the onset of the left St burst. At least 50 control cycles (i.e. without stimulation) were averaged and separated into these 10 bins according to the time they were evoked in the cycle to provide a template of baseline locomotor EMG (blEMG) during the step cycle (dotted line in Fig. 3). From the 120-200 stimulations, approximately 10-20 reflex responses were grouped in each of the 10 bins superimposed on the blEMG for that bin. Onset and offset of reflexes in extensors, delineated as a prominent negative or positive deflection away from the blEMG, were determined manually using pre-defined latencies as guidelines (Duysens and Stein 1978; Abraham et al. 1985; Pratt et al. 1991; Loeb 1993). We used previously described nomenclature (Duysens and Loeb 1980) where N and P respectively denote negative (inhibitory) and positive (excitatory) responses. The numbered suffix indicates response onset where 1 is ~10 ms and 2 is ~25 ms. Excitatory responses in ipsilateral extensors beginning at ~ 35 ms are sometimes termed P3 (Duysens and Loeb 1980) but for simplicity will be referred here as P2. EMG from onset to offset was rectified and integrated and the blEMG was subtracted from this value. The subtracted value was then divided by a 10 ms portion of the blEMG in the corresponding bin to provide a measure of reflex amplitude. Correlation coefficients (r) were calculated (Sigmaplot 9.0) to determine the strength of the linear association between the amplitude of N1 and P2 in ankle extensors of the same limb evoked by Tib nerve stimulation during the step cycle.

Statistics

A one-way analysis of variance (ANOVA) was used to determine the effects of Tib nerve stimulation when given at different times during the step cycle on the duration of the step cycle and selected EMG bursts across 5 cats. If significant, Dunnet's post-hoc test was performed against control (non-stimulated) values. An

ANOVA was also used to determine significant differences between the latency and duration of ipsilateral and contralateral responses of the same nature (e.g. inhibition or excitation) evoked by Tib nerve stimulation across 5 cats. The duration and latency of inhibitory and excitatory responses in extensors on the ipsilateral and contralateral sides were grouped separately to evaluate differences between both hindlimbs. For example, inhibitory responses in extensors of the ipsilateral limb were compared to inhibitory responses in extensors of the contralateral limb. Significance level was set at $p \leq 0.05$. Descriptive statistics are mean values ± the standard error of measurement (SEM).

Results

Crossed inhibition evoked by SP or Tib nerve stimulation

Short-latency inhibitory responses were observed in extensors of the left leg during stance of the left leg and in the right leg during stance of the right leg with stimulation of the Tib or SP nerves of the left leg, which were followed in both cases by longer-latency excitatory responses. For instance, figure 2 shows the effects of Tib nerve stimulation on selected EMG bursts (Srt and triceps surae muscles bilaterally) during locomotion in one cat. In the same session but at different times, the left Tib nerve was stimulated during stance of the left (Fig. 2A) and stance of the right (Fig. 2B) leg. In both instances stimulation evoked a very brief period of silence, or inhibition, of the ongoing EMG in ankle extensors of both legs. A closer examination in the right LG clearly shows that stimulating the left Tib nerve during stance of the right leg produces a period of inhibition starting approximately 12 ms following the stimulus (Fig. 2C).

In one session for one cat the left SP nerve was stimulated repeatedly at a fixed interval following the onset of the left St burst to elicit a large of number of responses while contralateral extensors were active. Figure 3 illustrates cutaneous reflex responses at short- and longer-latency in the right LG evoked by stimulating the left SP nerve approximately 100 times in one cat while the right hindlimb was in the latter half of stance. A short-latency crossed inhibition at ~13 ms of the ongoing

EMG lasting for ~14 ms is observed (grey area) followed by a longer-latency excitatory response at ~26 ms (black area), which lasts for ~14 ms. Crossed inhibitory responses were consistently observed in ankle and hip extensors but not in vastus lateralis, a knee extensor. Although preliminary results showed that Tib nerve stimulation evoked short-latency inhibitory responses during the hip flexor burst of the contralateral Srt, a bifunctional muscle, the presence of crossed inhibition in flexors and other muscles requires further investigation before a conclusive statement can be made.

To compare reflex responses evoked in extensors of the left and right leg, muscles were recorded bilaterally and stimulation was evoked at different times during the step cycle. Figure 4 shows the average of ~10 responses in each of the 10 phases of the step cycle, synchronized to the left St burst, to stimulation of the left Tib nerve in the left (Fig. 4A) and right (Fig. 4B) MG of the same cat. Stimulation of the left Tib nerve evoked a short-latency inhibition (~10 ms) and a longer-latency excitation (~35-40 ms) in the left MG. Stimulation of the same nerve likewise evoked a short-latency inhibition (~ 13 ms) and longer-latency excitation (~ 25 ms) in the right MG during locomotion. As can be seen, inhibitory responses in the right MG appear at a slightly longer latency and are of shorter duration than responses in the left MG. Response amplitudes in MG of both hindlimbs were modulated according to the phase of the step cycle. The background locomotor EMG is given on the far right for each muscle to illustrate the activity of these muscles during the step cycle. Responses were qualitatively similar in LG, soleus, and GM (not shown). For 5 cats, the mean latency of crossed inhibitory response evoked by Tib nerve stimulation, averaged across all ankle extensors muscles, was 13 ms with a duration of 11 ms. Crossed excitatory responses had a mean latency of 24 ms with a duration of 19 ms. On the left side, for these same muscles, inhibitory responses had a mean latency of 11 ms with a duration of 29 ms. Excitatory responses of the left leg had a mean latency of 38 ms with a duration of 20 ms. On average, inhibitory and excitatory responses in the right leg had a significantly longer and shorter latency of 2 ms and 15 ms, respectively, compared to the left side. Furthermore, the duration of

inhibitory responses on the left side was significantly greater than on the right side by 18 ms but the duration of excitatory responses was not significantly different ($p = 0.524$).

To assess the relationship between the amplitude of short-latency inhibition with the amplitude of longer-latency excitation, N1 and P2 responses were plotted and correlations were made. Upper panels of figure 5 show group data of N1 and P2 responses evoked in ankle extensors of the left (Fig. 5A) and right (Fig. 5B) hindlimbs with stimulation of the left Tib nerve at different times during the step cycle for 5 cats. Response amplitude was modulated throughout the step cycle. Bottom panels show by regression analyses that N1 and P2 amplitudes correlated strongly ($r = -0.85$) on the right side (Fig. 5D) but that there was no correlation ($r = -0.22$) on the left side (Fig. 5C). In other words, a large N1 amplitude will be associated with a large P2 amplitude and vice-versa in contralateral ankle extensors.

Effects of Tib nerve stimulation on step cycle and EMG burst durations

The effects of stimulating the Tib nerve on selected EMG bursts were assessed in 5 cats to determine if nerve stimulation produced concomitant increases in muscle bursts that are simultaneously active bilaterally. Across 5 cats (Figure 6), stimulation of the left Tib nerve prolonged the burst durations of the left Srt and right ankle extensors in phases 0.15 and 0.25, when these muscles were both active (Fig. 6A). The burst durations of ankle extensors of the right leg were unchanged from phases 0.75-0.95, when these muscles are active but the left Srt is silent. Thus, prolongation of the burst in ankle extensors of the right leg occurs only in phases where the burst or activity of the left Srt is also increased. There was no effect of Tib nerve stimulation on burst durations of the right Srt and left ankle extensors at any point during the step cycle (Fig. 6B). For the group, there was a strong correlation ($r = 0.91$) between the durations of the left Srt and right ankle extensors with Tib nerve stimulation at different times during the step cycle (Fig. 6C) whereas for the right Srt and left ankle extensors (Fig. 6D) the correlation was less strong ($r = 0.65$).

Across cats there were no significant effects of Tib nerve stimulation on step cycle duration ($p \geq 0.05$). However, there were significant shifts in the onset and offset of certain muscles relative to the onset of the left Srt but only in two specific parts of the step cycle, with the first and second parts corresponding to phases 0.15-0.35 (e.g. mid- to late flexion of the left leg) and 0.85 (early extension of the right leg), respectively (not shown). For instance, the left Srt burst had a delayed offset (16-44 ms) during phases 0.15-0.35 due to the increased burst duration of this muscle, which was accompanied by a delayed offset (17-22 ms) of right ankle extensors in the same phases. Onsets of left ankle extensors and right Srt were also delayed (4-10 ms) in phases 0.15-0.25 but their offsets were unchanged in these phases. In phase 0.85, the offset (28 ms) and onset (24 ms) of left and right ankle extensors, respectively, occurred earlier. The right Srt burst also finished earlier (3 ms). Therefore, stimulation can influence the timing and durations of hip flexors and ankle extensors bilaterally in specific phases of the step cycle.

Discussion

In the present study, short-latency inhibitory responses in extensors of the limb contralateral to SP and Tib nerve stimulation were evoked during locomotion. To the best of our knowledge no crossed inhibitory responses, evoked by stimulating cutaneous afferents, have been described in intact walking cats, showing that crossed inhibitory pathways described in anesthetized non-locomotor cats (Curtis et al. 1958; Arya et al. 1991; Edgley and Aggelopoulos 2006) operate during normal locomotion. Duysens and Loeb (1980) described in detail responses evoked in contralateral muscles by stimulating cutaneous nerves of the foot during locomotion in the cat. The reason for the absence of crossed inhibition in that paper is unclear but an inspection of their figure 3 would seem to indicate that a period of inhibition precedes the excitatory response in the contralateral MG. The lack of a template of background locomotor activity (e.g. non-stimulated trials) probably prevented a clear distinction of the crossed inhibition that was observed in the present study. The

crossed pathways responsible for these responses and their putative functions during locomotion are discussed.

Afferents mediating the responses

Stimulation of both SP and Tib nerves just above motor threshold evoked qualitatively similar short-latency crossed inhibitory responses in extensors indicating that some of these effects were mediated by large diameter cutaneous afferents. Although the Tib nerve innervates intrinsic muscles of the foot and contains group I and II muscle afferents the SP nerve at the level of the ankle is entirely cutaneous. Stimulating the Tib nerve at an intensity of 1.2-1.5 x the threshold for evoking small but consistent short-latency responses in the ipsilateral TA could have recruited group I muscle afferents and group II muscle and cutaneous afferents. Edgley and Aggelopoulos (2006) reported that IPSPs in contralateral extensor motoneurons evoked by stimulating SP or sural nerves appeared near the threshold of the most excitable fibers, suggesting that large diameter Aβ fibers, most likely mechanoreceptor afferents, were responsible. Smaller diameter afferents can also contribute to responses because increasing stimulus intensity evoked larger crossed inhibitory responses in anesthetized cats, indicating a convergence between large and small diameter afferents (Edgley and Aggelopoulos 2006). Irrespective of which afferent type mediated responses, it is clear that crossed inhibition can be evoked in the intact cat during locomotion and forms part of the crossed pathway (i.e. inhibition followed by excitation) for certain muscles.

Central pathways

The differences in latencies of inhibitory responses on the left and right sides were similar to those following stimulation of cutaneous afferents in anesthetized cats (Edgley and Aggelopoulos 2006). For instance, contralateral inhibition of EMG on average started ~2 ms later than ipsilateral inhibitory responses whereas Edgley and Aggelopoulos (2006) reported a difference of ~1 ms. The small difference between the two preparations could simply be due to the conduction distance from the spinal

cord to recording sites and because in the present study we recorded EMG activity whereas Edgley and Aggelopoulos (2006) recorded post-synaptic potentials in motoneurons.

Central pathways responsible for crossed inhibitory responses are probably the same described for anesthetized preparations (Jankowska et al. 2005b; Bannatyne et al. 2006; Edgley and Aggelopoulos 2006). For example, inhibitory interneurons in the dorsal horn of mid-lumbar spinal segments have wide ranging ipsilateral and contralateral projections to many regions of the spinal grey matter including connections with large cholinergic neurons in the ventral horn, most likely motoneurons (Bannatyne et al. 2006). Excitatory connections from primary cutaneous afferents to these inhibitory interneurons in the dorsal horn could mediate crossed inhibition during locomotion. Another pathway mediating crossed inhibition in anesthetized cats includes activation of contralateral Ia inhibitory interneurons by excitatory commissural interneurons (Jankowska et al. 2005b). Commissural interneurons, inhibitory and excitatory, can be excited by various afferents, including group II and cutaneous afferents (Jankowska et al. 2005a; Jankowska et al. 2005b; Edgley and Aggelopoulos 2006; Jankowska 2007).

Short-latency crossed inhibition was not present in the right VL following stimulation of the SP or Tib nerves of the left leg. The absence of crossed inhibition in VL could be due to the fact that motor pools of VL are located at L5-L6 whereas those of glutei and triceps surae muscles, which exhibited crossed inhibition, are found at L7-S1 (Vanderhorst and Holstege 1997; Yakovenko et al. 2002). This could suggest that relay neurons in the crossed inhibitory pathway observed in the present study are located more caudally within the spinal cord.

The strong correlation between the amplitude of crossed inhibition and excitation (Fig. 5) could mean that the short-latency inhibition controls the excitability of the longer-latency excitatory pathway without requiring inputs from supraspinal levels. It is likely that part of the excitatory response is mediated by post-inhibitory rebound (Abraham et al. 1985). However, because short- and longer-latency excitatory responses can be evoked during the swing phase of locomotion in ipsilateral ankle

extensors without preceding inhibition a longer latency reflex pathway must also be involved (Duysens and Loeb 1980). Therefore, excitatory responses are probably mediated by reflex pathways, which can be supplemented via post-inhibitory rebound.

Effects of stimulation and bilateral cycle adjustments

During fictive locomotion, stimulating the Tib nerve during ipsilateral stance increased the duration of the activity of ipsilateral extensors, whereas stimulation during the ipsilateral flexion phase terminated flexion and initiated extension (Guertin et al. 1995). In intact cats stimulation can advance or delay phases but abrupt terminations and initiations are rarely observed because this would disrupt ongoing locomotion. Instead, the activation of sensory pathways ensures proper interlimb coordination by adjusting the timing and durations of specific bursts. In a situation where speed is enforced by the treadmill, step cycle duration remains largely unaffected, although sub-components of the step cycle can be altered. For example, as shown previously (Duysens and Stein 1978) and in this study stimulating the Tib nerve during ipsilateral flexion prolonged ipsilateral hip flexor and contralateral ankle extensor bursts whereas stimulation during ipsilateral stance had little or variable (Duysens and Stein 1978) effect. Crossed pathways coupling both hindlimbs from cutaneous or muscle afferents could ensure that step cycle duration remains constant while at the same time varying the sub-phases (e.g. flexion and extension phases bilaterally) to ensure proper interlimb coordination.

It was also reported that most timing adjustments occurred around mid- or late swing (Forssberg et al. 1980), which in our preparation corresponds approximately to phases 0.25-0.35 where effects of stimulation on locomotor bursts were most evident. For example, stimulation of the left Tib nerve delivered during phases 0.25-0.35 delayed the offset of the left Srt and of right ankle extensors. The onsets of left ankle extensors and of the right Srt were also delayed. Thus, there are critical points during locomotion in which peripheral inputs can influence the step cycle, as shown previously during fictive locomotion (Saltiel and Rossignol 2004a; Saltiel and

Rossignol 2004b). Additionally, when the cat was in a double support phase stimulation of the left Tib nerve did not increase the burst duration of ankle extensors of the right leg, most likely because the left leg was also being supported. Therefore, biomechanical events can modify or 'override' some bilateral cycle adjustments (Saltiel and Rossignol 2004a; Saltiel and Rossignol 2004b). In a functional context during locomotion, a prolongation of swing of the left leg during a perturbation would require a concomitant increase in stance of the right leg so that progression and equilibrium are maintained.

Functional considerations

It has been proposed that decomposing the step cycle into several sub-phases, with each requiring the activation of a specific set of modules would simplify how descending systems modify limb activity (Grillner 1981; Grillner and Wallen 1985; Stein and Smith 1997; Lafreniere-Roula and McCrea 2005; Krouchev et al. 2006; Ivanenko et al. 2007). Although only a concept, these modules would undoubtedly be coupled by feedback from the periphery through various ipsilateral and crossed pathways. As such, inhibition in addition to excitation would provide more flexibility to this system. The burst durations of the left Srt and right ankle extensors (Fig. 6) were strongly correlated with stimulation of the left Tib nerve while these muscles were active suggesting that crossed pathways interconnect left hip flexor and right ankle extensor 'modules'.

Sherrington (Sherrington 1910b; Sherrington 1913) long ago suggested that inhibition in reflex pathways is an integral component of various reflex pathways in several behaviors, including locomotion, but the precise function of inhibition remains poorly understood. In particular, crossed inhibition of extensors while the ipsilateral leg is in flexion would tend to destabilize the animal during walking. Although we can only speculate as to the function of crossed inhibition during locomotion, when considered during the forward progression of stepping, crossed inhibition may serve to temporarily and very briefly "halt" or slow down forward progression. After all, crossed extension is only going to be useful if the ipsilateral

flexion actually frees the limb from the perturbation. Moreover, short-latency inhibition of extensors, ipsilaterally or contralaterally, could serve to delay the onset of excitatory responses to enable supraspinal structures sufficient time to influence these pathways. Therefore, even though the precise function of short-latency inhibition of contralateral extensors remains unclear what is certain is that crossed extensor reflexes are more complex than originally thought.

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Figures

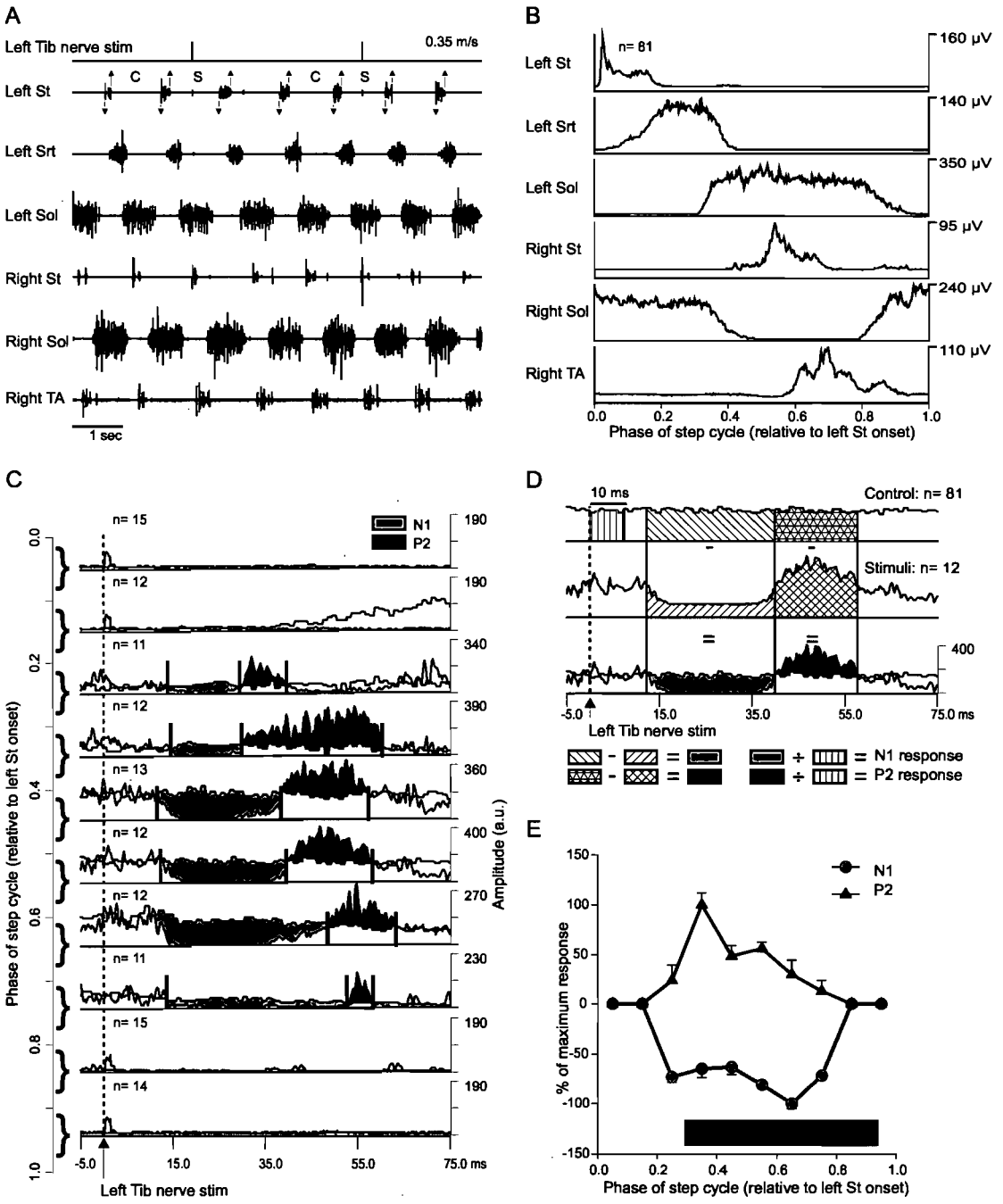

Figure 1.

Figure 1. Methodology for analysing reflex responses during locomotion in the cat. A) Raw EMG burst recordings of several hindlimb muscles during locomotion (an 8 second sequence is shown). A cycle is defined as the period between onsets of two successive St bursts. Stimuli (vertical lines in first trace) were given approximately once every three cycles at different delays following St burst onset using a pre-programmed sequence to evoke responses in different parts of the step cycle. Up and down arrows respectively indicate onset and offset of left St bursts during locomotion. Cycles are tagged as stimulated (S) if a stimulus was given whereas the preceding burst is generally tagged as control (C) cycle provided it did not follow a stimulated cycle. Stimulated cycles are grouped in one of 10 bins according to the time stimulation was given during the step cycle. For example, in a cycle lasting 1000 ms 10 equal bins of 100 ms would be generated. A stimulus given from 0-99 ms following St burst onset would be placed in the first bin and so on for each stimuli. B) A template of locomotor EMGs was generated from 81 control cycles beginning at left St burst onset and normalized to 1. Each template is separated into 10 bins and provides the background level of EMG (blEMG) in each phase of the step cycle. C) Stimulated cycle are grouped and averaged into one of 10 bins with the corresponding blEMG superimposed. Onsets and offsets (short vertical lines) of short- and longer-latency responses are determined manually for extensors. D) In each phase the blEMG occurring in the same time window as the response is subtracted from the response in the stimulated cycles illustrated by the integrated areas (short- and longer-latency responses are shown in grey and black, respectively). The subtracted value is then divided by a 10 ms block of blEMG in the same bin giving N1 and P2 response amplitudes. The division is necessary because inhibitory responses are a function of blEMG; meaning that subtracted values are larger if there is a greater level of blEMG and vice-versa. A fixed time window is used for all bins because if the same time window as the response was used the duration of the inhibition or excitation would be taken out of the equation. E) N1 and P2 responses are expressed as a percentage of the maximum response in one of the 10 bins. For example, the largest P2 response in

this case was in the 4th bin and every response is expressed as a percentage of that value. The black rectangle represents the activity of the muscle during the step cycle.

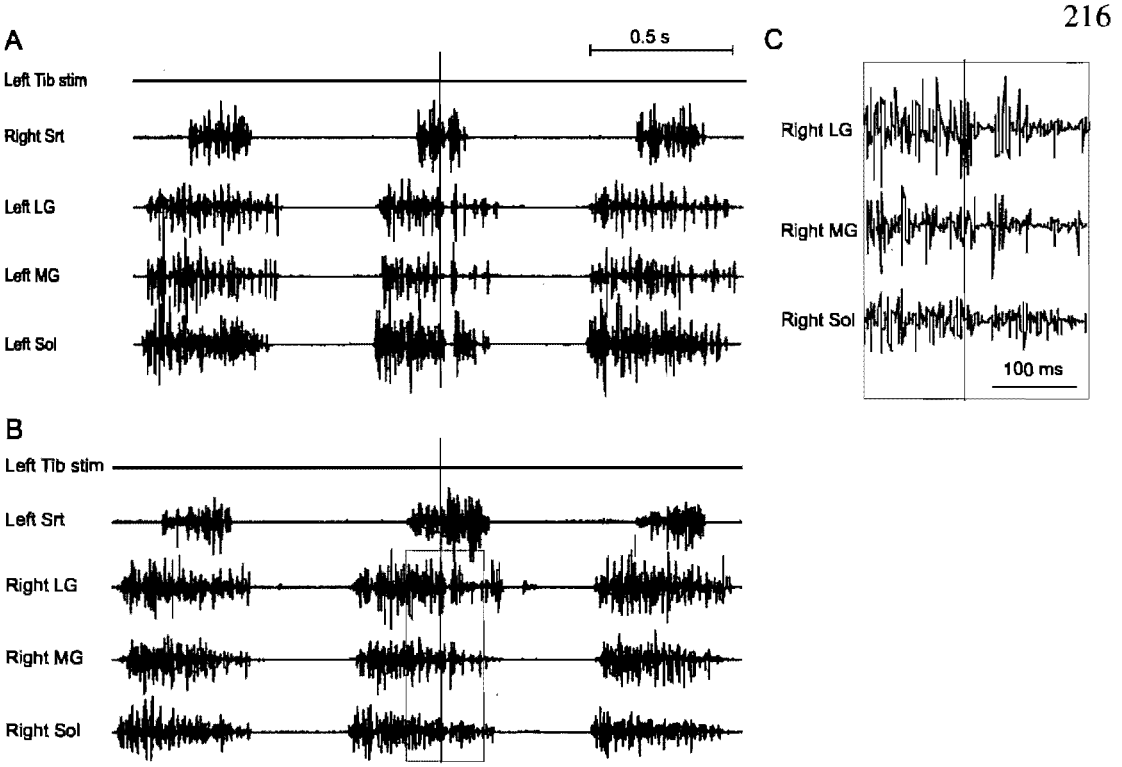

Figure 2. Effects of Tib nerve stimulation on the duration of selected locomotor bursts (Srt and triceps surae muscles bilaterally). Stimulation of the left Tib nerve was delivered at different times during stance of the left (Fig. 2A) and right (Fig. 2B) hindlimb in the same cat. A closer look at the crossed inhibition in the right triceps surae muscles is provided in Fig. 2C.

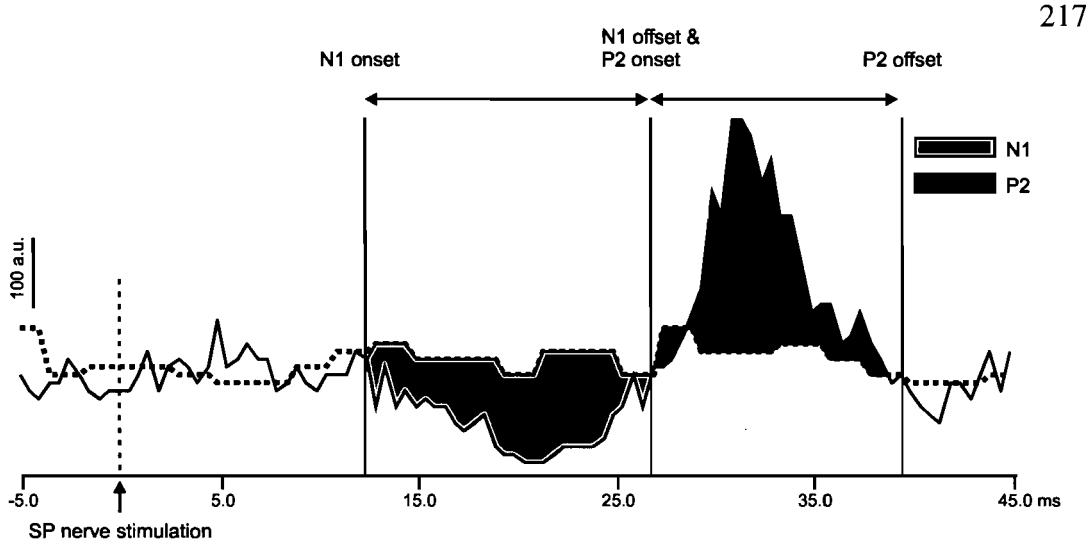

Figure 3. Cutaneous reflexes evoked by stimulating the left SP nerve in the right lateral gastrocnemius (LG) during the latter half of the extension phase of the right hindlimb. Stimulation of SP evoked a short-latency inhibition (~ 3 ms) followed by a longer-latency excitation (~26 ms). Onsets and offsets of both responses were determined and the areas of EMG activity were integrated in stimulated cycles (solid black line) and the locomotor template (dotted black line) to provide a measure of N1 (grey area) and P2 (black area) responses. Responses were then divided by a 10 ms block of the baseline locomotor EMG (blEMG), during the same phase of the step cycle, to give amplitudes of N1 and P2. Each line (template and stimulated cycles) is the average of approximately 100 cycles.

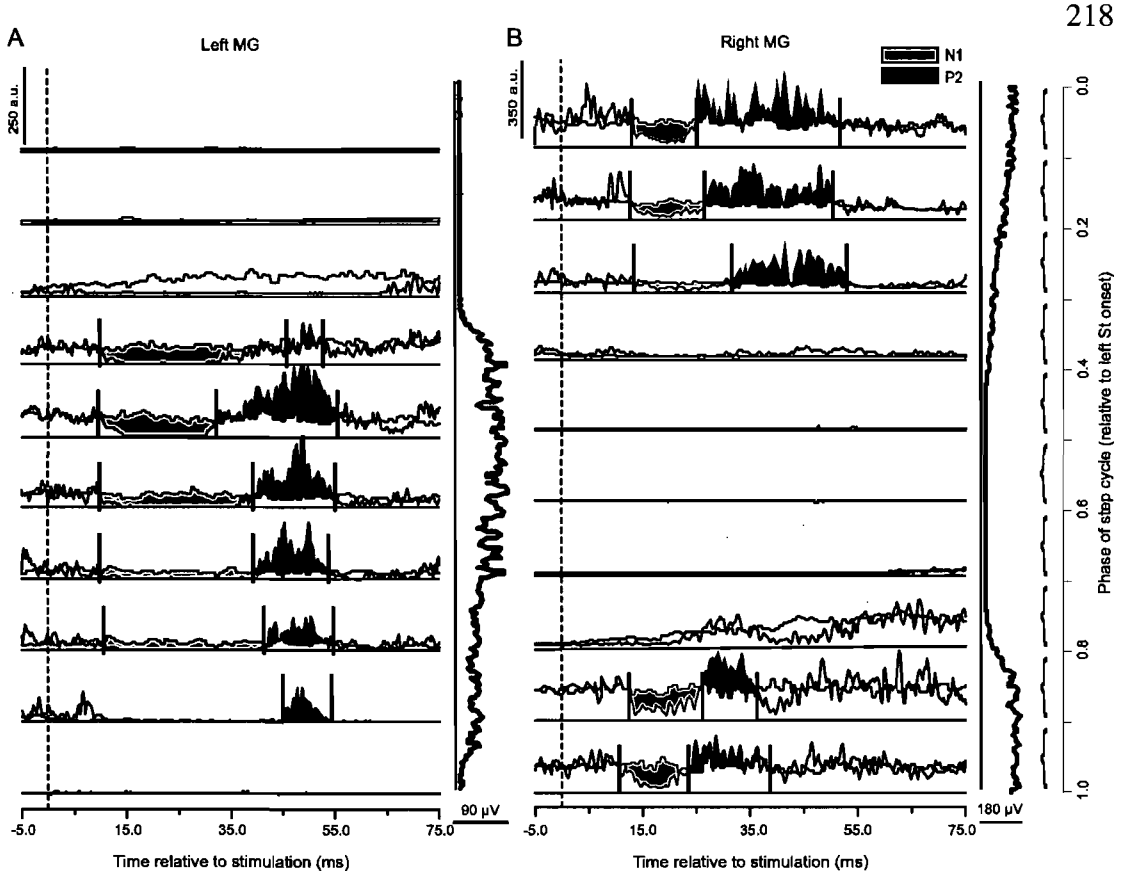

Figure 4. Cutaneous reflexes evoked in the left (Fig. 4A) and right MG (Fig. 4B) of the same cat by stimulating the left Tib nerve. Averaged reflex responses were separated and grouped into 10 phases according to the time they were evoked in the step cycle. Each line is the average of approximately 10 stimulations. On the far right of each panel is the rectified single EMG burst of the corresponding muscle during the step cycle. Each EMG burst line is the average of approximately 60 bursts.

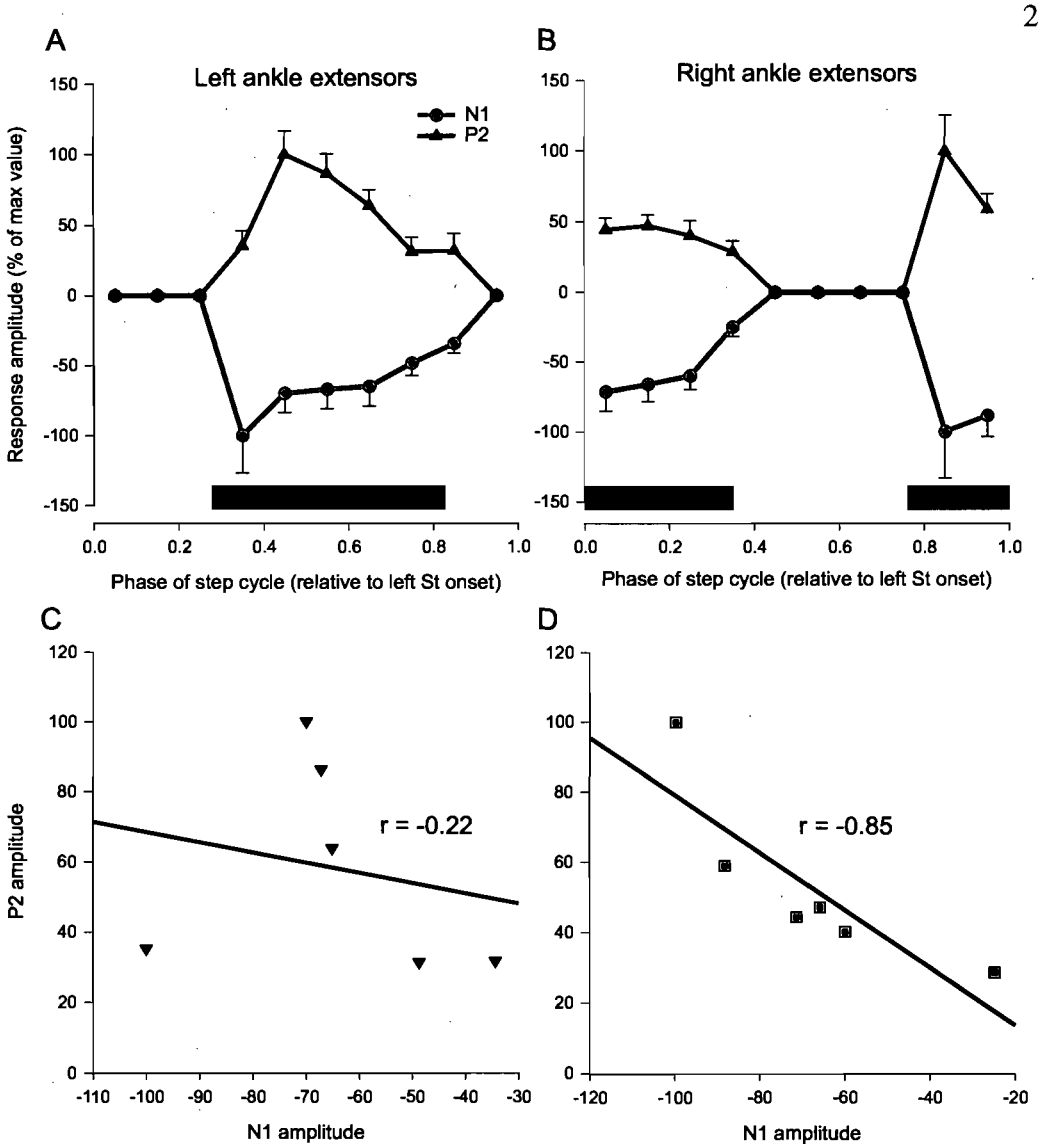

Figure 5. Upper panels show reflex amplitudes of N1 and P2 in ankle extensors of the left (Fig. 5A) and right (Fig. 5B) legs as a function of blEMG expressed as a % of the maximal value during the step cycle at the same scale. Each data point is the mean ± SEM of approximately 10 responses. Black horizontal rectangles represent the period of activity of each muscle during the normalized step cycle. Bottom panels show the relationship between these N1 and P2 amplitudes in ankle extensors of the left (Fig. 5C) and right (Fig. 5D) legs with corresponding correlation coefficients (r). Note that only those points where the muscle was active are included in the regression analyses.

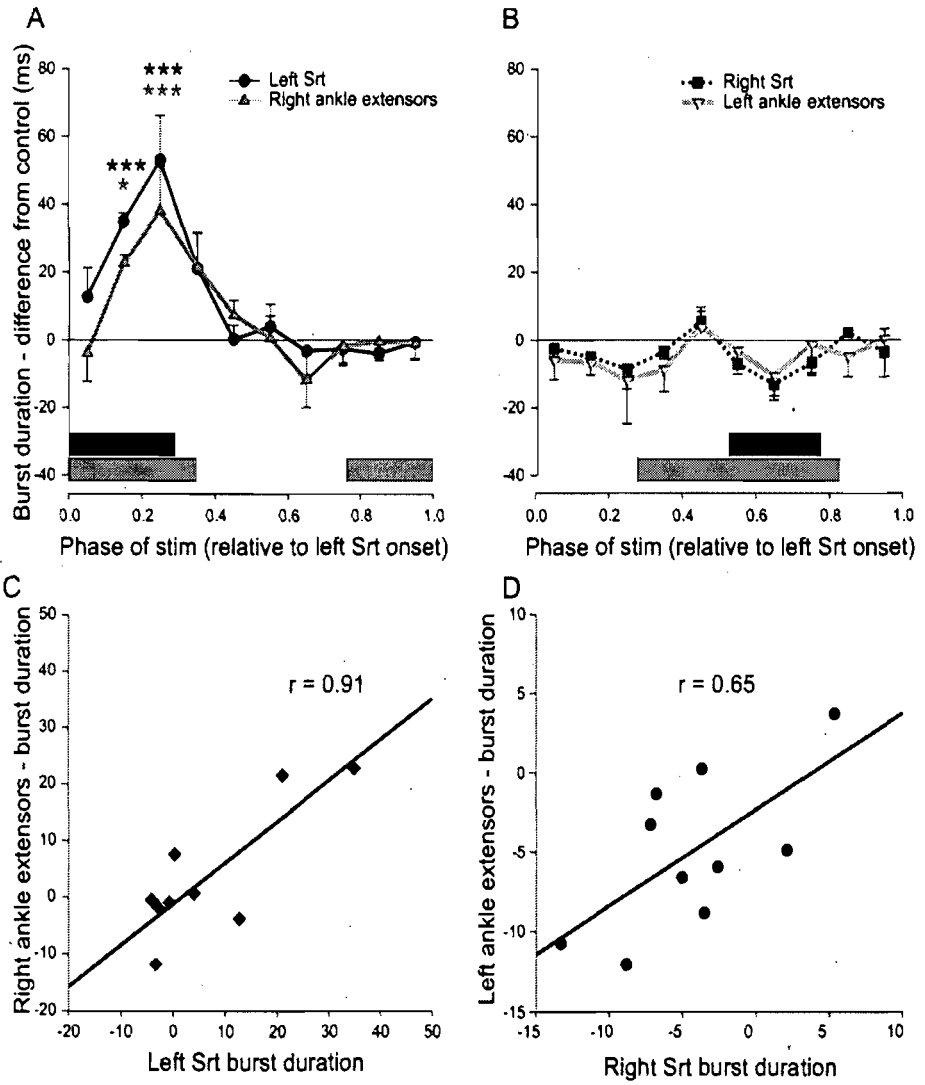

Figure 6. Upper panels show the effects of stimulating the left Tib nerve at different times during the step cycle on burst durations of the left Srt and right ankle extensors (Fig. 6A) and of the right Srt and left ankle extensors (Fig. 6B), expressed as the difference from control (i.e. non-stimulated bursts), across five cats at the same scale. Black and grey bars at the bottom of each graph show the period of activity for Srt and ankle extensors, respectively. Bottom panels show the linear relationship and coefficient of correlation (r) between burst durations of the left Srt and right ankle extensors (Fig. 6C) and of the right Srt and left ankle extensors (Fig. 6D) during locomotion. Each data point is the mean ± SEM where * is $p < 0.05$ and *** is $p < 0.001$.

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Functional organization of cutaneous reflex pathways during locomotion and reorganization following peripheral nerve and/or spinal cord lesions

par

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Chapter 7 - General conclusion

As discussed in the introduction locomotion is a complex task requiring dynamic sensorimotor interactions between the spinal CPG, descending supraspinal inputs, ascending and descending propriospinal inputs, and sensory feedback from the periphery (Rossignol *et al.* 2006). Removing inputs at any of these levels creates a new "state" within the spinal locomotor circuitry and consequently, the spinal CPG must optimize available inputs to maximize remnant locomotor capabilities (Rossignol 2006; Rossignol et al. 2008). In the introductory chapter it was stated that functional changes occurring within spinal reflex pathways (i.e. reflex gain, phase-dependent modulation) after lesions to the spinal cord or to peripheral nerves during locomotion are largely unknown. In chapters 2-6, cutaneous reflexes were recorded in the same cats before and after lesioning the spinal cord and/or the nerve supplying the left LGS to probe the functional organization and reorganization of sensorimotor pathways during locomotion and to determine whether changes in cutaneous reflex transmission could participate in the locomotor compensation after such lesions.

That recordings were made in the same cat before and after different lesion paradigms is important because reflexes can differ from one cat to another (Loeb 1993; Loeb 1999), which limits information drawn by comparing groups of intact and groups of lesioned cats. In all studies, reflexes were initially evoked and recorded in the intact state during locomotion. In one group of cats, reflexes were recorded before and after sectioning the left LGS nerve (Chapter 2) and following a period of recovery a complete spinalization was made at T13. Cats were then trained on a treadmill to express a spinal locomotion and recordings resumed once a 'stable' spinal locomotion was achieved (Chapter 5). In another group of cats, reflexes were again evoked in the intact state and after obtaining stable recordings a complete spinalization was made. Recordings resumed once a stable spinal locomotion was expressed and after baseline values were recorded (Chapter 4), the left LGS nerve was sectioned (Chapter 3). As a whole, cutaneous reflexes from the Tib nerve were studied in 5 states, which included 1) intact, 2) intact-denervated, 3) spinal, 4) spinal-

denervated and 5) denervated-spinal. The results in chapters 2-5 suggest that cutaneous reflex pathways are involved in the functional recovery of locomotion following spinal cord and/or peripheral nerve lesions and that the spinal locomotor network optimizes available inputs following injury. These issues and their implications are discussed in the following sections.

Mechanisms mediating functional recovery following a peripheral denervation

A partial denervation of ankle extensors in the intact [Chapter 2, (Frigon and Rossignol 2007a)] and spinal (Chapter 3) states produced an increased activity in multiple hindlimb muscles bilaterally, not just the primary remaining synergist, the medial gastrocnemius (MG), as reported previously (Pearson et al. 1999; Pearson 2000; Bouyer et al. 2001). It was hypothesized that the increased MG activity was mediated by enhanced transmission in MG group I pathways because this muscle was more stretched following the denervation and was required to produce more force (Pearson et al. 1999; Pearson 2000). However, although there is evidence that the efficacy of transmission in group I pathways from MG is modified after denervating adjacent ankle extensors (Whelan and Pearson 1997; Fouad and Pearson 1997) this increased efficacy does not appear to mediate the increased MG activity [discussed in (Frigon and Rossignol 2007a)].

In Chapters 2 and 3, cutaneous reflexes evoked by stimulating the Tib nerve during locomotion were modified after denervating the left LGS but changes in the gain of these pathways did not correlate with changes in the burst activity of several hindlimb muscles. It is unlikely that the increased muscle activity is mediated directly by changes in the gain of reflex pathways either from remaining synergists or cutaneous receptors. Instead, we hypothesized, as have others (Gritsenko et al. 2001; Pearson et al. 1999) that the increased muscular activity is due to a modified central drive from the spinal CPG for locomotion. How the spinal locomotor CPG rescales its activity remains unknown but inputs provided by reflex pathways from cutaneous, joint and muscle receptors could be important in signalling the CPG that changes in muscular activity are required to stabilize the locomotor pattern. In addition, changes

in reflexes, could serve to modify the locomotor pattern and palliate deficits proper to each cat.

Performing a lesion of the left LGS in the intact and spinal states also enabled us to determine structures involved in functional recovery. Denervating the left LGS in otherwise intact cats [Chapter 6; (Frigon and Rossignol 2007b)] or in already spinal cats [Chapter 3; (Bouyer et al. 2001)] produced only transient deficits and the pattern of locomotion adapted over time. Evidently, in the spinal state (i.e. denervation after spinalisation) the spinal cord along with peripheral inputs from the hindlimbs are sufficient to offset the loss of two ankle extensors but in the intact state, supraspinal inputs in addition to spinal mechanisms are probably involved in the recovery. In chapter 3 we showed that adaptive mechanisms after denervating the left LGS, such as changes in locomotor bursts and Tib nerve reflexes, in the intact and spinal states shared some similarities, indicating that the spinal cord possesses intrinsic mechanisms that can effect changes in locomotor bursts and reflex pathways but also differences showing that supraspinal inputs are involved.

Functional organization and reorganization of cutaneous reflex pathways during locomotion

Reflex responses evoked by stimulating cutaneous nerves of the foot during locomotion have been well described in cats and humans (Zehr and Stein 1999; Rossignol et al. 2006). Depending on the phase of the step cycle both inhibitory and excitatory responses can be evoked in ipsilateral extensors by stimulating cutaneous nerves whereas in ipsilateral flexors two excitatory responses are usually elicited. In flexors and extensors contralateral to the stimulation longer-latency excitatory responses at ~20-25 ms (P_2) were described (Duysens and Loeb 1980). However, in chapter 6 (Frigon and Rossignol 2007b) we showed that longer-latency excitatory responses in contralateral ankle extensors are almost always preceded by a short period of inhibition, providing evidence that crossed inhibitory pathways described in anesthetised cats (Edgley and Aggelopoulos 2006; Arya et al. 1991; Aggelopoulos et al. 1996; Curtis et al. 1958) operate during normal locomotion. The function of

crossed inhibition remains unclear but it could adjust the timing of muscle bursts bilaterally to ensure proper balance and a smooth forward progression following a perturbation.

Creating different states with different lesion paradigms enabled us to draw some general conclusions regarding the modulation and organization of cutaneous reflexes during locomotion. For example, following a denervation of the left LGS all connections from this mixed nerve are lost. Sensory afferents within the LGS nerve undoubtedly had excitatory and inhibitory connections with other other reflex pathways from the periphery, the spinal CPG, and ascending projections to supraspinal centres. As a result, the removal of LGS inputs created a functional reorganization, shifting the balance of excitation and inhibition normally provided by the LGS nerve (discussed in more detail later on). The most consistent effect following denervation of the left LGS in the intact state was an increase in short-latency excitatory responses (P_1) during the stance phase of locomotion in the left St, a knee flexor [(Frigon and Rossignol 2007a); Chapter 3]. It is possible that sensory inputs from the left LGS, which become active during loading of ankle extensors in the stance phase, normally suppress the excitatory pathway from the Tib nerve to the left St. After removing inputs from the left LGS, the Tib nerve pathway to the left St is dis-inhibited and P_1 responses appear or are greatly increased throughout stance. A return of P_1 responses toward pre-denervation values in some cats suggests that other inputs replace those of the left LGS over time.

Complete transection of the spinal cord at T13 also generated several changes in the gain of Tib nerve reflexes during locomotion (Chapter 4). For example, short-latency excitatory responses appeared during the stance phase in some ipsilateral ankle extensors, replacing the more commonly observed short-latency inhibition. Parallel inhibitory and excitatory pathways probably "compete" during locomotion and under normal circumstances (i.e. with an intact spinal cord) the inhibition has a stronger synaptic weight. However, after complete removal of descending inputs there is a shift in the balance between excitation and inhibition in favour of the excitation and as a result short-latency excitatory responses start to appear during

stance. The onset of longer-latency excitatory response (P_2) in ipsilateral extensors was earlier after spinalisation suggesting that in the intact state the inhibitory pathway actively suppresses the longer-latency excitatory pathway. Several changes in the gain of reflex pathways from the Tib nerve in ipsilateral and contralateral muscles show that supraspinal inputs are normally involved in the modulation of cutaneous reflexes during locomotion.

Following the loss of supraspinal descending commands or sensory information from ankle extensors, the optimization of remaining inputs appears functionally relevant for the recovery of locomotion. For example, the gain of reflex responses in ipsilateral flexors, such as St and Srt, was increased mostly during the swing phase of locomotion after spinalisation (Chapter 4). Increased sensory feedback in knee and hip flexors could assist the initiation and reinforce the swing phase. Likewise reduced inhibition and/or increased excitation from Tib nerve reflex pathways to ipsilateral extensors during stance could reinforce the support phase of spinal locomotion. Spinal sensorimotor circuits no doubt optimize available inputs during normal locomotion as well, not just following injury. For example, over the years the inputs most critical in generating the stance-to-swing transition has been debated (Rossignol et al. 2006). Some have argued that the most important signal mediating the transition from stance to swing is unloading of the ankle extensors (Ekeberg and Pearson 2005) whereas other have suggested that reaching a certain degree of hip extension is most critical (Grillner and Rossignol 1978). However, the most likely explanation is that both inputs are important and that the spinal CPG 'weighs' the available information contextually (Frigon and Rossignol 2006a; Rossignol et al. 2008). For instance, during swimming a swing phase can be initiated without unloading of the ankle extensors. In this form of locomotion inputs from the hip are probably critical to initiate swing. In other tasks such as decline walking where there is little hip extension, inputs from ankle extensors probably become more important. Therefore, as is the case following a lesion, the spinal CPG most likely optimizes available sensory inputs to generate an appropriate locomotor pattern.

Plasticity within spinal circuits after spinalization

It should be emphasized that although the spinal cord is capable of remarkable adaptive plasticity, as demonstrated by the expression of locomotion following a complete spinal transection in adult cats (Barbeau and Rossignol 1987), it also has limited adaptive abilities. For instance, after complete cutaneous denervation of the hindpaws in the spinal state plantigrade placement of the paw is permanently lost, whereas in the intact state the same denervation will only produce temporary deficits. This suggests that in the intact state descending supraspinal commands can select other peripheral inputs to adjust the placement of the foot and that at least some cutaneous information from the foot is required to place the paw during spinal locomotion (Bouyer and Rossignol 2003b; Bouyer and Rossignol 2003a).

Previous studies have shown that denervating the skin of the hindpaws (Bouyer and Rossignol 2003b) or some ankle flexors (Carrier et al. 1997) before spinalization in adult cats produced major deficits in the expression of spinal locomotion, even though these animals had initially recovered from the denervation. In chapter 5, we performed a complete spinalisation at T13 in cats that had "recovered" from a denervation of the left LGS to determine if descending commands from supraspinal structures were involved in maintaining adaptive mechanisms following the initial peripheral nerve lesion and to assess whether lesioning the left LGS in the intact state influenced the expression of spinal locomotion. The expression of locomotion varied greatly from one cat to another after complete spinalisation ranging from a well-organized bilateral locomotion to an inability to express spinal locomotion. In the one cat that recovered full hindquarter weight support during spinal locomotion, a large ankle yield appeared and persisted on the denervated side, which was not observed in the non-denervated leg. Perineal stimulation could reduce the magnitude of ankle yield on the denervated side but had little effect on the non-denervated leg indicating that the denervation reduced excitability within the spinal locomotor network. That the deficit did not completely disappear suggests that inputs from the LGS nerve do more than provide a non-specific excitability to the spinal CPG. For instance, in a previous study, clonidine

was administered in spinal cats with complete cutaneous denervations (Bouyer and Rossignol 2003b). Despite the increased spinal excitability provided by clonidine, which translated in increased hip and knee flexion, proper paw placement did not recover. These results and those of chapter 5 indicate that sensory inputs from the skin and ankle extensors provide more than a simple non-specific increase in spinal cord excitability. Sensory inputs from ankle extensors are required for the proper "development" of spinal locomotion as are other reflex pathways.

Experience-dependent mechanisms can modify the spinal circuitry. For instance, operant conditioning of the soleus H-reflex in adult rats, which requires descending inputs from the corticospinal tract, generates a reflex asymmetry within the spinal cord (Chen and Wolpaw 1997). The reflex asymmetry persists following complete transection of the spinal cord indicating that the operant conditioning produced long-lasting plasticity with spinal circuits. It is likely that a peripheral denervation induced changes in the spinal cord either directly by influencing spinal interneurons or indirectly by reorganizing the descending drive from cortical and subcortical structures on spinal circuits. Peripheral denervations, in particular appear to have potent effects on spinal circuits, which negatively influences the expression of locomotion following a complete spinal transection. However, not all lesions or past experiences will adversely affect the expression of spinal locomotion. For instance, locomotor training, which facilitates the recovery of locomotion following a complete spinalization (Barbeau and Rossignol 1987; Lovely et al. 1990; de Leon et al. 1998; Chau et al. 1998a) shapes the activity of reflex pathways investigated during fictive locomotion (Cote and Gossard 2004; Cote et al. 2003). After spinal cord injury, some reflex changes are undoubtedly detrimental to the recovery of locomotion, which has led some authors to suggest that locomotor training "normalizes" the activity in reflex pathways to facilitate the emergence of spinal locomotion (Cote and Gossard 2004; Cote et al. 2003; Frigon and Rossignol 2006b).

Furthermore, preliminary results demonstrated that performing a spinal hemisection at T10-T11 in adult cats followed by a period of recovery and a subsequent complete transection at T13 greatly facilitated the expression of spinal

locomotion (Rossignol et al. 2007). In some cats that were trained following the initial partial lesion a well-organized bilateral spinal locomotion with partial hindquarter support was expressed within 24 hours after complete spinalization and the quality of locomotion continued to improve over time. In normal spinal cats the expression of spinal locomotion takes approximately 2-3 weeks (Barbeau and Rossignol 1987). Why is it that a peripheral denervation before spinalization will negatively impact the expression of locomotion while abolishing descending supraspinal inputs on one side of the spinal cord will facilitate locomotor recovery after spinalization? The answer to that is not clear. Any changes within the nervous system implicate plasticity at multiple sites (Wolpaw and Tennissen 2001). For instance, there is evidence that corticospinal efficacy is increased with treadmill training after incomplete spinal cord injury in humans (Thomas and Gorassini 2005) and that intrinsic spinal mechanisms are modified following partial spinal lesions in adult cats (Rossignol et al. 2007). Although changes in sensory feedback from the periphery have not been reported after partial spinal lesions it is clear that the gain of cutaneous reflexes during locomotion is increased after complete spinalization in adult cats (Chapter 4). Similarly, after a peripheral denervation in adult cats, descending inputs from supraspinal structures are enhanced (Bretzner and Drew 2005a) as are cutaneous reflex responses (Bernard et al. 2007; Frigon and Rossignol 2007a). Therefore, the spinal CPG appears to optimize all available inputs following injury. However, if a complete spinalization is performed after the denervation increased efficacy of transmission in descending pathways is removed and the spinal CPG does not appear to have an adequate contingency plan to deal with the loss of supraspinal inputs. This indicates that the spinal cord has limited adaptive capabilities.

Another very possible explanation is that the availability of all sources of sensory feedback from the periphery (i.e. the integrity of reflex pathways) is critical for the proper expression of spinal locomotion. If denervations are performed after spinalization the spinal CPG can use alternate cues to adapt to the loss of sensory feedback but during the 'development' of spinal locomotion all sources of inputs

from the periphery are essential. In chapter 5 we found that reflex changes after spinalization in denervated cats were different than those of normal spinal cats (Chapter 4), indicating the cutaneous reflex pathways are reorganized in a different manner in the two conditions. It is clear that the adaptive plasticity within the spinal locomotor network following spinalization is not the same if a denervation is performed before spinalization and moreover that spinal plasticity is strikingly different in situations of peripheral nerve and spinal lesions.

Mechanisms of plasticity

The main mechanisms responsible for changes in cutaneous reflex pathways following lesions to the spinal cord and/or peripheral nerve could include a combination of physiological and structural alterations within spinal sensorimotor circuits.

A shift in the balance between excitation and inhibition

Proper functioning of the spinal locomotor network requires an optimal balance between excitation and inhibition. Disrupting this balance by removing certain structures, such as supraspinal descending pathways or peripheral nerves, undoubtedly engenders a reorganization of the synaptic weighting of excitatory and inhibitory connections within the spinal cord, thus shifting the spinal locomotor network into a new state requiring a new balance. Several mechanisms could be responsible for a shift in the balance between excitation and inhibition. For example, following peripheral nerve lesions, there is an early and progressive reduction of dorsal horn GABAergic interneurons (Castro-Lopes et al. 1993; Ibuki et al. 1997; Moore et al. 2002), and Renshaw cells (Sanna et al. 1993) and an increased excitatory drive from the motor cortex after cutaneous denervation of the hindpaws (Bretzner and Drew 2005a). The release of inhibitory and/or increased excitatory influences could explain increases in cutaneous reflex excitability from the Tib nerve during locomotion after removing adjacent cutaneous innervation (Bernard et al. 2007).

After complete spinal lesions in adult cats there are increased levels of GABA and glycine within the spinal cord (Tillakaratne et al. 2002; Edgerton et al. 2001) and a loss of excitatory inputs from supraspinal centres (Edgerton et al. 2004; Frigon and Rossignol 2006b). Increased inhibitory and/or decreased excitatory influences are likely substrates for reduced reflex excitability in the days following spinalization (i.e. spinal shock). However, over time reflexes become exaggerated, as spasticity ensues, which may result from a reduced effectiveness in inhibitory circuits and/or a bias towards excitation within spinal circuits. For example, crossed inhibitory responses in ankle extensor motoneurons evoked by stimulating cutaneous or group II afferents were less frequent and of smaller amplitude following complete spinal transection in anesthetised cats (Edgley and Aggelopoulos 2006; Aggelopoulos et al. 1996). Weakened cross inhibitory actions after spinal transection could involve modulation by monoamines because crossed IPSPs evoked by group II afferents could be re-established by activating serotoninergic receptors (Aggelopoulos et al. 1996). The simplest scenario to explain this is that, with the spinal cord intact, a tonically active descending serotoninergic pathway permits the expression of the crossed IPSPs and after spinal transection the loss of this tonic action reduces or abolishes crossed inhibition (Edgley and Aggelopoulos 2006). Injection of a serotoninergic agonist (quipazine) increases cutaneous reflex excitability in chronic spinal cats during locomotion (Barbeau and Rossignol 1990). Descending neurotransmitter systems, such as glutamatergic, noradrenergic, dopaminergic, and serotonergic all act on receptors that diminish or increase cellular excitability (Jordan et al. 2008). Spinal lesions of varying extents impact transmission in these descending systems and drastically change the balance between excitation and inhibition within the spinal locomotor network.

It is highly probable that the recovery of locomotion following spinal cord or peripheral nerve lesions involves a dynamic functional reconfiguration of the locomotor circuitry producing a long-term shift in the balance between inhibitory and excitatory synaptic transmission within the spinal locomotor network. Levels of GABA and glycine within the spinal cord following complete spinal lesions can be

modified with step training in adult cats (Tillakaratne et al. 2002; Edgerton et al. 2001; de Leon et al. 1999b; de Leon et al. 1999a), indicating that the balance between excitation and inhibition can be shaped by experience-dependent mechanisms.

A shift in the balance between excitation and inhibition within the spinal cord after peripheral nerve or spinal cord injury could also lead to the activation of weak or latent synapses, which could modify cutaneous reflex pathways. For instance, it was suggested that activating the relatively ineffective synapses that project rostral and caudal to receptive fields within the spinal cord could explain the somatotopic reorganization of cutaneous pathways despite an absence of collateral sprouting from intact primary afferents (Wilson and Kitchener 1996). Dis-inhibition of latent synapses could be due to the removal of a tonic or phasic presynaptic inhibition or an increase in the excitability of dorsal horn neurons resulting from changes in the balance of inhibitory and excitatory post-synaptic inputs (Wilson and Snow 1987), increased density of post-synaptic receptors and changes in membrane conductance (Hendry and Jones 1986; Welker et al. 1989; Jacobs and Donoghue 1991).

Structural reorganization

Collateral sprouting of primary afferents, descending and ascending fibres can lead to persistent changes in the somatotopy or in reflex transmission within the spinal cord by reorganizing or strengthening the connectivity. Sprouting of the central terminals of axotomized primary afferent fibres has been shown following peripheral nerve injury (Woolf et al. 1992; Woolf et al. 1995; Shortland and Woolf 1993; Koerber et al. 1994). For example, central terminals of injured myelinated Aβ fibres sprouted from their normal terminations in laminas III-V to partially invade laminas II and I, which normally receive inputs from C and Aδ fibres (Woolf et al. 1992). However, it does not appear that central sprouting of intact large primary myelinated afferents occurs to a large extent within the spinal cord in the adult following different lesions [for a discussion of this topic see (McMahon 1992; Wilson and Kitchener 1996; Navarro et al. 2007; Shehab et al. 2004)]. The acquisition of new receptive fields in the dorsal horn of the spinal cord following peripheral nerve

injury could be suggestive of sprouting of intact fibres into the denervated area but more likely implicates the activation of latent synapses and/or the reinforcement of relatively ineffective ones (Wilson and Kitchener 1996; Valero-Cabre et al. 2004). From a functional perspective, sprouting of cut afferents within the spinal cord is of little use unless the axon regenerates in the periphery and re-establishes a functional connection with its receptor. Therefore, collateral sprouting of intact primary cutaneous afferents is unlikely to significantly contribute to increased cutaneous reflexes following peripheral denervations.

Following spinal cord injury, damaged axons within the spinal cord can regenerate and spared axons can sprout in the adult nervous system [reviewed in (Maier and Schwab 2006; Steward et al. 2003). Providing a more permissive environment, by inactivating growth inhibitors (Fournier and Strittmatter 2001; Carulli et al. 2005) or chronically stimulating spared axons (Brus-Ramer et al. 2007) can promote axonal growth.. There is also evidence that these new connections are functional (Bareyre et al. 2004) but whether or not they mediate the recovery of motor functions is debatable because clearly demonstrating that axonal growth (i.e. regeneration and/or sprouting) is a causal factor in behavioural recovery is difficult.

Implications

It should now be a little clearer that the spinal cord possesses a rich and complex locomotor circuitry that can operate without descending inputs from supraspinal and long propriospinal sources and can readily adapt to the loss of two primary ankle extensors. The series of projects described in chapters 2-6 provided additional information regarding the organization of cutaneous reflexes during locomotion in the cat and how these reflex pathways are reorganized following different lesion paradigms. Cutaneous reflex pathways are particularly sensitive to changes in the state of the locomotor circuitry because large modifications in the gain of cutaneous reflex pathways were observed after denervating the left LGS in the intact and spinal states and by going from the intact to the spinal state. Inputs from

the skin should not be underestimated in the functional recovery of locomotion following lesions of different structures.

From a clinical perspective, it is encouraging to think that the adult nervous system is plastic enough to optimize remaining inputs after various lesions and that this optimization can be enhanced by different types of stimulation (Rossignol 2006). On the other hand this plasticity is limited following spinal cord injury and appears to depend critically on sensory feedback from the legs. After spinal cord injury, past experiences of the patient must be taken into consideration. For example, did the patient suffer from peripheral neuropathies before the spinal injury? Were peripheral nerves damaged at the time of spinal injury? The results of Chapter 5 and of others (Bouyer and Rossignol 2003b; Carrier et al. 1997) suggest that the integrity of reflex pathways following spinal lesions is a key determinant in the recovery of locomotor functions following spinal cord injury. Augmenting cutaneous feedback has already been shown to facilitate locomotor recovery following partial spinal lesions in animal models (Muir and Steeves 1995; Smith et al. 2006) and anecdotal evidence suggests a similar phenomenon in spinal cord-injured humans (Wernig and Muller 1992). Driving and shaping the functional plasticity in cutaneous reflex pathways using training and different modalities of stimulation, such as electrical, mechanical, and pharmacological hold much promise in facilitating the recovery of walking after spinal cord injury.

Future perspectives

Although, the present work recorded cutaneous reflexes before and after several different lesion paradigms we have only begun to explore the functional reorganization of reflex pathways in different locomotor states. For example, denervating the left LGS in the intact or spinal states modified cutaneous reflexes in the limb ipsilateral and contralateral to the stimulation but what about reflexes evoked by stimulating a nerve in the non-denervated limb? Sensory afferents project to the contralateral spinal cord and a peripheral denervation on one side could modify reflexes in the non-denervated limb. In a similar vein, are reflexes evoked and

recorded in the forelimbs (Drew and Rossignol 1987b; Drew and Rossignol 1987a) altered by lesioning a nerve in the hindlimb? Long ascending propriospinal connections relaying information from the hindlimbs to the forelimbs are probably altered following a peripheral denervation in the legs, which could effect changes in reflex pathways in the forelimbs during locomotion. Moreover, there is no data on changes in reflex pathways connecting the fore- and hindlimbs following different lesions. These "interlimb" reflexes are thought to coordinate activity in all four limbs during locomotion (Zehr et al. 2001; Miller et al. 1973) and are increased following cervical spinal cord injury in humans (Calancie et al. 2005) but whether these connections are altered following partial lesions at levels interposed between cervical and lumbosacral enlargements is unknown. Strengthening these connections following a partial lesion at thoracic levels could be critical for the recovery of quadrupedal locomotion.

As stated earlier, a partial spinal hemisection before spinalization greatly facilitates the expression of spinal locomotion in adult cats (Rossignol et al. 2007). However, there is no data with regards to changes in reflex responses in both hindlimbs following the spinal hemisection or after the complete spinalization. The spinal hemisection could induce an asymmetry in the phase-dependent modulation of cutaneous reflexes, which could persist or disappear after the spinalization. Furthermore, the normal phase-dependent modulation of H-reflexes in the legs during walking is lost following spinal cord injury in humans (Fung and Barbeau 1994) but H-reflexes have never been recorded in the same animal before and after a complete or partial spinal lesion in a locomotor condition. Proprioceptive reflexes might be reorganized differently compared to cutaneous reflexes following complete and/or partial spinal lesions. One only has to look at the literature to see that H-reflexes are difficult to obtain in cats but unpublished observations from our laboratory showed that consistent H-reflexes can be evoked in the vastus lateralis, a knee extensor, by stimulating the quadriceps nerve during locomotion. H-reflexes are clearly amenable to probe the functional organization and reorganization of proprioceptive inputs during locomotion in different states. In Chapter 2 we showed that cutaneous inputs

could compensate for the loss of proprioceptive information but is the converse also true? Can proprioceptive inputs compensate for the loss of cutaneous information? H-reflexes in vastus lateralis could be recorded in the same cat during locomotion before and after denervating cutaneous nerves of the paw. Additionally, in all these studies and those described in Chapters 2-5, the synaptic mechanisms mediating changes in reflex pathways need to be elucidated, which could be done using acute preparations and fictive locomotion.

In addition, besides a handful of studies (Bretzner and Drew 2005a; Keck et al. 1998; Pijnappels et al. 1998), the effects of supraspinal inputs on cutaneous reflex pathways in the legs during locomotion are largely unknown. Performing lesions of various cortical and subcortical structures could induce considerable changes in reflex pathways in the fore- and hindlimbs during locomotion. This could have important implications for the recovery of motor functions after stroke in humans. The effects of cortical lesions on spinal sensorimotor circuits need to be systematically investigated.

Cutaneous feedback facilitates the recovery of locomotion (i.e. swimming) following spinal lesions (Muir and Steeves 1995; Smith et al. 2006) but to date few studies have attempted to determine the mechanisms underlying this process or used this method to any great degree to promote the recovery of walking after spinal cord injury [see however (Wernig and Muller 1992; Fung and Barbeau 1994)]. Phasic stimulation of the skin or cutaneous nerve could induce long-lasting changes within spinal circuits that could stabilize or enhance the output from the spinal locomotor CPG and facilitate the recovery of walking after spinal cord injury in humans. Reflex pathways can be 'classically' conditioned in the spinal cat (Durkovic 1983; Durkovic 1975) but whether reflexes can be conditioned in a locomotor condition in the spinal state is unknown. Future studies could attempt classical conditioning of cutaneous reflex pathways to induce persistent changes within spinal circuits and determine whether this approach translates in a better recovery of locomotor functions. Operant conditioning of the soleus H-reflex in adult rats after partial spinal lesions can

improve locomotion (Chen et al. 2006) but whether this operant conditioning modifies cutaneous reflex pathways in parallel has not been tested.

Continued research of reflex pathways during locomotion is necessary to better understand the importance of sensory feedback during intact locomotion and following neurotrauma. The studies proposed here are but a few that can be performed to investigate the functional organization and reorganization of reflexes during locomotion in different states, which could have important ramifications in the recovery of locomotor functions following injury to the nervous system.

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Annex 1 - Functional plasticity following spinal cord lesions

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Abstract

Spinal cord injury results in marked modification and reorganization of several reflex pathways caudal to the injury. The sudden loss or disruption of descending input engenders substantial changes at the level of primary afferents, interneurons, and motoneurons thus dramatically influencing sensorimotor interactions in the spinal cord. As a general rule reflexes are initially depressed following spinal cord injury due to severe reductions in motoneuron excitability but recover and in some instances become exaggerated. It is thought that modified inhibitory connections and/or altered transmission in some of these reflex pathways after spinal injury as well as the recovery and enhancement of membrane properties in motoneurons underlie several symptoms such as spasticity and may explain some characteristics of spinal locomotion observed in spinally transected animals. Indeed, after partial or complete spinal lesions at the last thoracic vertebra cats recover locomotion when the hindlimbs are placed on a treadmill. Although some deficits in spinal locomotion are related to lesion of specific descending motor pathways, other characteristics can also be explained by changes in the excitability of reflex pathways mentioned above.

Consequently it may be the case that to re-establish a stable walking pattern that modified afferent inflow to the spinal cord incurred after injury must be normalized to enable a more normal re-expression of locomotor rhythm generating networks. Indeed, recent evidence demonstrates that step training, which has extensively been shown to facilitate and ameliorate locomotor recovery in spinal animals, directly influences transmission in simple reflex pathways after complete spinal lesions.

Keywords: Spinal reflexes, neuroplasticity, spinal locomotion, reflex pathways, spinal cord injury, spasticity.

1. General Introduction

After spinal lesions, there is gradual recovery below the lesion of some motor functions, including locomotion (Rossignol et al. 2002; Rossignol et al. 2000; Bélanger et al. 1996; Barbeau and Rossignol 1987). Such recovery undoubtedly results from the re-expression of genetically-determined intrinsic spinal circuitry called a Central Pattern Generator (CPG) that can manifest itself even when spinalisation is performed before having learned to walk (Forssberg et al. 1980b; Forssberg et al. 1980a) and can operate even in the absence of afferent feedback in curarised cats (Grillner and Zangger 1979; Grillner 1981; Rossignol et al. 1990) (see Figure 1). The underlying mechanisms for this recovery probably implicate interrelated anatomical, neurochemical and physiological changes in the spinal cord below the lesion. Indeed, post-spinal excitability changes in reflex pathways and the evolution in the expression of locomotion may in part be interrelated (Cote and Gossard 2004; Cote et al. 2003; Muir 1999). In order to better understand how these neuroplastic changes in the cord relate to functional recovery after injury, this review will concentrate firstly on observable modifications at the level of simple circuits such as spinal reflexes and secondly at the level of more complex locomotor pattern-generating networks. In a final section, changes in reflex excitability will be discussed and related to characteristics of spinal locomotion.

2. Changes in reflex pathways after spinal cord injury

It has been well established that following lesions of the spinal cord neural circuitry caudal to the injury undergoes substantial modifications in segmental connections due to the dramatic loss of supraspinal and propriospinal inputs (Sherrington 1899; Mendell 1984). As such, exteroceptive and proprioceptive reflex pathways display rapid, perdurable, and evolving plasticity after spinal injury.

Initially, spinal reflexes are substantially depressed due to the severe decrease in motoneuron excitability as synaptic input from supralesional centers is diminished or lost in complete or partially transected spinal cords (Barnes et al. 1962; Conway et al. 1988; Bennett et al. 2004; Bennett et al. 2001b; Walmsley and Tracey 1983; Kuhn

1950; Bennett et al. 1999; Hultborn 2003; Murray and Goldberger 1974). This period, defined as spinal shock, is characterized by muscular paralysis, hypotonus, and the abolition of spinal reflexes (Brown 1994; Ditunno et al. 2004; Ashby and Verrier 1975). It appears that afferent transmission in reflex pathways increases immediately after spinal lesions (Nelson et al. 1979; Baker and Chandler 1987b; Cook and Woolf 1985; Crenna et al. 1982; Li et al. 2004b; Lundberg 1982) since raising motoneuronal excitability to pre-injury levels by applying an excitatory conditioning stimulus to the motor pool produces enhanced reflexes (Bennett et al. 2004). However, without artificially augmenting motoneuron excitability, the loss of persistent inward currents (PICs) and plateau potentials obscures any increase in reflex amplitude. Gradually, as motoneuron excitability recovers and the ability to generate PICs and plateau potentials is re-established (Bennett et al. 2001a) another phase ensues highlighted by exaggerated reflex responsiveness, clonus, and hypertonus (Dimitrijevic and Nathan 1967b; Young 1994; Brown 1994) collectively defined as spasticity.

In man, the clinical hyperreflexia resulting from partial or complete spinal lesions has been extensively described (Dimitrijevic and Nathan 1968; Dimitrijevic and Nathan 1973; Dimitrijevic and Nathan 1971; Dimitrijevic and Nathan 1970; Dimitrijevic and Nathan 1967a; Dimitrijevic and Nathan 1967b) and the celerity of changes in input-output properties of spinal reflexes is attributed to the massive loss of control from descending and propriospinal sources (Liddell 1934; Dimitrijevic and Nathan 1967b; Bennett et al. 2004; Engberg et al. 1968). As such, several investigators have developed animal models to study changes in reflex pathways after lesions to the spinal cord. For example, animals with partial spinal lesions (Hultborn and Malmsten 1983a; Hultborn and Malmsten 1983b; Carter et al. 1991; Murray and Goldberger 1974; Aoki et al. 1976; Decima and Morales 1983), or complete transections (Bennett et al. 1999; Bennett et al. 2004; Bailey et al. 1980; Smith et al. 1983) display enhanced monosynaptic and/or polysynaptic reflex responses at segmental levels caudal to the lesion.

Plastic changes in a reflex arc can occur at primary afferents, interneurons, and motoneurons and it appears that disrupting or interrupting descending pathways results in marked reorganization at all three levels. Therefore, following injury to the spinal cord reflex circuits undergo anatomical and physiological changes and activating or modulating these pathways potentially could enhance functional motor recovery (Barbeau et al. 1999; Pearson 2001; Muir and Steeves 1997). It is imperative to understand the reorganization of spinal pathways after spinal cord injury (SCI) to design and improve training protocols and pharmacological treatments to ameliorate motor functions. The following sections describe changes in simple reflex pathways resulting from altered or abolished descending input after SCI in humans and animal models.

2.1. Stretch reflexes

The stretch reflex, mediated by group Ia and II afferents has both dynamic and static components and has received particular attention following SCI. Figure 1 represents pathways from group Ia and II fibers originating from different receptors in muscle spindles responsible for detecting changes in muscle length (II) and rate of change (Ia). Changes in stretch reflex pathways have been ascribed as a causal link for spasticity since SCI patients display increased resistance to muscle lengthening and especially to higher stretch velocity.

Consequently, the stretch reflex has been used to demonstrate neurophysiological changes after SCI in humans. Immediately following SCI, during spinal shock, stretch reflexes are absent or substantially depressed (Leis et al. 1996a) but recover progressively with time and as spasticity ensues they become exaggerated (Hiersemenzel et al. 2000). Indeed, it is generally reported that stretch reflexes are enhanced in chronic SCI patients (Dimitrijevic and Nathan 1967a; Taylor et al. 1984; Hiersemenzel et al. 2000; Calancie et al. 2004; Nakazawa et al. 2006b). For example, stretch reflexes of intact individuals were compared to those of spastic patients who had suffered a SCI (Dimitrijevic and Nathan 1967a). It was shown that although latency was unchanged the amplitude and duration of stretch reflexes were increased

after SCI. In addition, following a brief muscle stretch there is a silent period before the muscle becomes tonically active with an after-discharge that may spread to several muscles of both limbs. This widespread excitation is a hallmark of spasticity Moreover, changes in stretch reflex pathways are dependent on lesion extent. For example, stretch reflexes, evoked by patellar and Achilles tendon taps, were compared in motor complete and incomplete SCI individuals within the first five days post-injury (Calancie et al. 2004). It was found that motor incomplete, compared with motor complete patients had significantly larger stretch reflexes. As time after injury progressed stretch reflexes gradually increased in both groups and although the relative increase was larger in motor complete individuals, motor incomplete patients still displayed much larger stretch reflexes. That stretch reflexes are larger in incomplete rather than complete SCI has recently been confirmed for chronic patients (Nakazawa et al. 2006a). Spasticity generally results from asymmetric descending influences since animal models using partial lesions, in contrast to complete transections, produce exaggerated reflex responses ipsilateral to the injury (Hultborn 2003). In humans, in whom clinical signs of spasticity are frequent after SCI, complete loss of descending input is rare and extant projections from propriospinal and supraspinal sources remain, which may explain why spasticity is a frequent consequence.

Since stretch reflex pathways are reorganised after SCI in humans several animal models have replicated these changes to elucidate the mechanisms subserving this plasticity. Using partial lesions of the cat spinal cord, Murray and Goldberger evaluated stretch reflexes in both hindlimbs before and after injury (Murray and Goldberger 1974). Contrary to other reflexes, stretch reflexes showed little depression on the lesioned side immediately after injury. As time elapsed, however, induction threshold decreased and stretch reflex amplitude increased in the injured side. Reflex responses on the intact side were similar to pre-injury levels and thus acted as an appropriate reference. Furthermore, it was reported that increased reflex activity paralleled ameliorations in locomotor performance. In other studies, a model of stretch hyperreflexia was developed in cats by unilaterally sectioning the

dorsolateral column in the spinal cord at T13-L3 levels (Taylor et al. 1997; Taylor et al. 1999). Ramp and hold flexion of both ankle joints generated stretch reflexes in triceps surae muscles before and after injury in awake cats. To determine whether the lesion influenced stretch reflex threshold and gain, the force applied by the animal before the stretch was monitored, as an indicator of motoneuronal pool excitability, and matched prior to and after injury. Measures of force such as threshold angle (joint excursion producing a 50g force) and incremental dynamic stiffness (slope of relationship between dynamic force and joint angle) were used to infer changes in the length-tension relationship and stretch reflex gain. It was shown that incremental dynamic stiffness was increased and threshold angle reduced after spinal lesions at matched pre-stretch background force levels on the side ipsilateral to the lesion only, indicating that at a given joint excursion the stretch-evoked response generated more force. The EMG response to stretch in soleus was also greater after injury on the injured side. Intrinsic muscle stiffness was ruled out since ketamine administration, which severely depresses reflex activity, completely abolished the increased stiffness associated with the lesion. Thus, the authors concluded that increased stiffness was due to enhanced reflex activity.

Recently, a model of spasticity was developed using a sacral SCI in the adult rat, which does not interfere with bladder, bowel, or hindlimb locomotor function (Bennett et al. 1999). The spinalisation was performed at S2 and therefore only influenced cutaneous and muscular regions of the tail. The rat was then inserted inside a hollow plexiglass tube with the tail freely hanging out the back. This model enabled changes in various reflex pathways of the tail to be investigated, in awake rats, before and after complete sacral transections. A rotating manipulator was developed to generate rapid downward or upward rotations, which induced stretch reflexes to extensor or flexor muscles, respectively. In intact rats stretch applied to either flexors or extensors generated little or no stretch reflex response, indicating that under normal circumstances supra-lesional centers powerfully inhibit these pathways. However, after SCI following spinal shock in which the tail remained flaccid and non-responsive the tail subsequently became hyperreflexive to muscle stretch

approximately fourteen days after sacral transection. In these rats with a spastic tail, stretches identical to those performed pre-injury produced large and sustained muscle responses resulting from phasic and tonic stretch reflexes. Therefore, similar to humans, in animal models of spasticity stretch reflexes appear to have lower induction thresholds and greatly exaggerated responses after SCI.

2.2. H-reflexes

The H-reflex, which bypasses muscle spindles and the fusimotor drive provided by gamma motoneurones by directly activating Ia afferents (see stimulation symbol on the 1a afferent on the extensor side of Figure 1), has proven very useful in evaluating, both in clinical and research settings, the central component of the stretch reflex pathway during different conditions and pathologies (Schieppati 1987; Zehr 2002; Misiaszek 2003).

In humans, H-reflexes, primarily evoked by stimulating the tibial nerve in the popliteal fossa, and recording electromyographic activity in soleus have been studied in acute and/or chronic SCI to evaluate changes in transmission to motoneurons from group Ia afferents (Leis et al. 1996a; Leis et al. 1996b; Cadilhac et al. 1977; Taylor et al. 1984; Ashby and Verrier 1975; Ashby et al. 1974; Hiersemenzel et al. 2000). In acute SCI, during spinal shock, despite the loss of stretch reflexes, H-reflexes are generally present albeit reduced (Weaver et al. 1963; Leis et al. 1996a; Diamantopoulos and Zander 1967; Ashby et al. 1974; Little and Halar 1985; Cadilhac et al. 1977). Contrary to stretch reflexes, H-reflexes recover rapidly after SCI and as spasticity ensues H-reflex amplitude increases further (Hiersemenzel et al. 2000). Recently, changes in H-reflex excitability were evaluated in complete spinal adult rats to study reorganization of reflex pathways after SCI (Valero-Cabre et al. 2004). Intramuscular recordings of multiple hindlimb muscles were made before and after a T9 spinal section in rats. H-reflexes were evoked in tibialis anterior, gastrocnemius, and plantar muscles by stimulating the sciatic nerve. Although M-wave amplitude transiently decreased after SCI and recovered towards pre-operative values, H-reflex amplitude, expressed as the H:M ratio, was significantly enhanced in all three

muscles studied immediately after spinal transection and remained elevated for the remainder of the study (60 days). In addition, H-reflex recruitment curves were facilitated, induction threshold was reduced, and onset of responses was delayed for all three muscles post-spinalisation.

Although these studies point to increased excitability of H-reflex pathways after SCI, some studies in humans (Taylor et al. 1984; Schindler-Ivens and Shields 2000; Schindler-Ivens and Shields 2004; Ashby et al. 1974) and animals (Thompson et al. 1992; Lee et al. 2005; Chen et al. 2001) show that H-reflex amplitude (H:M ratio) and threshold (Boorman et al. 1996) are not altered after SCI. For example, Schindler-Ivens and Shields (2004) demonstrated that soleus H-reflex threshold, gain, and amplitude were unchanged in clinically complete chronic SCI patients exhibiting signs of spasticity compared to intact individuals. A possible explanation for these discrepant findings is that the degree of injury influences H-reflexes. This does not seem to be the case since no difference in H-reflex amplitude was found in incomplete and complete SCI patients(Nakazawa et al. 2006c). Moreover, severity of injury (mild, moderate, and complete) on plantar muscle H-reflexes, was assessed in adult rats at varying times after SCI injury under chloral hydrate anesthesia, which similar to ketamine as negligible effects on H-reflexes (Lee et al. 2005). The amplitude of baseline H-reflexes tested at 0.1 Hz did not change consistently after SCI. However, similar to other studies (Thompson et al. 1992; Skinner et al. 1996) rate-sensitive depression at high stimulation frequencies was altered after SCI.

Indeed, the frequency of stimulation appears to be a critical factor when assessing modifications in H-reflexes after SCI. It is well established that increasing the frequency of stimulation evokes rate-sensitive depression of H-reflexes, attributed to neurotransmitter depletion at Ia afferent terminals (homosynaptic depression) (Hultborn et al. 1996; Kohn et al. 1997) or to increased presynaptic inhibition (Schindler-Ivens and Shields 2000; Thompson et al. 1992; Calancie et al. 1993). However, this 'normal' rate-sensitive depression is altered after SCI in rats (Thompson et al. 1992; Skinner et al. 1996) and humans (Ishikawa et al. 1966; Calancie et al. 1993; Schindler-Ivens and Shields 2000), which may lead to specious

conclusions concerning changes in H-reflex amplitude or gain. For example, Thompson et al. (1992) using a spinal contusion injury in rats measured the rate-sensitive depression of H-reflexes at different stimulation frequencies. In intact rats and within six days of SCI, H-reflexes were attenuated at frequencies greater than 0.3 Hz and as stimulus frequency increased so did H-reflex depression. However, twenty-eight days post-injury the rate-sensitive depression of H-reflexes was severely diminished and in some animals reflexes were potentiated with increasing stimulus frequency. This impairment in rate-sensitive depression after SCI was maintained for the duration of the study. In another study, rate-sensitive depression was assessed after SCI in humans (Schindler-Ivens and Shields 2000). Soleus H-reflexes were evoked at different stimulation frequencies in intact, acute and chronic SCI individuals. Although H-reflex amplitude was diminished in all three groups with increasing stimulation frequency, the H-reflex depression was considerably less in chronic SCI patients. Moreover, rate-sensitive depression was gradually reduced in an acute SCI individual tested for several weeks reaching levels similar to chronic SCI. These results in rats and humans indicate that impaired rate-sensitive depression does not occur immediately post-injury, evolving and persisting over time. Consequently, changes in H-reflex amplitude after SCI should be interpreted with caution since putative increases in H-reflex amplitude or gain might simply result from altered rate-sensitive depression if reflexes are evoked at stimulation frequencies sensitive to this effect. To avoid effects from rate-sensitive depression, which is altered after SCI, H-reflexes should be elicited at stimulation frequencies no greater than 0.1 Hz.

H-reflex excitability has also been evaluated during walking in intact and SCI patients. In intact humans soleus H-reflex excitability exhibits phase-dependency during the step cycle progressively increasing from early to late stance and becoming quiescent during the swing phase (Capaday and Stein 1986; Crenna and Frigo 1987; Simonsen and Dyhre-Poulsen 1999; Rossignol et al. 2006). This pattern of modulation persists in incomplete SCI patients showing weak signs of spasticity but changes dramatically in more severe cases of spasticity (Fung and Barbeau 1994). In

the latter, modulation during stance and inhibition during swing are abolished. Furthermore, conversely to intact and SCI individuals with mild spasticity, which show marked attenuation of soleus H-reflexes during walking compared to standing, severe spastic SCI patients have ambulatory soleus H-reflexes of greater or similar value relative to standing. Therefore, phase- and task-dependent modulation of H-reflexes is impaired after SCI and alleviating or reducing spasticity appears to restore this modulation.

2.3. Intracellular recordings and group Ia afferents

Intracellular motoneuron recordings following peripheral nerve stimulation have been investigated in a few studies after SCI in animal models (Munson et al. 1986; Nelson and Mendell 1979; Mayer et al. 1984; Hochman and McCrea 1994a). Acute experiments performed under anesthesia, which abolish some motoneuronal properties such as plateau potentials (Guertin and Hounsgaard 1999; Hultborn 2003), might mask alterations in reflexes but enable determination of changes at pre-motoneuronal levels. For example, Hochman and McCrea (1994a) compared Ia EPSPs of lumbar ankle extensor motoneurons, evoked by peripheral nerve stimulation, in non-lesioned and chronic cats spinalised at L1-L2 six weeks prior to the acute experiment. It was found that Ia EPSPs in lateral gastrocnemius (LG) and soleus motoneurons after stimulating the homonymous nerve (LGS) were significantly greater in spinal cats compared to non-lesioned animals and had reduced rise times. However, medial gastrocnemius (MG) Ia EPSP amplitude and rise time were largely unchanged in response to MG nerve stimulation, corroborating earlier reports (Munson et al. 1986; Nelson and Mendell 1979; Mayer et al. 1984), which indicates that not all motoneuronal pools are influenced similarly by spinal cord transection. Furthermore, heteronymous rather than homonymous monosynaptic connections from Ia afferents became stronger following spinalisation. For example, stimulation of LGS and MG nerves with recordings in MG and LG motoneurons, respectively, were twice as large in chronic spinal cats compared to non-lesioned cats. This indicates a reorganization of synaptic connections between synergists after

spinalisation, and not an alteration in passive membrane properties since a general change in motoneuron excitability would influence all responses similarly (Hochman and McCrea 1994b). The authors concluded that larger homonymous and heteronymous Ia EPSPs could in part mediate increased ankle extensor stretch reflexes in chronic spinal cats.

2.4. Mechanisms subserving changes in reflexes from group Ia afferents

Changes in reflexes from group Ia afferents can result from altered gamma-motoneuron activity, presynaptic inhibition (PSI) or homosynaptic depression (HD) at Ia terminals, and/or changes in motoneuron excitability (see figure 1). Modified alpha-motoneuron excitability is discussed later on since it impacts all reflex pathways.

There is evidence to suggest that diminished stretch reflexes during spinal shock result from depressed gamma motoneuron activity. For example, several studies report that H-reflexes, which are not influenced directly by fusimotor drive, are present during spinal shock (Leis et al. 1996a; Diamantopoulos and Zander 1967; Ashby et al. 1974; Little and Halar 1985; Cadilhac et al. 1977; Weaver et al. 1963), whereas stretch reflexes are absent or severely depressed (Leis et al. 1996a), indicating that gamma motoneuron activity is reduced immediately after SCI. Although fusimotor drive recovers, enhanced activity does not appear to contribute to increased stretch reflexes after SCI since primary muscle spindle responses are depressed or unchanged in chronic spinal cats (Bailey et al. 1980). In this study, intact cats were compared with acute and chronic spinal cats at various epochs post-spinalisation. Muscle spindle activity was recorded in medial gastrocnemius Ia fibers in dorsal roots in response to static stretch. It was shown that acute spinal cats (3 days post-spinalisation) were hypotonic and slightly hyperreflexic and that spindle responses were significantly depressed. In chronic spinal cats the limb was hypertonic and hyperreflexic, reaching a peak 30 days post-spinalisation, and although muscle spindle activity recovered it was still lower than control levels. These results indicate that depressed motoneuron excitability observed in spinal

shock could in part be mediated by decreased facilitatory inputs from muscle spindles but that exaggerated stretch reflexes in spasticity are not attributed to increased fusimotor activity.

Altered PSI and/or HD at Ia terminals are attractive candidates since these mechanisms are under control from descending pathways (Rudomin and Schmidt 1999). In acute stages of injury PSI or HD appear to be greatly increased, which may contribute to depressed motoneuronal excitability (Ashby et al. 1974; Ashby and Verrier 1975; Calancie et al. 1993). For example, soleus H-reflexes were evoked with or without homonymous tendon vibration to assess changes in PSI and/or HD in acute and chronic SCI (Ashby et al. 1974; Ashby and Verrier 1975). It was found that conversely to intact individuals, vibration completely abolished H-reflexes in acute SCI, indicating that PSI and/or HD are heightened shortly after SCI. However, contrary to acute stages, PSI and/or HD become considerably diminished in chronic SCI patients (Taylor et al. 1984; Faist et al. 1994; Levin and Chapman 1987; Calancie et al. 1993; Nielsen et al. 1993). Homosynaptic depression, assessed at different stimulation frequencies or by applying a preceding muscle stretch, is thought to result from reduced transmitter release due to prior activation of Ia afferents and is modified with changes in descending input in humans (Calancie et al. 1993; Ishikawa et al. 1966; Nielsen et al. 1995; Schindler-Ivens and Shields 2000; Nielsen et al. 1993) and rats (Thompson et al. 1992; Skinner et al. 1996). Indeed activation history influences soleus Ia afferent pathway excitability since stretching the soleus has considerably less effect in reducing H-reflexes in chronic SCI patients compared to intact participants (Nielsen et al. 1993). In other studies H-reflexes evoked at different stimulation frequencies were used to assess HD. For example, H-reflexes in intrinsic foot muscles, elicited by stimulating the tibial nerve, were evaluated before and after a contusion injury at the 8th thoracic level at different frequencies of stimulation at various times after SCI (Thompson et al. 1992). At six days post-injury no rate-dependent depression, as a function of intact controls, was found. However, by twenty-eight days H-reflexes evoked at stimulation rates above 1 Hz were considerably greater than controls and this reduction in HD persisted for the duration

of the study. Similar findings have recently been demonstrated in humans (Schindler-Ivens and Shields 2004). These results indicate that diminished HD evolves gradually after SCI and persists with time.

To solely assess changes in PSI at Ia afferent terminals soleus H-reflexes were conditioned by stimulating the femoral nerve, which facilitates soleus H-reflexes by reducing PSI at Ia afferent terminals (Hultborn et al. 1987) in intact and chronic SCI participants (Faist et al. 1994). It was found that facilitation of soleus H-reflexes was considerably greater in SCI patients compared to intact participants suggesting that presynaptic inhibitory control is impaired with spinal lesions. In another study soleus H-reflexes were conditioned by stimulating the common peroneal nerve at C-T intervals known to evoke PSI of the soleus Ia afferent pathway in intact and complete chronic SCI individuals (Levin and Chapman 1987). In intact individuals CP conditioning elicited profound attenuation of soleus H-reflexes, whereas, in SCI patients this depression was significantly reduced, indicating that PSI of the soleus H-reflex pathway is altered after SCI. Therefore, these two studies provide evidence that PSI at Ia afferent terminals is impaired and diminished following SCI.

2.5. Recurrent inhibition

Renshaw cells, which mediate classic recurrent inhibition of alpha-motoneurons (Renshaw 1941), also strongly project to gamma-motoneurons, Ia inhibitory interneurons (see figure 1) and other Renshaw cells. Since segmental and descending pathways project to Renshaw cells (McCrea et al. 1980; Pratt and Jordan 1980; Katz and Pierrot-Deseilligny 1998) their activity may be modified following SCI promoting widespread changes in interneuronal and motoneuronal activity.
Indeed, using a conditioned H-reflex technique (Pierrot-Deseilligny and Bussel 1975) it was demonstrated that recurrent inhibition is consistently increased in SCI patients (Shefner et al. 1992). In this study, stimulating the tibial nerve in the popliteal fossa, to evoke soleus H-reflexes was followed by a supramaximal pulse 10 ms after, allowing investigation of recurrent inhibitory pathways (see Shefner et al. for full methods). No significant differences in H:M ratios between intact and SCI

individuals were found. However, marked differences were found for the conditioned H-reflex (H'), which negatively correlates with the level of recurrent inhibition. In all intact individuals the H' response was clearly present, whereas in most SCI patients this response was absent or very small. This indicated that recurrent inhibition, which is normally under inhibitory control from brainstem centers (Fung et al. 1987), is augmented following interruption or disruption of descending inputs to Renshaw cells. Interestingly, with multiple testing sessions the appearance of H' responses, or reduced recurrent inhibition, in SCI patients was associated with clinical improvements, particularly a decrease in stiffness.

Increased recurrent inhibition has also been demonstrated on the lesioned side of hemisected cats (Hultborn and Malmsten 1983b). In this study, stimulating cut dorsal roots evoked test reflexes and discharges were recorded in intact ventral roots. Recurrent inhibition was evoked by conditioning test reflexes with antidromic volleys in peripheral motor nerves, which reduce the excitability of alpha-motoneurons. It was demonstrated that recurrent inhibition was predominantly larger on the hemisected side compared to the non-lesioned side.

These studies in humans and cats indicate that Renshaw cell activity, and thus recurrent inhibition, is heightened after SCI. Although increased recurrent inhibition would depress motoneuronal excitability and therefore prevent hyperreflexia their powerful connections to other interneurons, especially Ia inhibitory interneurons should be considered.

2.6. Reciprocal inhibition

Reciprocal inhibition, mediated by disynaptic connections from homonymous Ia afferents to antagonist motoneurons (see Figure 1), via the Ia inhibitory interneuron, normally prevents simultaneous contraction of agonist and antagonist during movement (Baldissera et al. 1981).

Ia reciprocal inhibition is generally reduced (Dimitrijevic and Nathan 1967b; Yanagisawa and Tanaka 1978; Boorman et al. 1996; Crone et al. 2003; Xia and Rymer 2005; Okuma et al. 2002) in complete and incomplete SCI humans, resulting

in abnormal motor behavior characterized by increased coactivation of agonists and antagonists. Indeed, it was reported that spontaneous lower limb movements in SCI humans were delineated by simultaneous activation of agonists and antagonists (Dimitrijevic and Nathan 1967b). Furthermore, this coactivation could persist for several minutes indicating that normal reciprocal inhibition between antagonistic muscle groups is severely impaired after SCI. Since these early studies, others have shown that instead of normal reciprocal inhibition between agonist and antagonist a reciprocal excitation or facilitation in spinal spasticity potentially mediated by an oligosynaptic excitatory pathway emerges (Crone et al. 2003; Xia and Rymer 2005; Okuma et al. 2002).

For example, soleus H-reflexes were conditioned by stimulating the common peroneal nerve which, in normal individuals, evokes reciprocal inhibition of the group Ia afferent pathway of the soleus (Crone et al. 2003). At a condition-test interval of 2-4 ms common peroneal nerve stimulation elicited short-latency inhibition of soleus H-reflexes in intact individuals, which is mediated by a disynaptic reciprocal Ia inhibitory pathway (Crone et al. 1987; Crone and Nielsen 1989; Crone et al. 1994). However, in SCI patients, this inhibition is replaced by marked facilitation of soleus H-reflexes. The authors concluded that this facilitation was mediated by an oligosynaptic group I afferent pathway, which is normally under powerful inhibitory control from supraspinal structures. Moreover, the time-course of this facilitation was associated with the emergence of hyperactive stretch reflexes, which indicates that changes in reciprocal pathways may be one of the underlying causes of spasticity. Recently, reciprocal pathways were evaluated by evoking stretch reflexes in ankle flexors and extensors of incomplete and complete SCI patients by applying a tap to the tibialis anterior and Achilles tendon, respectively (Xia and Rymer 2005). In both groups there was evidence of reciprocal facilitation. In other words, responses in antagonists were greater than the muscle being stretched. Moreover, the incidence of reciprocal facilitation was considerably greater in incomplete spinal individuals. These two studies provide strong evidence that reciprocal pathways are deeply reorganised after SCI and that varying degrees of change in Ia reciprocal

pathways depend on the extent of the lesion since the prevalence of reciprocal facilitation is greater in incomplete SCI (Xia and Rymer 2005). This indicates that remaining descending connections have more disruptive effects on the control of reciprocal inhibition than complete lesions.

In another study using cases of asymmetric spinal spasticity, where one side exhibits more clinical signs, the side displaying better recovery and less spasticity was associated with more pronounced Ia reciprocal inhibition (Okuma et al. 2002). For this, soleus H-reflexes were preceded (0.5-10 ms) by a conditioning stimulation consisting of single pulses to the common peroneal nerve to induce reciprocal inhibition of the soleus group Ia afferent pathway. This was done in both legs to determine if there was asymmetric reciprocal inhibition. Compared to intact individuals, spinal cord injured patients had much greater H:M ratios but no differences were seen between the two limbs in the injured group. However, significant differences in the level of reciprocal inhibition were found between the legs of spinal patients, whereas intact individuals showed no side-to-side differences. In all patients studied, reciprocal inhibition was greater on the less affected side and in some instances a reciprocal facilitation was observed on the spastic side. The authors thus suggested that the level of reciprocal inhibition could be a good indicator of the clinical severity of spasticity and that restoring reciprocal inhibition benefits functional recovery.

Besides the emergence of an oligosynaptic pathway, another potential mechanism for reciprocal facilitation is increased Renshaw cell activity (Shefner et al. 1992), which exerts powerful inhibitory actions on Ia inhibitory interneurons (Fedina and Hultborn 1972; Hultborn et al. 1971). Indeed increased recurrent inhibition from Renshaw cells on Ia inhibitory interneurones after SCI would lead to decreased reciprocal inhibition and potentially to an increased likelihood of co-contraction (see the third section and Figure 2).

Therefore, absence, impairment, or asymmetries of descending inputs to Ia inhibitory interneurons result in reorganization of reciprocal reflex pathways between antagonistic motor pools.

2.7. Nonreciprocal group I pathway

Ib afferents from Golgi tendon organs mediate disynaptic inhibition of homonymous muscles via Ib interneurons (see Figure 1) and transmit information related to contraction-generated tension (Jami 1992). However, other segmental afferents, such as group Ia and cutaneous afferents, project to Ib interneurons (see Figure 1) and as such nonreciprocal group I pathway is considered a more appropriate term (Jankowska 1992). The group I pathway exhibits a reflex reversal (e.g. switches to a disynaptic excitatory connection) under certain circumstances, such as during locomotion (Gossard et al. 1994; McCrea et al. 1995; Gossard and Hultborn 1991; Prochazka 1996; Pearson and Collins 1993). This is represented in Figure 1 by the inhibitory interneurons inhibiting the black inhibitory interneurons. Thus, the nonreciprocal group I pathway exerts powerful actions on locomotor networks. Cortico- and reticulo-spinal tracts have strong effects on Ib interneurons (Schomburg 1990; Jankowska 1992) and it would thus be anticipated that disruption of these pathways engenders a reorganization in nonreciprocal group I pathways.

However, in humans interrupting or disrupting these pathways does not seem to influence transmission from group Ib afferents (Downes et al. 1995). To demonstrate this, soleus H-reflexes were conditioned by stimulating the medial gastrocnemius nerve at a condition-test interval of 6ms in intact and SCI individuals, which has previously been shown to evoke group Ib afferent disynaptic inhibition in humans (Pierrot-Deseilligny et al. 1981; Pierrot-Deseilligny and Morin 1979; Delwaide et al. 1991). As there were no changes in Ib-mediated inhibition between the two groups it was concluded that reflex effects from Ib afferents are unchanged after SCI in humans (Downes et al. 1995). However, since transmission from other afferent inputs, which converge on Ib interneurons, is altered following SCI it is probable that considerable modifications occur in group I nonreciprocal pathways. Alternatively, that the nonreciprocal group I pathway is largely immutable following SCI may highlight its significance for functional motor recovery.

2.8. Group II pathway

Group II afferents mediate non-monosynaptic stretch reflexes, flexor reflexes and some postural reflexes and an increase in the gain of group II reflex pathways is thought to contribute to spasticity following SCI (Eriksson et al. 1996; Jankowska and Hammar 2002; Jankowska 1992). Jankowska and Hammar (2002) posit that group II reflexes exert such powerful actions in spasticity that interneuronal contribution from group Ia reciprocal and Ia/Ib nonreciprocal pathways are comparatively negligible. The evidence for reorganisation of group II pathways following SCI is sparse due to the greater difficulty in selectively activating these pathways. However, pharmacological agents that alleviate spastic symptoms are thought to selectively target group II afferent pathways (Bras et al. 1990; Jankowska and Hammar 2002; Jankowska et al. 2000). For example, systemic or intrathecal administration of the noradrenergic (NA) agonists clonidine and tizanidine, or the NA precursor L-DOPA, abolishes activation of interneurons in group II reflex pathways and reduces spastic signs. Thus, in cats the loss of NA-releasing descending tract neurons, which exert powerful inhibitory control of interneurons intercalated in group II pathways may promote the characteristic exaggeration in reflex responsiveness (Jankowska and Hammar 2002). Therefore, hyperreflexia in spinal spasticity may partly be due to modifications in descending inhibitory control of group II afferent pathways.

Although changes in group II pathways has not been investigated with SCI, there is evidence that altered descending input to spinal centres alters transmission in these pathways in humans (Marque et al. 2001; Maupas et al. 2004). In one study, transmission in group II pathways was assessed by conditioning the quadriceps H-reflex with a stimulus to the common peroneal nerve in hemiplegic post-stroke patients with clinical signs of spasticity (Marque et al. 2001). In intact individuals this conditioning stimulus has been shown to evoke an early (10-12 ms C-T interval) and a late (15-20 ms C-T interval) facilitation of the quadriceps H-reflexes via group I and group II afferent activation, respectively (Marque et al. 1996; Simonetta-Moreau et al. 1999). It was shown that the spastic limb had considerably greater

group I and II mediated facilitation compared to the non-spastic side and matched controls. There were no significant differences between the non-spastic side and control participants. The authors attributed the greater facilitation to enhanced transmission in group I and II pathways resulting from changes at pre-motoneuronal levels due to altered descending control. In a subsequent study, the same group used tizanidine, an alpha-2 noradrenergic agonist known to selectively depress transmission in group II pathways, to evaluate its effects on the facilitation of quadriceps H-reflexes by common peroneal nerve conditioning in hemiplegic spastic post-stroke patients and intact individuals (Maupas et al. 2004). Oral administration of tizanidine considerably reduced the group II-, and to a lesser extent, group I-mediated facilitation of quadriceps H-reflexes so that no significant differences were apparent between spastic and unaffected sides.

Therefore, these results show that altered descending input modifies group II afferent transmission and that administration of NA agonists in part reduces spasticity by selectively targeting these pathways.

2.9. Cutaneous pathways

Cutaneous reflexes, via polysynaptic pathways, predominantly modify ongoing movement and participate in positioning the foot during locomotion (Zehr and Stein 1999; Rossignol et al. 2006; Bouyer and Rossignol 2003b; Bouyer and Rossignol 2003a). For example, stimulation of the dorsal surface of the hindpaw during stepping generates a coordinated reflex response of the hindlimb musculature allowing the perturbed limb safe passage over an impeding obstacle (Forssberg 1978; Forssberg 1979). These responses are preserved after spinalisation (Forssberg et al. 1975). Since spinal interneuronal networks mediating cutaneous reflexes receive strong projections from descending sources (Schomburg 1990) lesions of the spinal cord greatly impact their behavior. This section will predominantly discuss changes in cutaneous reflex pathways originating from low-threshold cutaneous afferents. High-threshold cutaneous and/or nociceptive afferents are discussed later on.

Changes in cutaneous pathways projecting to motoneuronal pools has been investigated by stimulating low threshold cutaneous afferents and observing modulatory effects on soleus H-reflexes in intact and SCI individuals (Lebizec et al. 1983; Levin and Chapman 1987; Fung and Barbeau 1994; Knikou and Conway 2001). What becomes evident is that not all cutaneous pathways are similarly influenced by altered descending input.

For example, in intact individuals, a conditioning stimulus to the sural nerve has been shown to facilitate the soleus H-reflex (Hugon and Delwaide 1969) and has been attributed to supraspinal influences (Delwaide et. al. 1981). To test whether supraspinal sources were responsible for this facilitation, Lebizec et al. (1983) studied sural nerve conditioning of soleus H-reflexes in complete SCI patients. It was found that facilitation persisted in SCI individuals and that the magnitude of increase was similar to previously studied intact individuals. What differed, however, was that progressive increases in conditioning intensity did not inhibit soleus H-reflexes, which normally occurs in intact individuals (Delwaide et al. 1981). In other studies, the conditioning effects of medial plantar nerve (MPN) stimulation, which innervates the sole of the foot, on soleus H-reflex excitability was assessed by electrical stimulation (Fung and Barbeau 1994) and by mechanical loading of the foot (Knikou and Conway 2001) in intact and SCI individuals. It was found that MPN conditioning of the soleus H-reflex, which produces a clear inhibition of the H-reflex, did not differ in intact and SCI individuals under resting conditions. Moreover, Fung and Barbeau (1994) further tested MPN conditioning during locomotion. In intact individuals, MPN conditioning modulated soleus H-reflex excitability phase-dependently with the inhibition being greater during early stance and swing phases. Although severe spastic SCI patients did not exhibit phase-dependent modulation of soleus H-reflexes during locomotion the conditioning stimulation from MPN was modulated similarly to intact subjects. The authors thus suggested that deficient Ia modulatory mechanisms during walking could be restored with conditioning stimuli mimicking foot contact.

In another study the effects from the superficial peroneal nerve (SPN) onto the soleus H-reflex pathway were investigated in intact and SCI individuals (Levin and Chapman 1987). In intact individuals, a conditioning stimulus to the SPN, which activates primarily low threshold Aβ cutaneous afferents, at C-T intervals ranging from 30-190 ms generally facilitates the soleus H-reflex. Conversely to sural nerve stimulation (Lebizec et al. 1983), no facilitation from the SPN was observed in SCI patients regardless of C-T intervals and moreover, in roughly half the SCI individuals tested, SPN conditioning produced an attenuation of soleus H-reflex amplitude rather than facilitation. In intact individuals, inhibitory actions from cutaneous afferents are typically associated with noxious stimulation (Delwaide et al. 1981). These results indicate that facilitatory actions from the SPN projecting to the soleus H-reflex pathway are altered following SCI.

These studies in human SCI patients indicate that not all cutaneous pathways are similarly impacted following injury. Thus, although supraspinal influences are not required for sural-induced facilitation or MPN-induced inhibition of soleus H-reflexes, it does appear that the loss of supraspinal control generates some changes in cutaneous pathways and that activating preserved pathways might benefit functional motor recovery. This is in agreement with a recent study showing that step training in spinal cats differentially influences transmission in several cutaneous pathways (Cote and Gossard 2004). Thus, plastic changes in reflex pathways either resulting from SCI or training are not uniformly distributed amongst cutaneous pathways.

Changes in transmission of cutaneous pathways have been shown to occur after SCI in animal models (Baker and Chandler 1987b; Afelt 1970; Bennett et al. 1999; Bennett et al. 2004; Valero-Cabre et al. 2004). For example, to study changes in the transmission of cutaneous afferents after complete spinal transection intracellular recordings of hindlimb triceps surae motoneurons were made at various epochs following injury in response to sural nerve stimulation in cats (Baker and Chandler 1987b). Similar to Lebizec et al. (1983), there were no changes in short or long latency post synaptic potential patterns or latencies, although response amplitudes were markedly greater in chronic compared to acute spinal cats. This

confirmed that cutaneous pathways from the sural nerve after spinalisation persisted over time after SCI but that transmission was enhanced. It was established that augmented reflex responses were the result of reorganised synaptic connection at interneuronal levels since no changes in motoneuron membrane properties were observed (Baker and Chandler 1987a), although this was done under anesthesia which abolishes certain properties such as plateau potentials. However, whether or not responses in chronic spinal cats were greater than pre-injury levels was not evaluated. To address this point, Valero-Cabre et al. (2004) stimulated the tibial nerve at the ankle at various stimulus intensities to selectively activate different afferent populations before and following T9 spinal transections in rats. Based on onset latencies it was possible to distinguish three components mediated by different afferents (C1: Aαβ; C2: Aδ; C3: C fibers) and to determine their changes after SCI. Ipsilateral and contralateral responses were recorded in biceps femoris and tibialis anterior muscles, respectively. It was found that immediately after injury the ipsilateral C1 component was largely unaffected whereas C2 and C3 components were completely abolished. At 14 days post-injury, C2 and C3 reappeared but remained diminished for the rest of the study, while the C1 component was markedly increased (peak 300% increase) compared to pre-transection levels maintaining elevated values for the duration of the study. The crossed spinal reflex (stimulation of the tibial nerve on the left side recorded in the tibialis anterior of the right side) was abolished for all three components one hour post-injury. Whereas C1 and C3 showed some recovery, the C2 component reappeared in a few rats during the 60 days post-transection. In another study, cutaneous responses to light touch were small or absent in intact rats but following sacral transection (> 14 days post-injury) these responses were greatly enhanced in both amplitude and duration. To quantify these responses electrical stimulation of the distal part on the dorsal surface of the tail was used to evoke a predominantly pure activation of low-threshold cutaneous afferents (Bennett et al. 1999; Bennett et al. 2004). In both intact and acute spinal rats this stimulation induced a short latency inhibition, which lasted up to 500 ms, followed by an excitation, which could not be potentiated with repetitive stimuli. However, in

chronic spinal rats, the same stimulation produced a pure short latency excitation and repetitive stimulation evoked progressively larger responses. Thus, after sacral SCI the short latency inhibitory cutaneous reflex reverses to an excitatory response and due to the lack of inhibition repetitive stimuli can generate increased and sustained muscular activityresulting from altered interneuronal connections and recovered motoneuron properties such as plateau potentials.

These results in humans and animal models show that cutaneous transmission is altered following spinal transections and that reflexes mediated by distinct afferent populations are modified and recover differently after SCI. Moreover, crossed reflex responses show marked differences in reorganisation compared to ipsilateral pathways. Since various reflex pathways are differently influenced a general change in motoneuronal excitability cannot constitute the primary mechanism. Thus alterations at interneuronal levels are undoubtedly occurring.

2.10. Interlimb reflexes coupling upper and lower limbs

Interlimb reflexes evoked by cutaneous and/or muscle nerve stimulation of the lower limbs and recorded in upper limb musculature, or vice-versa, is postulated to participate in the coordination of the four limbs during locomotion in intact humans (Gassel and Ott 1973; Kearney and Chan 1979; Kearney and Chan 1981; Meinck and Piesiur-Strehlow 1981; Piesiur-Strehlow and Meinck 1980; Zehr et al. 2001). Following cervical SCI interlimb reflexes are more prevalent with consistently earlier onset latencies (Calancie 1991; Calancie et al. 2005; Calancie et al. 2002; Calancie et al. 1996) compared to the intact state (Zehr et al. 2001). For example, interlimb responses recorded in upper limb muscles evoked by stimulating the tibial nerve in the popliteal fossa do not emerge prior to six months after cervical SCI (Calancie et al. 2002) and responses become increasingly prevalent in individuals as time elapses subsequent to the lesion (Calancie et al. 2005). Furthermore, interlimb reflexes, which are more prominent in proximal muscles in intact individuals (Zehr et al. 2001), are rare in proximal motor pools after SCI, becoming more evident in distal musculature (Calancie et al. 2002). As such, few interlimb responses are evoked in

proximal upper arm musculature compared to muscles of the forearm and hand after SCI (Calancie et al. 2005). Interestingly, in intact individuals, interlimb reflex activity is characterized by an initial inhibitory response followed by an excitatory one (Zehr et al. 2001) whereas, after SCI interlimb responses are almost exclusively excitatory (Calancie et al. 1996). Thus, taken together, these observations provide strong evidence that interlimb reflexes, mediated by propriospinal connections coupling cervical and lumbosacral motor pools, are deeply reorganised and are constantly evolving following SCI.

2.11. High-threshold afferents

High-threshold afferents, via polysynaptic connections from muscle, cutaneous, or nociceptive receptors, are involved with posture and locomotion (Lundberg 1979; Grillner 1981), and mediate several motor behaviors such as the classic withdrawal reflex (Sherrington 1910). Since supraspinal centres exert powerful actions on these pathways (Schomburg 1990) the loss of descending control induces marked reorganisation.

In intact humans, activation of high threshold afferents consistently evokes a short latency flexion response (Hugon and Delwaide 1969; Pedersen 1954), which becomes infrequent and/or absent after motor complete SCI (Roby-Brami and Bussel 1987; Knikou et al. 2005). However, in the latter group a longer latency reflex arises conducted by low-threshold cutaneous and/or muscle afferent pathways (Dimitrijevic and Nathan 1968; Roby-Brami and Bussel 1987; Shahani and Young 1971; Andersen et al. 2004; Knikou et al. 2005). In one study, Roby-Brami and Bussel (1987) electrically stimulated tibial and sural nerves to evoke flexion reflexes in complete SCI patients. It was found that early latency reflexes were infrequent in SCI patients whereas longer latency reflexes (> 120 ms) were consistently observed. The onset of late reflexes become progressively delayed with increasing stimulus intensity and occurs independently of the early response. Afferents mediating early responses are thought to inhibit the pathway responsible for the late response since longer latency reflexes are more prominent and/or prevalent if the early component is suppressed or

weakened (Anden et al. 1966b; Anden et al. 1966a; Jankowska et al. 1967a; Jankowska et al. 1967b). Indeed, in the acute or chronic spinal cat the late reflex surfaces with DOPA injection, suppressing the early response in the process (Anden et al. 1966b; Anden et al. 1966a; Jankowska et al. 1967a; Jankowska et al. 1967b). It had previously been observed in spinal cats that late reflexes appeared provided the early flexion reflex was weak (Anden et al. 1966b), which might explain why in man the late flexion reflex occurs without drug administration (Roby-Brami and Bussel 1987). The emergence of a polysynaptic pathway has also been demonstrated in spinal rats (Bennett et al. 2004). In this study stimulating the tip of the tail evoked polysynaptic latency responses, which became significantly more common and amplified after chronic spinal transection (Bennett et al. 2004). The longer latency component has been implicated in the generation of spinal locomotion in the cat (Jankowska et al. 1967a; Schomburg et al. 1998) and may therefore represent a pathway released from inhibition by the loss of descending signals since it is absent in intact individuals (Roby-Brami and Bussel 1987).

In more recent studies, electrical stimulation of afferents or manipulation of the hip joint has been shown to modulate the late flexion reflex in spinal cord injured humans (Knikou et al. 2005; Knikou and Conway 2005). Flexion reflexes were evoked by electrically stimulating the sural nerve below the lateral malleolus at high intensities. Like previous reports (Roby-Brami and Bussel 1987) short-latency reflexes were rare after SCI, whereas long latency flexion reflexes were consistently evoked. It was demonstrated that hip flexion depressed the late flexion reflex, corroborating earlier findings (Dimitrijevic and Nathan 1968), while extension of the hip produced facilitation. A similar depression of the late flexion reflex was observed with functional electrical stimulation of the rectus femoris, a hip flexor (Knikou and Conway 2005). These effects highlight the importance of sensory feedback in modulating spinal interneuronal systems, which may prove useful for rehabilitative strategies (Knikou et al. 2005; Knikou and Conway 2005).

Other studies have shown that other parameters of high threshold afferent pathways are altered following SCI. For example, spinal lesions modify receptive

fields of nociceptive withdrawal reflexes in rats (Schouenborg et al. 1992) and humans (Andersen et al. 2004). In these studies nociceptive withdrawal reflex receptive fields are expanded after SCI and reflex threshold is decreased. For example, electrical stimulation at various sites on the plantar surface of foot, just above threshold for tibialis anterior responses, was used to elicit flexion reflexes in intact and complete SCI individuals (Andersen et al. 2004). Response amplitude in tibialis anterior and soleus were generally augmented in the SCI group compared to intact individuals at virtually every site tested, indicating that receptive fields for these muscles had expanded into previously absent regions. Moreover, ankle flexion in SCI patients following electrical stimulation was considerably greater than intact individuals. These results provided quantitative evidence that nociceptive reflex receptive fields are expanded in agonist and antagonist muscles after SCI and that reflex amplitude is enhanced.

Another novel property of high threshold afferent pathways after SCI is that successively applied stimuli generate windup, a considerably potentiated response (Hornby et al. 2003; Bennett et al. 1999). For example, a supra-threshold stimulus to the plantar skin of the foot followed by successively applied stimuli at intervals less than three seconds generate windup of flexion reflexes in SCI patients (Hornby et al. 2003). A similar phenomenon is found in sacral spinal rats following light brushing of tail hairs, which induces a powerful flexor response after SCI no seen in the intact state, which can be greatly potentiated by applying three consecutive stimuli (Bennett et al. 1999). This windup of flexion reflexes in rats has been linked to the development of unusually long (200-500 ms) polysynaptic excitatory (pEPSPs) inputs to motoneurons after SCI (Li et al. 2004a). These pEPSPs in conjunction with the loss of inhibitory control of PICs and plateau potentials in turn produce slow maintained motoneuronal depolarizations, which cause spastic reflexes (Bennett et al. 2004).

Furthermore, under normal circumstances, as described by Sherrington (Sherrington 1910) and termed local sign, contraction of a particular muscle withdraws the skin region receiving the nociceptive stimuli (Schouenborg and Weng

1994; Andersen et al. 1999). However, following SCI this property is altered. For example, local sign withdrawal, a reflex directing the limb away from noxious stimuli, was evaluated in intact and complete SCI patients (Schmit et al. 2003). Noxious cutaneous stimuli applied over various sites in the lower limb produced local sign withdrawal in intact individuals but SCI patients had flexion responses irrespective of stimulus location. Flexion was observed at the hip and ankle regardless of whether stimuli were applied to regions of the foot, ankle, or lower leg. The authors concluded that interneuronal processing of cutaneous stimuli is altered following SCI either directly by the loss of descending input or indirectly via plastic changes in afferent pathways.

Therefore, along with the emergence of novel or latent polysynaptic pathways these various changes imply that high threshold or nociceptive reflex pathways are deeply reorganised following injury to the spinal cord.

2.12. Mechanisms mediating plastic changes in reflex circuitry

Although alterations in reflex pathways after SCI are well known phenomena, there is a paucity of information with regards to precise mechanisms subserving this plasticity. Changes in reflex pathways after SCI can transpire in numerous ways. The first one implicates changes in motoneuron excitability either by pre- or post-synaptic mechanisms, which have recently been reviewed after SCI (Heckmann et al. 2005) and are briefly summarized here. Persistent inward currents (PICs), which normally generate sustained depolarizations called 'plateau potentials', are sharply reduced with the loss of descending monoaminergic inputs during acute SCI (Hounsgaard et al. 1988; Conway et al. 1988; Bennett et al. 2001b), resulting in marked decreases in motoneuronal and reflex excitability. Subsequent increases in reflexes are made possible by the recovery of motoneuron excitability and the ability to generate PICs and plateau potentials (Bennett et al. 1999; Bennett et al. 2001b; Bennett et al. 2001a; Li and Bennett 2003), which are suggested to reappear because of a supersensitivity to remnant monoamines. Although PICs with chronic SCI recover to levels similar to those recorded in intact motoneurons (Li and Bennett

2003; Bennett et al. 1998; Lee and Heckman 1998) the loss of descending control that normally turn off motoneurons could account for abnormal prolonged motoneuronal firing, as observed in spasticity (Li et al. 2004a). Moreover, although the focus has been on changes in PICs influencing motoneuronal excitability, little is known about similar phenomena impacting interneurons. It may be the case that PICs throughout the interneuronal relay of a reflex pathway are modified following SCI. Therefore changes in PICs and hence plateau potentials appear to considerably influence reflex behaviour after SCI and are thought to largely contribute to the development of spasticity.

Altered afferent transmission, which influences motoneuron excitability, can result from changes in presynaptic inhibition and/or homosynaptic depression. As previously mentioned, early after injury PSI and/or HD of the Ia afferent pathway is increased, which may contribute to depressed motoneuronal excitability and hyporeflexia (Ashby et al. 1974; Ashby and Verrier 1975; Calancie et al. 1993). However, PSI and/or HD gradually diminishes with chronic SCI, which may partly subserve enhanced reflexes (Taylor et al. 1984; Faist et al. 1994; Levin and Chapman 1987; Calancie et al. 1993). Changes in PSI and synaptic transmission after SCI are certainly not limited to group Ia afferents and are undoubtedly present for other primary sensory afferents and interneurons interposed in these pathways. Furthermore, modifying synaptic input from sensory afferents has been shown to influence plateau potential thresholds (Bennett et al. 1998). Therefore, a combination of pre- and post-synaptic changes at the motoneuronal level influences excitability of the motor pool

Plasticity in reflex pathways can also occur through the emergence of new connections either by activating latent pathways released from inhibitionor by establishing new synapses through collateral sprouting. Due to the rapid nature of the plasticity, and the abrupt emergence of new reflex pathways after spinalisation (Bennett et al. 2004), activation of latent connections is a likely candidate (Wall 1975; Merrill and Wall 1989). Moreover, collateral sprouting of dorsal horn afferents, spinal interneurons and extant descending projections has been implicated

in reorganizing reflex pathways (Helgren and Goldberger 1993; Murray and Goldberger 1974; Raineteau and Schwab 2001). Indeed, SCI has been shown to trigger sprouting of primary afferents in the spinal cord (Krenz and Weaver 1998) and studies have attempted to delineate molecules involved in initiating or promoting this phenomenon (Schnell et al. 1994; Buchli and Schwab 2005; Li et al. 2005; Reier 2004). However, to what extent these new connections are functional is unclear.

Another means of inducing plastic changes in spinal reflexes is by strengthening extant or newly formed connections. Indeed, modified synaptic transmission through long-term potentiation- or depression-like phenomena has been shown to occur at various sites in the central nervous system, including the spinal cord (Randic et al. 1993; Lozier and Kendig 1995; Liu and Sandkuhler 1995; Pockett 1995; Pockett and Figurov 1993). Thus, altered synaptic transmission through LTP or LTD could enhance or depress excitability of reflex pathways after SCI and/or training.

Furthermore, biochemical properties of the spinal cord change and evolve over time after SCI and with step training (reviewed in (Edgerton et al. 2004)). For example, it has been established that certain pharmacological agents are more effective at specific stages after a lesion, indicating that the sensitivity of the spinal cord to certain drugs is altered and continues to evolve following SCI (Giroux et al. 2003; Chau et al. 2002). Indeed, there is an upregulation of certain receptor types in the injured spinal cord, which return to control values thereafter (Giroux et al. 1999; Roudet et al. 1996). Moreover, step training influences the biochemical milieu of the spinal cord. For example, studies have shown that strychnine or bicuculline, a glycinergic agonist and GABAA receptor antagonist respectively, which were ineffective in improving stepping in trained spinal cats, substantially ameliorated stepping ability of non-trained spinal cats (de Leon et al. 1999b; Edgerton et al. 1997). These studies indicated that training modulates inhibitory connections within the spinal cord thus facilitating the expression of spinal locomotion.

Although considerable more work is required to elucidate the precise mechanisms subserving changes in reflex pathways after SCI it is evident that

modifications in spinal reflexes occur at several loci and at different levels, which evolve temporally and with various interventions, such as locomotor training.

3. Locomotor recovery after spinal cord lesions

The above sections described changes in more or less simple reflex pathways after spinalization. One of the major findings however has been that, after a complete spinal transection, complex motor patterns such as locomotion can recover, involving the reactivation of more elaborate spinal circuits. This second part first summarizes aspects of this functional locomotor recovery after partial or complete spinal sections in cats, then attempts to explain characteristics of spinal locomotion as a function of post-spinal excitability changes in simple reflex pathways described in the first section.

3.1. Recovery of locomotion after partial spinal section at the last thoracic segment in cats

Figure 1 only indicates that supraspinal structures in the brain stem and telencephalon exert an influence on spinal mechanisms generating locomotion (more details are found in (Rossignol et al. 2006). Obviously, supraspinal descending pathways exert powerful effects on spinal mechanisms and are capable of initiating or stopping locomotion and modifying it to reach a target or avoid obstacles. It is of interest to determine if some descending pathways play a sine qua non role in evoking voluntary quadrupedal locomotion. As will be seen, probably none of the descending pathways are uniquely necessary since cats recover voluntary locomotion after various lesions of dorsal or ventral spinal tracts, albeit some deficits may persist in relation to the specific pathways or groups of pathways lesioned. This undoubtedly results from the remarkable compensation from remaining descending pathways.

As an example, previous work suggested that medial and medio-lateral pathways are essential for locomotion (Eidelberg 1981) since sparing a small part of one ventrolateral quadrant allowed recovery of locomotion in chronically lesioned cats (Eidelberg et al. 1981a; Contamin 1983; Afelt 1974; Eidelberg et al. 1981b).

However, we and others have indicated that cats (Gorska et al. 1990; Gorska et al. 1993a; Gorska et al. 1993b; Brustein and Rossignol 1999; Rossignol et al. 1999; Zmyslowski et al. 1993; Bem et al. 1995; Brustein and Rossignol 1998) and even monkeys (Vilensky et al. 1992) can walk with all four limbs after large lesions of medial and mediolateral pathways that carry vestibular and reticulospinal tracts. Our experiments (Brustein and Rossignol 1998; Brustein and Rossignol 1999) showed that after large ventral and ventrolateral lesions at T13 cats initially behaved as complete spinal cats for 3-6 weeks but all cats eventually walked voluntarily with all limbs. Coupling between the hindlimbs remained stable but cats often walked with a more crouched posture and coordination between the hindlimbs and forelimbs was at times irregular. HRP was injected below the spinal lesion and the number and location of surviving cells with spinal projections were evaluated. In the Pontine Reticular Formation and the Medullary Reticular Formation (MRF), the number of labeled cells amounted to 5-48 % of normal values depending on the lesions; there were no vestibulospinal neurons. Rubrospinal cells whose axons course through the dorsolateral funiculi were either normal or decreased in number depending on the amount of encroachment of the lesion on the rubrospinal tract. HRP labeled cells appeared to be slightly more numerous and distributed somewhat differently in the motor cortex compared to control cats (Rossignol et al. 1999). Therefore, locomotor adaptation may have been achieved by remaining corticospinal pathways or from remaining reticulospinal and rubrospinal cells. Unfortunately, propriospinal neurons were not studied although they could have a role in such compensation (Jordan and Schmidt 2002).

Cats with lesions of dorsal and dorsolateral pathways, destroying among others the corticospinal tracts,can also walk over ground with all four limbs (Gorska et al. 1993b; Zmyslowski et al. 1993; Bem et al. 1995; Jiang and Drew 1996). After a period of impaired voluntary quadrupedal locomotion for a few days, cats continued to walk with a more crouched position for a few weeks with an increased step cycle duration. Cats had a significant persistent foot drag and could not correctly modify their gait to step over obstacles on a treadmill; instead the dorsum of the foot hit the

obstacle (Drew et al. 1996). Despite these deficits, cats performed remarkably well on the treadmill.

3.2. Recovery of locomotion after complete spinal section at T13 in cats

In numerous animal species hindlimb locomotion on a treadmill can recover after complete spinal cord sections (Delcomyn 1980; Grillner 1981; Rossignol et al. 1996; Rossignol 1996; Rossignol et al. 2000; Sherrington 1910; Shurrager and Dykman 1951). Grillner and colleagues studied quantitatively the kinematics and electromyographic (EMG) activity of locomotion after spinalisation, especially in kittens (Grillner 1973; Forssberg et al. 1980b; Forssberg et al. 1980a) and should be credited for the surging interest in functional capacities of the spinal cord for the last 35 years. Besides illustrating that spinal cats can walk with the hindlimbs, this group established the key concept that spinalised kittens prior to "learning" to walk can express a locomotor pattern, which can be maintained for several months, thus showing that the locomotor circuitry is genetically determined and extant within the spinal cord.

Within the context of plasticity, it is important to ask which spinal levels are implicated in the expression of locomotion since these levels might be critical after spinalisation. This became apparent with pharmacological experiments using intrathecal cannulae implanted chronically in spinal cats to deliver various drugs (Giroux et al. 2001; Chau et al. 1998b) to evaluate their effects on the initiation or characteristics of locomotion. A post-mortem study of the cannulae revealed that drugs acting over restricted portions of the cord (midlumbar L3-L4) had major effects. Further studies showed that intraspinal microinjections at these specific and restricted levels of a noradrenergic agonist such as clonidine and an antagonist, yohimbine, could respectively induce or block spinal locomotion in cats spinalized at T13 one week before these acute experiments (Marcoux and Rossignol 2000). Furthermore, we studied the effect of a second more caudal spinal lesion in cats first spinalized at T13 after having recovered locomotion (Langlet and Rossignol 2002). Whereas a second spinalisation at the levels of L2 or rostral L3 did not abolish

locomotion, lesions at caudal L3 and L4 prevented locomotion entirely despite several weeks of additional locomotor training. Since these segments are rostral to the motoneurons of the hindlimbs (Vanderhorst and Holstege 1997), it was concluded that these mid-lumbar segments were crucial in spinal locomotion. This was in keeping with the idea that important interneurons are located in these pre-motoneuronal segments and discharge during fictive locomotion (Jankowska and Edgley 1993; Jankowska et al. 2003; Davies and Edgley 1994; Shefchyk et al. 1990; Edgley et al. 1988).

Hindlimb locomotion thus recovers after complete spinalisation in adult cats (Barbeau and Rossignol 1987; Rossignol et al. 2000; Bélanger et al. 1996; Rossignol et al. 2002). After 2-3 weeks of treadmill locomotor training cats can walk with plantar foot contact and support the weight of their hindquarters. Although young animals possess better potential for expressing spinal locomotion (Bregman and Goldberger 1983; Howland et al. 1995b; Howland et al. 1995a; Smith et al. 1982; Bradley and Smith 1988), adult cats can also express a full pattern of hindlimb locomotion. We and others (Edgerton et al. 1991; Edgerton et al. 1997; Roy et al. 1998; de Leon et al. 1998b; de Leon et al. 1998a) have clearly established that, when trained several times a week on a treadmill, adult spinal cats gradually recover regular, bilateral, weight bearing steps. Moreover, we have found that after 2-3 months, locomotor performance is remarkably stable from one day to the next when measuring cycle duration at a given fixed treadmill speed, suggesting that even if the animals have regained plantar foot placement and weight support, there are still spinal processes evolving to stabilize the spinal circuitry (Barbeau and Rossignol 1987).

4. Reflex pathways and spinal locomotion

4.1. Effects of post-spinal changes in reflex pathways on spinal locomotion

In the first section of this review as well as in (Rossignol et al. 2006) contributions of reflexes to different aspects of normal locomotion (foot placement, force generation, cycle timing) have been summarized. What is the importance of these sensory inputs to spinal locomotion?

The importance of cutaneous feedback for the full expression of spinal locomotion in cats was recently addressed (Bouyer and Rossignol 2003b)(Bouyer and Rossignol 2003). For example, intact and spinalised cats are capable of compensating for the progressive loss of cutaneous nerves in the hindpaw (Bouyer and Rossignol 2003b; Bouyer and Rossignol 2003a). Functional deficits, such as inappropriate paw placement, only become apparent once all cutaneous nerves are cut indicating that a modicum of cutaneous information is necessary for normal locomotion. However, if a complete cutaneous denervation is performed prior to spinalisation than the spinal animal never recovers proper paw placement or weight bearing despite several weeks of rigorous step training. These studies indicate that some cutaneous information is required for flawless locomotion and that at least some intact cutaneous pathways are critical for expressing spinal locomotion.

Muscle proprioceptors also play an important role. Indeed, activation of load pathways appears critical for functional locomotor recovery following spinal transection since they exert powerful actions on rhythm generating networks of several species, including humans (Prochazka 1996; Duysens et al. 2000; Harkema et al. 1997). Progressive increases in weight bearing improve stepping in chronic spinal cats (Barbeau et al. 1987; Barbeau and Rossignol 1987) and recent findings indicate that the level of hindlimb loading markedly influences the quantity and quality of locomotion in spinal rats (Timoszyk et al. 2005), indicating a strong contribution of these reflex pathways in the recovery process. This is not surprising since group I afferents reinforce ongoing extensor activity during the stance phase (Conway et al. 1987; Guertin et al. 1995; Pearson and Collins 1993; Rossignol et al. 2006) and are ideal substrates in assisting force generation.

As detailed earlier, significant changes in reflex pathways occur and how these changes may actually be reflected in some aspects of spinal locomotion relative to normal locomotion is of interest. Figure 2 compares characteristics of locomotion in the same cat before and 41 days after a complete section of the spinal cord at T13. In Fig. 2A and B are represented, as figurines, the swing and stance phases in intact and spinal states, respectively. After spinalization the overall step length is decreased

although the cat walks at the same speed (0.4 m/s) in both cases. Shortening of the step cycle may result from hyperexcitable muscle stretch pathways that provide strong signals to terminate the stance phase. Hyperactivity in muscle stretch pathways might also account for the more "spastic" gait adopted by the spinal cat. Indeed, during stance, the knee and ankle yield vary little at stance onset and remain strongly extended throughout stance in the spinal state. After spinalization the hip joint has a slightly smaller overall excursion but, more remarkably, the hip joint clearly moves in a more extended range. Some of these changes might also be seen in a few muscle discharges (although only a limited sample is shown here). For instance, iVL, a knee extensor, exhibits a gradually increasing activity up to the end of stance and the overall period of activity of coGL, an ankle extensor, is prolonged after spinalization. Another interesting aspect is that following spinalization periods of co-contraction between antagonist muscles become increasingly frequent. Here, clearly the ankle extensor iGL is coactive for a short period with the ankle flexor iTA, which might result from reduced reciprocal inhibition reported in the first part of this review.

Obviously some of these considerations are speculative but nevertheless of interest since it could eventually be possible to see how modifying specific reflex pathways (by training, for instance) translates into changes of locomotor characteristics in spinal animals.

4.2. Normalization of reflex pathways after spinal cord injury with locomotor training

As we have seen SCI results in dramatic reorganisation of reflex pathways caudal to the injury. Consequently, transmission to and from the spinal cord is markedly altered and may impair functional motor recovery. However, different paradigms, such as operant conditioning (Segal and Wolf 1994; Chen et al. 1996), step training (Cote and Gossard 2004; Cote et al. 2003; Trimble et al. 1998), and cycle training (Skinner et al. 1996; Kiser et al. 2005; Reese et al. 2005) have been shown to modify transmission in reflex pathways after SCI. It may be the case that in

order to re-express motor programs, such as locomotion, that modified afferent input to the spinal cord incurred after injury must be shaped or normalized.

Locomotor training after spinal transections in chronic adult spinal cats is an established method to improve the rate and quality of stepping recovery. What is unclear, however, are the mechanisms subserving this functional recovery. Several authors have suggested that stepping provides spinal locomotor rhythm generating networks appropriate sensory cues that serve to entrain the locomotor pattern (Lovely et al. 1986; Rossignol 1996; Harkema 2001). Although, plastic changes in locomotor rhythm generating networks are undoubtedly occurring recent evidence indicates that transmission in afferent pathways from load and cutaneous receptors are modified by step training after SCI (Cote and Gossard 2004; Cote et al. 2003). To demonstrate step training-dependent changes in afferent transmission after SCI, Cote and Gossard (2003, 2004) compared a group of cats that were spinalized and not trained (shams) with cats that were spinalized and step trained (trained). After a period of 3-4 weeks, an acute experiment was performed to evaluate afferent transmission using intracellular recordings of hindlimb motoneurons in response to peripheral nerve stimulation in shams and trained cats. It was shown that step training reduced the amplitude of monosynaptic excitation and disynaptic Ib inhibition and that these changes were not due to similar modifications in motoneuronal properties since AHP duration was unaltered with one month of training (Cote et al. 2003). Decreased monosynaptic reflexes were attributed to increased PSI at Ia terminals and the authors posited that step training potentially reduces spasticity by decreasing Ia afferent transmission. The authors also postulated that a normalization of inhibitory control systems in the spinal cord might be critically important for locomotor recovery. Transmission in these load pathways were also assessed during fictive locomotion since disynaptic Ib inhibition exhibits a reversal to a polysynaptic excitation during locomotion (Gossard and Hultborn 1991; Gossard et al. 1994; McCrea et al. 1995). It was shown that trained cats possessed greater amplitude polysynaptic excitation than shams, which may be useful for the recovery of weight bearing in spinal locomotion. In a subsequent study (Cote and Gossard 2004) cutaneous transmission projecting to

hindlimb motoneurons was tested in a similar fashion. It was found that training did not change the distribution of the various responses evoked by cutaneous stimulation at rest but that in the majority of cases training decreased mean cutaneous response amplitudes, in particular transmission in the MPN. Therefore, these results demonstrated that step training in chronic adult spinal cats influences transmission in several afferent pathways and that functional locomotor recovery may in part be mediated by a normalization of exaggerated or erroneous reflex information, which occurs after SCI..

The authors thus suggested that hyperexcitability of reflexes after spinal transection is counter-productive for the expression of locomotion and that step training provides a 'normalization' of sensory feedback. Clonidine, which exerts powerful actions on locomotor circuits in spinalised cats(Barbeau and Rossignol 1987), similar to step training depresses transmission in cutaneous (Barbeau et al. 1987; Chau et al. 1998b; Chau et al. 1998a) and group I pathways (Cote et al. 2003). Therefore, as previously proposed pharmacological agents and/or step training may aid locomotor recovery by normalizing activity in reflex pathways after SCI (Naftchi 1982; Robinson and Goldberger 1986; de Leon et al. 1999a; Cote and Gossard 2004; Cote et al. 2003).

Using a different protocol it was shown that training spinally transected adult rats modified H-reflex excitability (Skinner et al. 1996). In this study, adult rats were completely spinalised at T10 and five days after surgery a three-month training regimen was initiated. Training consisted of passive cycling with rats suspended and hindfeet strapped onto pedals. H-reflexes in plantar muscles and their sensitivity to high frequency stimulation, evoked by stimulating the tibial nerve were compared in three groups at the conclusion of training. In the control group (no transection), high frequency stimulation resulted in marked depression of H-reflexes, which was considerably reduced in the transected group, corroborating an earlier report (Thompson et al. 1992). This frequency-dependent depression has previously been attributed to PSI or HD at Ia afferent terminals. However, in the trained spinal rats this frequency-dependent depression returned to normal values, suggesting that

training restores or reorganizes presynaptic inhibitory mechanisms. In a subsequent study, the same group demonstrated that rate-sensitive depression could be increased as early as fifteen days after initiating training but that full restoration emerged gradually over time (Reese et al. 2005).

Similar to animal models, locomotor training can significantly influence gating in transmission of the H-reflex pathway after SCI in humans (Trimble et al. 1998; Kiser et al. 2005). Using a similar paradigm to Skinner et al. (1996) a motorized bicycle was used to restore normal rate-sensitive depression of the H-reflex and thus improve spasticity in a C7 SCI patient (Kiser et al. 2005). Training consisted of passive cycling, five times a week for 13 weeks. Cycle training restored normal rate-sensitive depression of soleus H-reflexes, which paralleled improvements in spasticity. However, four weeks after training cessation rate-sensitive depression began to decrease and spastic signs returned indicating that cycle training must be maintained to retain ameliorations. In another case study of an incomplete SCI man, rate-sensitive depression of soleus H-reflexes was assessed before and after four months of treadmill training (Trimble et al. 1998). Soleus H-reflex recruitment curves were elicited at 0.1 and 1 Hz to compare the effects of low frequency depression in the SCI man to intact individuals. Low-frequency depression (1 Hz) of H-reflexes in the SCI patient were considerably less before training compared to intact individuals but significantly increased after training, which paralleled ameliorations in locomotor performance. Although these are only case studies, it does suggest that training, similar to animal models, normalizes afferent input to the spinal cord, which may benefit functional motor recovery. Considerable more research using larger sample sizes is required to elucidate the neurophysiological mechanisms subserving functional motor recovery in humans with SCI.

Therefore, although training benefits may stem from other plastic changes in spinal circuitry, locomotor improvements appear to be generated, in part, by normalizing erratic or disorganized sensory feedback incurred after SCI.

5. Concluding remarks

The spinal cord after a lesion provides the opportunity to study changes in relatively simple sensorimotor systems. And yet, these changes are complex and involve several mechanisms, which impact the behavior of these "simple circuits". We have attempted to summarize how changes in reflex pathways may account for various symptoms, such as spasticity, incurred after spinal injury. Furthermore, we have tried to relate these changes in reflex excitability to behavioral characteristics in more complex networks underlying locomotion, which utilize in part elements of these simple reflex circuits. This leads to the notion that perhaps rehabilitative techniques to reinstate function after spinal cord injury may rely on or be manifested in the normalization of these reflex pathways. Having a clearer view of the relation between characteristics of simple reflexes and more complex behaviors such as locomotion may generate more focused rehabilitative approaches as well as reflex testing as a tool to assess the progression of therapies or the prognosis of spinal injury. What is clear however is that the spinal cord, like the brain is reprogrammed after spinal cord injury and optimizes remnant functions. This demonstrated spinal plasticity proffers an even clearer basis for influencing and directing this neuroplasticity present after spinal injury through rehabilitative approaches.

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Figures

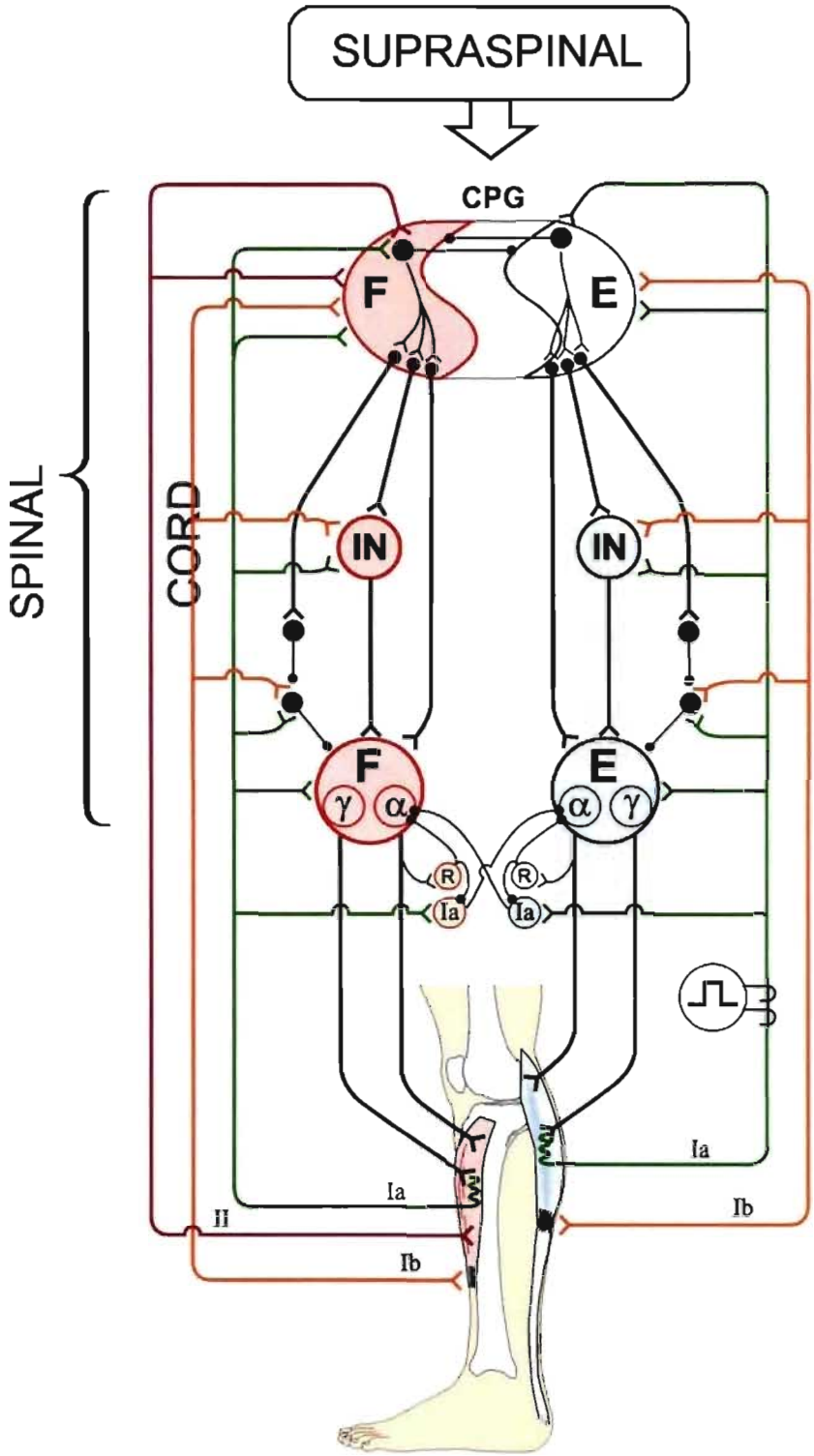


Figure 1.

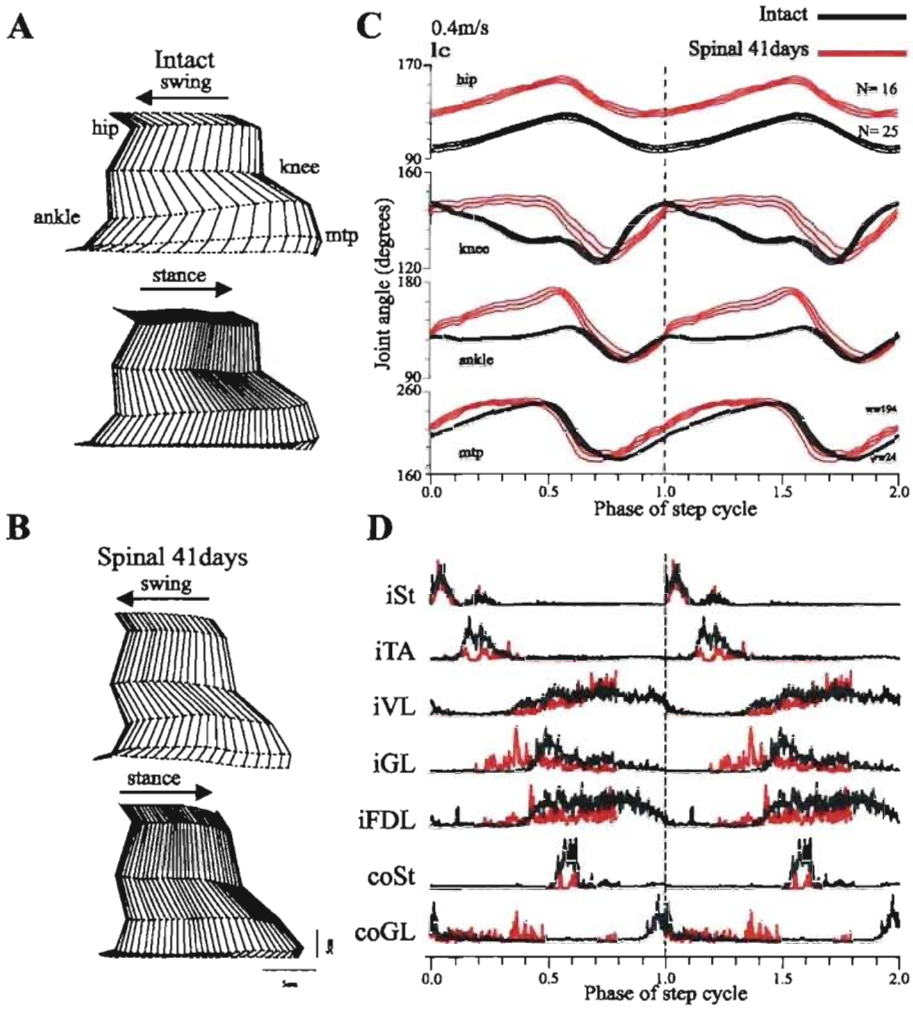
Figure 1 - General scheme of reflex pathways and spinal locomotor control
This scheme is subdivided in 3 parts.

The supraspinal levels includes various descending pathways from the telencephalon and brain stem involved in activating, stopping or modulating characteristics of the spinal central pattern generator (CPG) for locomotion as well as the excitability of transmission in reflex pathways at motoneuronal or premotoneuronal (presynaptic and/or interneuronal) levels. The large arrow emerging from the Supraspinal level encompasses all these functions without any further details.

The spinal cord level includes the CPG with a generally reciprocal activity between the Flexor (F) and Extensor (E) side. These two antagonist phases of the CPG circuitry are separated to indicate that each part may exert a function on other spinal mechanisms (represented by 3 output neurons emerging from each part of the CPG) as well as interact between each other (inhibitory connections between F and E). The Interneurones are represented by two large pink and blue interneurones [IN] which are interposed between afferents and motoneurons in disynaptic pathways as well as other more specific inhibitory interneurons (in black) representing disynaptic inhibitory pathways (such as Ib inhibitory interneurones) which can also be inhibited by other interneurons in certain tasks such as locomotion. Finally, motoneuron pools include both α-motoneurons projecting to extrafusal muscle fibers and γ-motoneurones projecting to intrafusal muscle fibers. Recurrent inhibition ® through Renshaw cells inhibit α-motoneurons (represented) and γ-motoneurons (not represented) and Ia interneurons responsible for reciprocal inhibition between α-motoneurons.

In the periphery, one ankle flexor muscle (pink) and one extensor muscle (blue) are represented with a spindle in both. Group Ia and II represent sensory fibers from spindles and are responsible for indicating rate and amount of muscle stretch, respectively. The stimulation symbol on the Ia fiber from the extensor illustrates direct stimulation of Ia afferents as performed during H-Reflex studies. Ib fibers

originate from Golgi tendon organs, which measure the force output of the muscle. Connectivity of the various afferents is partial and is largely based on that established in (Rossignol et al. 2006).


A
Intact
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B
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swing
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C
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Ic
Intact
Spinal 41days
hip
knee
ankle
mtp
N= 16
N= 25
Joint angle (degrees)
Phase of step cycle
D
iSt
iTA
iVL
iGL
iFDL
coSt
coGL
Phase of step cycle

Figure 2.

Figure 2 - Comparison of locomotion in the intact and spinal states in the same cat

A- Stick figures of the swing and stance phases reconstructed from videos taken at 60 frames/sec. Each frame is de-interlaced to extract each video field and light reflecting markers placed on the various joints are software detected and used to reconstruct the movement as stick figures for each joint as indicated. The angles measured are indicated and the arrows show the direction of limb movement. The stick figures are displaced from one another by a value corresponding to the foot displacement in the X direction so that each stick is clearly individualized. The length calibration found in B is therefore different in the X and Y axes.

B- Same as in A but 41 days after spinalisation with daily locomotor training on the treadmill. Note that in this case the limb is somewhat extended throughout the cycle and that overall step length is reduced compared to A. The calibration is explained in A.

C- Joint angular displacements are illustrated for the intact state (black, 25 cycles) and spinal state (red, 16 cycles). Note the shift of the hip in extension (shift of the hip trace) and of the knee and ankle extension during stance. The cycle is normalized to unity.

D- Electromyographic (EMG) recordings of the same sections as in C. Ipsilateral (i) refers to the side of the video camera while (co) is contralateral. Semitendinosus (St) is mainly a knee flexor but also acts as a hip extensor; Tibialis Anterior (TA) is an ankle flexor; Vastus Lateralis (VL) is a knee extensor and Gastrocnemius Lateralis (GL) an ankle extensor; Flexor Digitorum Longus plantar flexes the digits and is active during stance. EMGs are largely superimposed in both conditions although some changes are distinguishable and discussed in the text. EMGs have also been normalized to unity.

Annex 2 - Experiments and models of sensorimotor interactions during locomotion

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Abstract

During locomotion sensory information from cutaneous and muscle receptors is continuously integrated with the locomotor central pattern generator (CPG) to generate an appropriate motor output to meet the demands of the environment. Sensory signals from peripheral receptors can strongly impact the timing and amplitude of locomotor activity. This sensory information is gated centrally depending on the state of the system (i.e. rest versus locomotion) but is also modulated according to the phase of a given task. Consequently, if one is to devise biologically relevant walking models it is imperative that these sensorimotor interactions at the spinal level be incorporated into the control system.

Keywords: locomotion, sensorimotor interactions, model, simulation, feedback,

Introduction

Locomotion is a complex task requiring interactions at various levels of the nervous system and as such it proffers several challenges for the design of computer simulations and/or robots emulating walking biological systems. There are obviously several ways by which complex robots or even simple toys can "walk" from one point to another. One can design machines that may use completely different principles, and in some cases more efficient, than those that have evolved in biology. However, if one is to design an accurate and adaptable robot based on models of locomotion that incorporate available biomechanical and physiological data several sensorimotor interactions must be considered. Such an approach is undoubtedly more complex and more costly but it would probably benefit both neuroscientists and robot engineers and provide a useful common theoretical framework. Therefore, we will aim here at describing some essential components of the sensorimotor interactions that take place during locomotion and extract some principles that may be useful in designing walking machines. Some of these ideas are briefly explored in a recent review (Pearson et al. 2006).

At the heart of sensorimotor interactions is the spinal cord where the central pattern generator (CPG) for locomotion is located. The CPG provides the basic locomotor rhythm and integrates powerful commands from various sources that serve to initiate or modulate its output to meet the requirements of the environment (Figure 1). It is thought that the CPG elegantly controls muscle activation and hence movement of the mechanical system during walking by adjusting its output in response to a plethora of sensory inputs. Signals from supraspinal, spinal, and peripheral structures are continuously integrated by the CPG for the proper expression and adaptation of locomotion. The present review will firstly focus on empirically derived sensorimotor interactions between sensory afferents from the periphery and the CPG within the spinal cord. A detailed review of these sensorimotor interactions was recently published (Rossignol et al. 2006) and only some highlights will be reported here. For instance, proprioceptors may exert control over the rhythmic pattern being selected, the pattern frequency (speed of walking),

the duration of component sub-phases, as well as the amplitude of muscle bursts and the force generated. Cutaneous inputs on the other hand can trigger various specific rhythmic patterns with some similarities to locomotion (scratching, fast paw shake), control foot positioning during locomotion, and can serve to step over obstacles that may occur in different phases of the locomotor cycle and in different modes of locomotion (i.e. forward or backward walking). These are but a few examples of how sensory inputs can adapt the locomotor pattern to the real world and undoubtedly some of the principles and mechanisms uncovered in these regulatory sensory pathways would be useful in the design of versatile walking machines to cope with real environments.

The second part reviews how some of these principles have been incorporated in models of locomotion developed in recent years and how other elements could be implemented to create more efficient and realistic walking machines. Computer simulations have been performed to simulate locomotion in lamprey (Ekeberg and Grillner 1999; Ekeberg 1993; Ekeberg et al. 1995), insects (Cruse et al. 1995; Cruse et al. 1998; Ekeberg et al. 2004), cats (Wadden and Ekeberg 1998; Yakovenko et al. 2004; Ivashko et al. 2003; Ekeberg and Pearson 2005e), and humans (Taga et al. 1991; Taga 1995a; Taga 1995b; Taga 1998; Paul et al. 2005; McFadyen et al. 1994). The present review will focus mainly on conceptual models of cat locomotion since much is known about sensorimotor interactions of these animals during locomotion (for reviews see (Rossignol et al. 2006)) but models of human walking will also be discussed. Several aspects of locomotor control in cats and humans have been investigated using computer simulations including rhythm generation (Ivashko et al. 2003; Yakovenko et al. 2002; Rybak and McCrea 2005), reflex function (Yakovenko et al. 2004; McCrea et al. 2004), stance-to-swing transitions (Ekeberg and Pearson 2005d), and obstacle avoidance (Taga 1998; McFadyen et al. 1994). Insights provided by these models should enable us to further our understanding of locomotion.

Therefore, section 1 describes various sensorimotor interactions occurring during locomotion based on experimental evidence derived primarily from cats and

underscores how these interactions should be implemented in theoretical models of locomotion, to generate more effective and functional walking systems. The second section reviews some of the theoretical models that have been developed to conceptualise cat and human locomotion, particularly how sensory inputs are integrated and transformed, highlighting key findings and limitations of these models.

Section 1: Sensory interactions with the spinal CPG during locomotion

In recent years, major reviews (Rossignol et al. 2006; Duysens et al. 2000; Zehr and Stein 1999; Pearson 2000) and a book (Orlovsky et al. 1999) have covered extensively the topic of sensorimotor interactions during locomotion and therefore the present review will only summarize some key aspects. At first, it should be recalled that real locomotor movements can still be expressed after removing sensory afferents through a dorsal rhizotomy, which removes all sensory afferents (Grillner and Zangger 1984; Wetzel et al. 1976; Goldberger 1977; Goldberger 1983; Giuliani and Smith 1987; Mott and Sherrington 1895; Twitchell 1954; Taub and Berman 1968; Taub 1976) or pyridoxine intoxication, which destroys large caliber afferents (Pearson et al. 2003). Moreover, after neurochemical blockade, which prevents all movements and therefore all phasic locomotor-related afferent feedback, a detailed rhythmic pattern called "fictive locomotion" can be recorded from muscle nerves (Grillner and Zangger 1979; Pearson and Rossignol 1991). As such, sensory feedback is not necessary for generating the basic locomotor output due to the presence of a spinal CPG for locomotion (Grillner 1981). However, this does raise questions as to the roles played by sensory afferent feedback in controlling the CPG.

Essentially, sensory feedback adapts output of the CPG in the real world. In other words, specific sensory inputs can have a global influence so that some rhythmic patterns are selected, permitted and/or abolished, thus acting akin to switches that trigger certain patterns or set their range of operation. Once the movements themselves have been triggered sensory afferents can set the pattern frequency, regulate the structure and transition of sub-phases, modify the amplitude of the electromyographic activity underlying the locomotor pattern, strongly assist

foot positioning on irregular terrains, and also correct the pattern when obstacles appear in the path of progression. Such adaptation not only provides great versatility to the pattern but also affords a great deal of flexibility within the multitude of spinal and supraspinal circuits carrying sensory information that interact dynamically with the CPG. It will be shown that dynamic interactions between afferent inputs and the CPG vary according to the task (task-dependence) but also in relation to the phases of the task (phase-dependence). Thus afferent signals exert context-dependent effects indicating that a multiplicity of spinal and supraspinal pathways as well as numerous mechanisms are involved in the selection and/or modulation of the excitability of these various pathways.

Proprioceptive inputs

Muscle afferents appear to play three major roles during locomotion. Firstly, locomotor movements can be initiated or blocked by some proprioceptive afferent inputs. For example, stimulating hip joint proprioceptors by extending the hindlimbs can initiate air stepping in spinal cats (Sherrington 1910b). In chronic spinal cats capable of walking on a treadmill flexion of one hip joint abolishes stepping on that side whereas the other side can continue to walk. When extending the hip approximately to the angle normally attained at the end of stance the held limb resumes stepping provided the other hindlimb is in a phase where it can accept the weight of the animal (Grillner and Rossignol 1978). Similar to the hindlimb, a maintained protraction of the shoulder on one side of a decorticate walking cat can altogether abolish forelimb locomotion whereas a tonic retraction of the shoulder can increase the vigor of locomotion, especially in ipsilateral flexors and contralateral extensors (Saltiel and Rossignol 2004a; Rossignol et al. 1993). In "fictive locomotion", we also showed that the degree of hip extension greatly influences the locomotor pattern (Pearson and Rossignol 1991). Thus proprioceptive information from proximal joint (hip, shoulder) exerts strong influences on the spinal locomotor CPG. Another powerful signal to the locomotor CPG originates in the ankle extensors. For instance, it was shown that loading the ankle extensors during

decerebrate walking in cats markedly increased the extensor bursts while diminishing the flexor bursts (Duysens and Pearson 1980). As such, it was concluded that load signals from ankle extensor muscles inhibit flexor components of the locomotor pattern and that unloading of ankle extensors is essential to initiate swing. In this model, force seems to play a larger role than actual muscle length (Donelan and Pearson 2004b; Donelan and Pearson 2004a).

Secondly, proprioceptive afferents may participate in adapting walking speed, in determining overall cycle duration, and in regulating the structure of the step cycle's sub-phases (i.e. swing, stance), which is required for speed adaptation and interlimb coupling. For example, increasing speed on a treadmill shortens the stance phase while the swing phase remains relatively constant across a wide range of walking speeds in both normal and spinal cats (Grillner 1981; Halbertsma 1983; Forssberg et al. 1980a; Barbeau and Rossignol 1987). Moreover, when the hindlimbs of a spinal cat are walking at two different speeds (split treadmill) each hindlimb adapts the structure of its step cycle to match its respective speed (Forssberg et al. 1980b). It is thought that proprioceptors from the moving legs provide the main inputs responsible for adapting the speed of each hindlimb. Indeed, the fictive locomotor rhythm recorded from cut peripheral muscle nerves can be powerfully entrained by sinusoidal movements of the hip joint at different frequencies (Andersson and Grillner 1983; Kriellaars et al. 1994).

There is evidence that both positive and negative feedbacks arising from hip afferents influence muscle timing. In one study, when ramp movements of the hip were applied early during ankle flexor nerve activity (tibialis anterior, TA) there was an increase in the amplitude of TA (positive feedback) but a decrease in its duration (Andersson and Grillner 1981). When the same flexion was applied at a slightly later time, both the amplitude and duration of TA were increased but if it was imposed during late extension the extensors were turned off and the next swing phase was generated earlier (i.e. it was phase-shifted). In another study, during fictive forelimb locomotion, perturbations in the direction of the expected movements in a given phase shortened that phase and the converse occurred in the opposite phase

(Rossignol et al. 1993; Saltiel and Rossignol 2004b). Such negative feedback was seen with specific stretches of hip muscles in decerebrate walking cats (Lam and Pearson 2001). For instance, during swing hip protraction shortened the flexion phase whereas resistance to hip flexion increased the amplitude and duration of bursts in Iliopsoas (Ip) and Sartorius (Srt). It was also shown that repetitive stretches of flexor muscles (usually in combination) can entrain the walking rhythm (Hiebert et al. 1996) as do sinusoidal stretches of extensors, in spinal paralyzed cats injected with DOPA, entrain the fictive locomotor rhythm (Conway et al. 1987).

The role of afferents in structuring the step cycle is seen not only in experiments where afferents entrain the rhythm but also in experiments where stimulating afferents actually resets the step cycle (see (Rossignol et al. 2006) for a review including experiments in humans and animals). For example, in experiments designed to identify afferents responsible for the effects exerted by the hip, electrical stimulus at a strength of 1.6T and higher of a hip flexor nerve (Sartorius) can reset the cycle suggesting that Srt actions on the rhythm generator is mediated by group Ia and Ib (polysynaptic) pathways (Lam and Pearson 2002). In addition, stimulating group Ia (Hiebert et al. 1996) as well as group II afferents (Schomburg et al. 1998) of the ankle flexor EDL during the extension phase can reset the rhythm to flexion. Stimulation of specific muscle afferents (Golgi tendon organs of extensors) can also reset the locomotor cycle (Conway et al. 1987) (see also Figure 1).

Thirdly, proprioceptive afferents are involved in setting the level of muscle activity through various reflex pathways (Donelan and Pearson 2004a; Donelan and Pearson 2004b).

The simplest pathways are undoubtedly activated by stretch (Group Ia, II). Thus, when extensor muscles are stretched during stance these pathways via their simple connections can increase synaptic inputs to extensor motoneurons (see Figure 1). The importance of the activation of these receptors can be shown in cats in which the limb is unloaded as in "foot-in-the hole" experiments where cats make one step through a trap (Gorassini et al. 1994) or walk on a peg lower than expected (Donelan and Pearson 2004b). When the sensory feedback is decreased from ankle extensors

(ankle extensors are stretched much less or not at all) the activity in these muscles is reduced. In humans, unloading using a harness, which can support the whole body during walking, produces a major reduction in burst discharge amplitude of antigravity muscles (Dietz and Colombo 1998; Dietz and Duysens 2000; Harkema et al. 1997; Sinkjaer et al. 2000). Similarly, if ankle extensors are unloaded during stance by mechanically extending the ankle using an external ankle brace there is a 50% decrease in the EMG amplitude of ankle extensor muscles. Although stretch receptors might be the most obvious source of excitatory feedback to motoneurons it turns out that afferent feedback from load receptors (Ib from GTOs) also provides an excitatory feedback to motoneurons during locomotion. This represents a clear case of a dynamic state-dependent modification in sensory transmission where the usual autogenic inhibition from group Ib afferents observed at rest is changed to a disynaptic excitation during locomotion by activating specific spinal pathways(Gossard and Hultborn 1991; Gossard et al. 1994; Quevedo et al. 2000; McCrea et al. 1995a; Angel et al. 1996). This is represented in Figure 1 where inputs from Ib afferents can be switched to excitatory or inhibitory interneurones and this concept of state-dependent reversal from autogenic inhibition to excitation from Golgi tendon organs is clearly presented in a recent review (Hultborn 2006a) .

Another example of proprioceptive feedback modulation occurs when responses to afferent stimulation change in a phase-dependent manner. A prime example is the cyclical modulation of monosynaptic reflexes (H-reflexes in humans) throughout the step cycle. Moreover, studies on ankle stretch during walking in humans (Yang et al. 1991; Andersen and Sinkjaer 1999; Andersen and Sinkjaer 1999) suggest that responses to stretch are twice as large during stance compared to swing and that 30-60% of soleus muscle activity might result from the activation of its stretch receptors during stance (Stephens and Yang 1999; Stein et al. 2000). Since ankle extensors are maximally stretched during swing it would have been expected that monosynaptic reflexes would reach their peak amplitude in this phase but instead these reflexes are profoundly inhibited during this period probably as a result of antagonist reciprocal inhibition and/or presynaptic inhibition. Reflex enhancement

during stance is thought to stem from increased fusimotor drive (Prochazka et al. 1985; Prochazka 1989).

In conclusion, proprioceptive pathways can modulate the locomotor pattern (cycle duration, sub-phase durations, amplitude) via various pathways such as monosynaptic to motoneurons, excitatory disynaptic pathways open during locomotion, and polysynaptic excitatory pathways acting through the CPG (see (Hultborn 2006b) for a recent historical review of these various pathways). In turn, the transmission in these reflex pathways is controlled by the CPG so that sensory afferents and the CPG are in constant dynamic interaction. The reader is referred to more complete description of such complex interactions in a previous review (Rossignol et al. 2006).

Cutaneous Inputs

The role of cutaneous inputs to locomotion has often been neglected but recent work on denervation of the foot pads in the cat has shed some light on putative functions (Bouyer and Rossignol 2003b; Bouyer and Rossignol 2003a). After denervating all cutaneous nerves innervating the hindpaws of cats with an intact spinal cord normal walking on a treadmill returned but precision walking on a ladder was abolished for several weeks. After spinalization at T13, however, the same cats that had recovered a normal locomotion permanently lost the ability to place the foot correctly on the plantar surface, the paw dragged during swing, and the steps were much shorter. In chronic spinal cats with intact cutaneous innervation, these deficits are only observed transiently in the early stages of spinal locomotion (Bélanger et al. 1996; Rossignol et al. 2000).This illustrates that intact cats can use alternate inputs to compensate for the loss of cutaneous inputs but that in spinal cats cutaneous inputs are critically important for locomotion when the brain cannot select other sources of cues.

As is the case for proprioceptive inputs, some cutaneous inputs also have major effects in triggering or halting locomotion. For example, perineal stimulation can facilitate the expression of spinal locomotion (Rossignol et al. 2000; Sherrington

1910a). Stimulation of some specific skin areas can also evoke rhythmic patterns such as fast paw shake (Barbeau and Rossignol 1987; Pearson and Rossignol 1991; Langlet et al. 2005). Tonically stimulating the skin of the back for instance can also inhibit real or fictive locomotion (Viala and Buser 1974; Viala et al. 1978).

Probably the most obvious role for cutaneous inputs was demonstrated by electrical and/or mechanical stimulation of the skin of the foot during walking. These experiments showed that cutaneous inputs during locomotion could generate corrective responses which were appropriate to the locus as well as the phase of stimulation. Early studies performed on chronic spinal cats (Forssberg et al. 1975; Forssberg et al. 1976; Forssberg et al. 1977) and intact cats (Forssberg 1979) showed that contacting the dorsum of the foot during swing triggered a prominent knee flexion which withdrew the foot away from the impact followed by a flexion of the ankle and hip to step over the obstacle. The same contact during stance did not evoke flexion but an extension or a short latency inhibition of extensors. A similar mechanical stimulus applied to the dorsum of the foot during backward walking in intact cats (Buford and Smith 1993) evoked a different type of response, but appropriate to remove the foot. Thus, different spinal reflex pathways appear to be selected during forward and backward walking.

Electrically stimulating the skin or cutaneous nerves during the swing phase generates a short latency excitatory response (P1, ~ 10 ms) in the knee flexor Semitendinosus (Duysens and Loeb 1980) and very often a second excitatory response (P2, ~ 25 ms) appears in chronic spinal and intact cats (Forssberg 1979) (see also Figure 2). Often these two responses are independently modulated in amplitude (Abraham et al. 1985; Forssberg 1979; Duysens and Loeb 1980), as also seen in Figure 2. The period of reflex responsiveness of a muscle does not necessarily correspond to its period of activity and indeed, sometimes it can be completely out of phase with its duty cycle (Drew and Rossignol 1987). Also, during fictive locomotion (LaBella et al. 1992) the amplitude of short latency reflexes exhibited phase-dependent modulation suggesting that the phase-dependency was not solely related to interactions between afferents but to central mechanisms as well. Recent papers by

McCrea and colleagues (Quevedo et al. 2005a; Quevedo et al. 2005b) have shed new light on the pathways implicated in stumbling corrections by recording such responses in conditions of fictive locomotion. Indeed, the detailed timing of functional stumbling responses can be recorded during MLR-evoked fictive locomotion. Cutaneous nerve stimulation applied during swing evoked responses in flexors whereas during stance it evoked excitatory responses in ongoing extensor muscle nerves, consistent with the notion that cutaneous afferents also have short latency connections to ankle extensors (LaBella et al. 1992). Work by others have also clearly established that for a given cutaneous input reaching two different synergistic muscles active during fictive locomotion in the same phase, the responses in the two muscles can vary because the same input is redirected through different interneuronal routes (Fleshman et al. 1984; Schmidt et al. 1988; Moschovakis et al. 1991; Burke 1999; Burke et al. 2001; Moschovakis et al. 1992; Degtyarenko et al. 1996).

In man, non-nociceptive electrical stimulation during walking also yields phase-dependent responses that induce withdrawal responses during swing and stabilizing responses during stance (Zehr et al. 1997). Tibial nerve stimulation, which mimics a stimulation of the plantar surface of the foot evokes an ankle flexion at the stance-to-swing transition to remove the foot whereas it produces an ankle extension in late swing to accelerate foot placement on the ground (Zehr et al. 1997; Duysens et al. 1992). Therefore, cutaneous inputs are greatly involved in the overall expression of locomotion (start, stop) but also in foot placement during normal or obstructed locomotion.

All these biological observations aver that models for walking (theoretical or robots) should incorporate not only afferent feedback but also the rules that govern them during locomotion such as state- and phase-dependent modulation. Afferent signals can be involved in selecting the appropriate rhythmic pattern (walking, scratching, paw shake, struggling) and can also adjust and limit the operating range of these patterns (limits of joint motions). Once in operation, afferent feedback can participate in setting the overall frequency of the pattern, thus adjusting the different

sub-phases of the pattern. This is particularly important for interlimb coupling when turning or walking at two different speeds. Afferent feedback may also be used to adjust the amplitude of discharges of several muscles in various phases. To accomplish this a sophisticated apparatus is required to change the excitability of afferents in different phases (fusimotor drive, presynaptic inhibition). Afferent feedback can participate in the correct placement of the foot and in responding to obstacles presented in various phases of walking and in different modes of walking (forward, backward). This again requires sophisticated mechanisms of interneuronal selection to redirect sensory inputs to appropriate pathways in order to generate corrective responses, which are congruent with the ongoing phase of the movement.

Section 2: Modeling sensorimotor interactions during locomotion

Before we discuss models of locomotion and how sensory inputs can be integrated, a brief overview of models of the actuators moving the limbs during movement and receptors signaling changes in muscle length or force is provided since these are necessary for effectively and realistically simulating locomotor control.

Movement is produced by moving linked (joints) segments (bones) by actuators (muscles) driven by command signals (neural impulses). Muscles pose a major design problem since they are not linear force generators whereby an increase in neural drive corresponds to a linear increase in muscle force. Both muscle length and shortening velocity greatly influence force generation and as such these relationships must be considered when simulating movement to closely mimic normal motor control. Keeping these relationships in mind, several models have been devised to mimic mammalian muscle properties (Brown et al. 1996; Brown et al. 1999; Brown and Loeb 2000; McMahon 1984; Hill 1938; Zajac et al. 2003a; Zajac 1989). The design of mathematical models of muscles is discussed at length in other articles of this special issue and will not be describe here. However, we will mention that incorporating muscle actuators derived from some of these models have already

been implemented in simulations of locomotion and as described below these simulated muscles can generate realistic limb movements during walking.

Another major hurdle in designing accurate computer models of locomotion is to determine how command signals are generated and modified. In other words how is sensory information integrated within the CPG to generate an appropriate motor output? We will now describe some models of limb proprioceptors that convey information related to muscle velocity, length, and force and later on how they can modify ongoing movement.

Models of sensory receptors in the hindlimbs

Muscle spindles

As discussed in section 1 limb proprioceptors can greatly influence locomotion and as such accurate physiological models of these receptors that can readily be incorporated in computer simulations of walking are required. In muscle spindles, the central sensory region transmits changes in muscle velocity and length via group Ia or II afferents and contractile fibers can alter the sensitivity of this central region by receiving gamma innervation (Hunt 1990). These different elements provide the spindle with a range of firing properties and as such it is important to appropriately conceptualize these regions. Several models of muscle spindles have been developed over the last few decades to capture the range of responses exhibited by these receptors under various conditions (Matthews and Stein 1969; Schafer 1974; Chen and Poppele 1978; Houk et al. 1981; Hasan 1983; Mileusnic et al. 2006). Although an exhaustive review of muscle spindle models is outside the scope of this paper we will describe the basic properties of a recent model (Mileusnic et al. 2006) to highlight the functioning of these receptors and we will then discuss how some of the previous models compared to biological data recorded during locomotion (Prochazka and Gorassini 1998b).

The mathematical model we will describe was based on feline muscles and attempted to mimic the structure and physiology of primary and secondary muscle

spindles (Mileusnic et al. 2006). The basic structure of the spindle is similar to a previous model (McMahon 1984) and consists of a sensory (transduction) region with afferent endings intertwined around it and a polar (contractile) region. The sensory region is modeled as a spring whose stretch is proportional to afferent firing. Once a threshold length is reached the sensory region discharges and above this value afferent firing is scaled linearly with stretch magnitude. The polar region is composed of a passive spring in parallel with a contractile element, which can be influenced by fusimotor activation (see also Figure 1). Thus, the polar region can modify the sensitivity of the sensory region and hence afferent firing, as is the case in physiological spindles.

Different intrafusal fibers and gamma-innervations are incorporated in the model. For instance, the spindle is comprised of efferent and afferent connections to and from, respectively, the three different types (bag1, bag2, and chain fibers) of known intrafusal fibers. Each type of intrafusal fiber responds to changes in fascicle length and can be influenced by fusimotor drive (dynamic or static depending on fiber type). The model generates two outputs: primary (group Ia) afferents respond to input from all three types of intrafusal fibers while secondary (group II) afferents receive input only from bag2 and chain fibers. The model accounted for distinct activation dynamics of bag2 and chain fibers by providing these two types of fibers with different fusimotor saturation points and temporal properties. In addition, the model captured well secondary afferent behavior by using static fusimotor drive to bag2 and chain fibers. Overall the model faithfully reproduced known physiological data under various kinematic and fusimotor conditions.

Predicted primary spindle activity of previous models (Matthews and Stein 1969; Chen and Poppele 1978; Houk et al. 1981; Hasan 1983) has been compared to firing profiles of primary spindle afferents of hamstring (Prochazka and Gorassini 1998b) and triceps surae (Prochazka and Gorassini 1998a) muscles recorded in cats during overground locomotion. It was shown, comparing modeled and actual data, that during locomotion muscle velocity is the key variable modulating primary afferent activity in hamstring muscles and that it mostly precludes contributions from

other variables such as muscle length, phasic fusimotor drive, tendon strain and intramuscular mechanical effects (Prochazka and Gorassini 1998b). Despite fundamental differences in the design of these models, all, except for one (Matthews and Stein 1969), gave a good prediction of hamstring Ia firing during the step cycle at different velocities. In particular, the model by Chen and Poppele (Chen and Poppele 1978), as well as the two modified by the authors provided the best fits. Phasic modulation of fusimotor activity did not improve the accuracy of the models in predicting hamstring Ia afferent activity although a static contribution is required since recorded primary afferent firing is quite high and is never completely silenced during the step cycle. This suggests that a tonic static fusimotor drive is active throughout the step cycle. Moreover, in triceps surae muscles the addition of phasic fusimotor activity is required for models to approximate Ia afferent firing (Prochazka and Gorassini 1998a). This may be because the triceps surae muscles exhibit greater recruitment and undergo relatively larger length changes during simple locomotion than hamstring muscles.

In the case of secondary afferents better fits are obtained when the dynamic component is altogether removed (Prochazka and Gorassini 1998a). In other words, unlike primary Ia afferents which almost exclusively respond to the velocity of muscle stretch, the discharge rate of secondary afferents is determined almost entirely by muscle length. Furthermore, similar to primary afferents, adding tonic static fusimotor activity and a phasic fusimotor effect improves secondary afferent model fit in triceps surae but not hamstring muscles.

Therefore if one is to study the neural control of locomotion using 'in silico' preparations accurate models of muscle spindles tested rigorously against empirical data are required to reproduce the powerful signals and wide range of responses generated by these receptors under a variety of different conditions.

Golgi tendon organs

Afferent input from muscle spindles is not the only proprioceptive signal capable of influencing locomotion. Input from Golgi tendon organs (GTOs) via Ib

afferents (see Figure 1), which transmit information related to force-generated tension (Jami 1992), as described in section 1, strongly impact locomotor control (Duysens et al. 2000). A few models of GTOs are currently available and attempt to explain the complex behavior of these receptors during simple contractions (Anderson 1974; Houk and Simon 1967; Gregory and Proske 1981; Mileusnic and Loeb 2006). Only the most recent model (Mileusnic and Loeb 2006), which was based on the cat medial gastrocnemius (MG) muscle, will be described to illustrate the complexity of these receptors and the signals they provide during ongoing movement.

The mathematical GTO model (Mileusnic and Loeb 2006) consisted of several muscle fibers originating from different motor unit (MU) types and was structured with bypassing (attached to muscle fiber inserting into receptor but not intertwined with afferent endings) and innervated collagen (loosely- and densely-packed), which were interconnected with two common collagen networks. Each common collagen network comprised a spring in parallel with a damper (rearranges collagen following stretch) connected in series with another spring, delineating the sensory region (impulse generating site). Ib afferent firing depended on the stretch of this sensory region and on the flower-shaped cross-sectional area of collagen in the common collagen network. The two independent generating sites were in competition whereby the stronger output site completely suppressed activity in the other (complete occlusion). Thus only the dominant common collagen network provided output activity for the GTO. Although the model made several assumptions in design such as the flower-shaped cross-sectional area, complete occlusion, and the existence of multiple generating sites, thanks to these properties the model was able to capture several phenomena observed empirically. For example, self- and cross-adaptation whereby previous activation of the GTO decreases its responsiveness to subsequent changes in tension generated by the same MU or a different MU, respectively (Gregory and Proske 1979; Gregory et al. 1985), was shown. Moreover, nonlinear summation, which is a Ib response smaller than the linear sum of two separate MU inputs (Gregory and Proske 1979), was also

demonstrated. The model was also able to show the dynamic and static responses of Ib firing. Thus the response properties of this model resembled those of physiological GTOs.

To the best of our knowledge only one model (Houk and Simon 1967) has been tested to predict the firing rate of GTOs during locomotion (Prochazka and Gorassini 1998a). Using the inverse of the Houk and Simon transfer function it was shown that the predicted force from triceps surae Ib afferents closely resembled the actual measured muscle force. This strongly implies that GTOs can faithfully signal whole muscle force during locomotion. However, the contribution of GTOs to locomotor control has not been modeled as of yet although, as discussed later on, Ib input has been incorporated in some simulations.

Therefore, realistic models of GTOs are now available and implementing these principles will undoubtedly ameliorate the simulation of movement by providing accurate force signals to the CPG or neural controller.

Cutaneous receptors

Another important receptor for the control of locomotion lies embedded in the skin. As discussed in section 1, cutaneous receptors can influence rhythmicity in various ways but during locomotion they are especially important in correcting limb trajectory and foot positioning. Although models of cutaneous receptors are available (Wu et al. 2004) there have been no attempts to incorporate them in the context of locomotor control. Instead, most locomotor models incorporate cutaneous information simply as a foot contact sensor (Wadden and Ekeberg 1998; Ivashko et al. 2003) or as a force (load) transducer (Paul et al. 2005) without taking into account the diverse effects of these receptors on locomotion. Due to the wide range of deficits incurred by modifying or abolishing cutaneous information during locomotion (Bouyer and Rossignol 2003b; Bouyer and Rossignol 2003a) it is imperative that more accurate models incorporate these receptors to provide the control system with this critical input.

Summary

Therefore, models of muscle or skin receptors can provide the nervous system with information regarding muscle length, velocity, force, and touch but exactly how this input is collated into a sense of limb position and/or locomotor movement is largely unknown. What we do know is that this sensory information can shape the locomotor pattern. As we saw in section 1 a given sensory input can influence motor output in different ways depending on the phase and the state of the system. Thus, not only must accurate models of limb receptors form an integral part of any physiologically accurate locomotor control system but it is also critical that the CPG be capable of integrating this sensory information in an appropriate and dynamic manner through various pathways, as suggested in Figure 1, for the flawless and context dependent execution of locomotion.

Models of locomotion

Now that we have reviewed sensorimotor interactions occurring during locomotion and models of limb receptors that give rise to sensory information, we will describe some models of locomotion that have attempted to implement some of these principles into their control system. Although simulating locomotion in mammals is still in its infancy the last decade or so has seen the number and complexity of different models increase considerably. Conceptual models of mammalian locomotion have been devised using principles derived from some of the simulations described above and based on the abundance of biological evidence obtained primarily in the cat. Several aspects of locomotor control using the cat as a model have been investigated using computer simulations including pattern generation (Ivashko et al. 2003; Yakovenko et al. 2002; Wadden and Ekeberg 1998; Rybak and McCrea 2005), reflex function (Yakovenko et al. 2004; McCrea et al. 2004), and stance-to-swing transitions (Ekeberg and Pearson 2005c). To closely approximate locomotor control it is imperative that models include a system analogous to the CPG, which can receive feedback from simple reflex pathways originating from muscle and/or cutaneous receptors. Using this minimal system as

the basic framework for simulating locomotor control more complex biomechanical and neurophysiological features of locomotion can then gradually be integrated.

The control of phase transitions

Before we discuss interactions between the CPG and sensory events from the periphery we will describe how phase transitions are governed during overground locomotion. For simplicity, the step cycle of the cat is usually broken down into four different phases: stance, liftoff, swing, and touchdown with each phase characterized by the activation of a different set of muscles (e.g. muscle synergies). However, as we saw in section 1, how the switch is made from one phase to another can be complex. As such, an instrumental concept in the design of locomotor models has been the development of finite state or conditional rules to control phase transitions based on data recorded in animals (Prochazka 1996). For example, the rule for switching from stance to swing is IF stance AND ipsilateral leg is unloaded AND ipsilateral hip is extended, THEN initiate swing (Granat et al. 1993; Prochazka 1993; Prochazka 1996). Moreover, the rule for the swing to stance transition is IF swing AND ipsilateral hip is flexed AND ipsilateral knee is extended, THEN initiate stance. More rules can be applied to include the contralateral leg such as IF stance AND contralateral swing THEN delay flexion and prolong stance. This ensures that ipsilateral flexion does not occur while the contralateral leg is still in swing, which would cause the animal to collapse. Several more rules can be assigned to generate a myriad of phenomena observed during locomotion, such as phase-dependent reflex reversal, gallop, backward walking, stumble corrective response, etc. (Prochazka 1996). In a biological system, as stated in section 1, these rules are likely mediated by sensory receptors, which interact with the CPG to govern phase transitions. Evidently, regulating phase transitions is a crucial part of any realistic locomotor modeling effort.

To better understand how phase transitions are achieved during locomotion models can be developed to systematically investigate the contribution of various

sensory inputs in governing these finite state rules. In section 1 we outlined that two important sensory mechanisms regulate the transition from stance to swing in the cat hindlimb during locomotion. Stretch-sensitive afferents in hip flexors and force-sensitive afferents in ankle extensors influence activity of hindlimb flexors and thus are thought to control the transition from stance to swing. The relative role of these two mechanisms was recently investigated by simulating the cat hindlimbs during locomotion using either of these mechanisms in isolation or by combining the two (Ekeberg and Pearson 2005b).

The three-dimensional model developed by Ekeberg and Pearson consisted of two hindlimbs, governed by separate finite-state controllers that could be coupled (i.e. mutual inhibition) or uncoupled, and two stiff front legs working as frictionless struts. Several muscles actuated each of the three-segment hindlimbs, which produced a force linearly proportional to the activation level provided by the controller. The hindlimbs progressed repeatedly through a sequence that included liftoff, swing, touchdown, and stance. The transition between these four states was accomplished using sensory signals from the legs, which activated different muscle synergies in each state. Different rules were applied based on joint angles and muscle activity to determine the transition from one state to another. For example, liftoff (stance-to-swing transition) was initiated when ankle extensor force was low enough (unloading rule) and hip extension reached an arbitrary value (hip extension rule), which simultaneously activated muscles involved in liftoff. A linear combination of these two factors could initiate liftoff at high ankle extensor force provided hip extension was sufficiently large enough, or vice-versa. The model also tested either the unloading or hip extension rules in isolation to determine if liftoff could be generated solely by either of these mechanisms. Transition between the other states is described in detail in the original paper and is not reiterated here. The simulation was first made using the combined rules and then when walking had stabilized a bilateral switch to one of the two rules was performed. Switching to the unloading rule did not alter coordinated stepping in the two legs but a switch to the hip extension rule generated an unstable gait and the model eventually tripped or fell. This instability

with the hip extension rule was attributed to a progressive shift in the reciprocal coordination of the two legs away from 0.5. If the controllers were coupled, as is the case in normal animals, the unloading rule generated identical results to uncoupled controllers and although the hip extension rule was now capable of producing a stable gait the reciprocal coordination between the hindlegs remained abnormal.

Although the model suggested that unloading of the ankle extensors was more important for phase transitions than hip angle it did not attempt to show how these sensory signals interact with a locomotor CPG. Instead, the rhythmic activation of muscles was controlled by sensory signals from the legs, as was proposed early in the 20th century by Sherrington (Sherrington 1910a). Biologically relevant views of locomotor control assert that locomotion is not produced by chains of reflexes but by a CPG, which sets the basic locomotor rhythm, although its output can be modified by sensory signals. It is more likely that the two mechanisms (hip extension and unloading rule) act in concert during normal locomotion and that their relative contribution varies according to the task or its context. For example, during rhythmic behaviors where loading of the ankles provides a strong signal, such as level stepping, it would be anticipated that the weight of this cue is important for phase transitions. However, if ankle extensor loading is low, such as during swimming, scratching or air stepping as extreme examples, than signals from hip afferents would become increasingly critical for phase transitions. It is also known that below knee amputees can walk despite no feedback from ankle extensors. In addition, since locomotion can be achieved when all sensory feedback is abolished (Goldberger 1987) or when large caliber afferents from the limbs have been destroyed (Pearson et al. 2003) it is unlikely that sensory input is essential for phase transitions. Thus without sensory feedback the CPG can generate phase transitions but it is evident that sensory input can shape and strongly influence the pattern. Therefore, what is clear is that phase transitions can be accomplished without unloading of the ankle extensors (or sensory input) and that one mechanism is more important for all forms of rhythmic behavior or even all modes of overground walking is highly improbable

based on the available data. To closely mimic normal control and to elucidate sensorimotor interactions during locomotion a CPG is required.

Motoneuron activation and the basic locomotor pattern

It should be noted that a CPG is not essential to generate a walking system since models and robots lacking a CPG can still produce stable locomotion, as exemplified by the Ekeberg and Pearson model (Ekeberg and Pearson 2005a). However, if one is to accurately model locomotion and to understand how sensorimotor interactions are performed then a CPG should be a sine qua non element of such a control system. The CPG is thought to turn muscles on and off thereby generating a rhythmic pattern but the general organization of this system has been the source of debate for decades. Some argue that the CPG consists of half-centers (Jankowska et al. 1967; Lundberg 1981), whereas others view it has a system composed of multiple, coupled, unit burst generators (Grillner 1981). Another theory indicates that the CPG because of recorded phenomena such as deletions or resetting (Lafreniere-Roula and McCrea 2005) possesses two layers composed of a rhythm generator that provides the basic locomotor rhythm and a pattern formation network that distributes and coordinates the activity of motoneuron pools (Rybak and McCrea 2005). However, with current techniques, identifying large ensembles of neurons involved in rhythm and/or pattern generation is impossible (Kiehn 2006). Thus, only theories abound as to the internal organization of the CPG. To circumvent this problem we can model the CPG in varying forms using computer simulations to draw some general principles and insights from available data. One of the first steps that should be undertaken in such an effort is to gain an understanding of the complexity of motoneuron activation during the locomotor step cycle. Recent studies, although they do not provide a working CPG model, elegantly accomplished this by showing the spatiotemporal activation of motoneuron pools during locomotion in the cat (Yakovenko et al. 2002) and in humans (Ivanenko et al. 2006).

In the cat (Yakovenko et al. 2002), this was done by combining known distributions of motoneuron pools innervating numerous hindlimb muscles within the

spinal gray matter (Vanderhorst and Holstege 1997) with previously recorded EMG activity profiles of these muscles during locomotion (Rossignol 1996; Abraham et al. 1985; Bélanger et al. 1988; Buford and Smith 1990; Carrier et al. 1997; Engberg and Lundberg 1969; Halbertsma 1983; Pratt et al. 1991; Wand et al. 1980). It was shown that the pattern of activity within the spinal cord proceeded in a rostrocaudal direction during the step cycle. During stance the caudal half of the enlargement was active before abruptly switching to the rostral half during liftoff and swing. Thus, unlike the lamprey where activity progresses rostrocaudally as a traveling wave (Orlovsky et al. 1999; Wallen and Williams 1984), this suggests that in cats discrete CPGs are involved and activate muscles more like light switches, thus providing a clue as to the organization of the mammalian CPG. The authors also indicated that extensor-flexor antagonist motoneuron pools were widely spaced from one another and that connectivity would require long and overlapping propriospinal neurons.

In a similar vein Ivanenko and colleagues (Ivanenko et al. 2006) recorded EMG activity from several muscles of the limbs and trunk on the right side of the body during human walking and combined these profiles with known cervical to sacral segmental organization of motoneuron pools (Kendall et al. 1993; Sharrard 1964). They showed that during the step cycle the pattern of activity shifted twice from rostral to caudal segments on the right side of the body. For example, just before and during foot contact activity is seen in the upper lumbar segments and then shifts caudally at mid-stance. At the transition from stance to swing activity shifts rostrally before jumping caudally at the end of swing. These results are also consistent with discrete pattern generators whereby different spinal segments control specific phases of the step cycle.

Although these spinal maps in cats and humans accounted well for the location and segmental distribution of motoneuron pools activated during locomotion they provided few insights as to the general organization, loci, or number of CPGs. Moreover, what is lacking is any information on the multitude of interactions and connections between different "modules" (Schouenborg 2002; Stein and Daniels-McQueen 2002; Bizzi et al. 1995) or "unit burst generators" (Grillner 1981) that

control discrete muscles or synergies during different phases of the step cycle. However, if one is to model the CPG with the complexity which is thought to exist within biological systems this then becomes an increasingly daunting task. Instead, studies have implemented simplified CPG circuitry within their locomotor models to generate the basic rhythm and to study sensorimotor interactions very simple reflex pathways have been integrated.

The CPG and sensorimotor interactions

To evaluate sensorimotor interactions during locomotion studies have modeled relatively rudimentary CPGs with simple reflex pathways. For example, Wadden and Ekeberg (Wadden and Ekeberg 1998) modeled a single leg of a cat controlled by a CPG (they termed this neural phase generator or NPG) which received sensory information from the periphery and a "supraspinal" command that served to initiate and select the movements akin to the MLR described in cats (Shik et al. 1966a). The NPG consisted of four interconnected modules each one controlling a separate phase (stance, liftoff, swing, and touchdown) of the step cycle with each module consisting of three neurons. The hold (H) neuron keeps the stepping generator in a specific phase and has excitatory connections with the quash (Q) and transfer (T) neurons. Whereas Q inhibits all other phases T activates H of the next phase and inhibits Q of its own phase. The smooth sequence of locomotor events is ensured because the excitatory connection from H to T has a longer time constant than H to Q and because T only excites the H of the next phase. Sensory feedback related to hip position and ground contact is also provided to the network. The sensory neuron signaling hip extension activates the T neuron of the stance phase and motoneurons of liftoff thus promoting a switch from stance to swing. Conversely, the sensory neuron activated by hip flexion excites the T neuron of the swing phase and motoneurons of touchdown therefore signaling the transition from swing to stance. In addition, the neuron which senses ground contact indirectly inhibits and excites motoneurons responsible for swing and stance, respectively, thus assuring that swing is not initiated while the leg is still in contact with the ground. Stretch reflexes to hip

and knee extensors were also incorporated into the system to increase stiffness at these joints during touchdown but the functioning of these pathways was not based on physiological parameters. The model produced stable rhythmic locomotor patterns by changing the active elements during different phases and stepping velocity could be altered by modifying the strength of the supraspinal command. Although this system was relatively rudimentary it did show that interconnecting elements of a distributed network could effectively control muscle activation and phase transitions in one leg, thus providing a good first step in approximating a CPG for mammalian locomotion.

More recently, a model of cat locomotion consisting of two coupled CPGs driving muscle activity in several muscles for each hindlimb, which received proprioceptive reflex feedback from the hindlegs was devised (Ivashko et al. 2003). Each hindlimb comprised three rigid segments connected to the pelvis, which was fixed to the trunk. The spinal cord circuitry consisted of separate modules with each module including an alpha-motoneuron controlling one muscle, a Renshaw cell, and Ia and Ib interneurons. Each module was driven by its respective CPG and by reflex feedback. Two types of CPG neurons were included in the model. "Principal" CPG neurons activated alpha-motoneurons in a given module during a specific phase of the step cycle. For example, some CPG neurons activated alpha-motoneurons for muscles active during stance, while others controlled muscles active during three different stages of the swing phase. "Switching" CPG neurons, on the other hand, controlled phase transitions by turning off principal CPG neurons. Activation of these switching elements was controlled by proprioceptive feedback from both hindlimbs and from touch sensors that signaled foot contact. Several established features were incorporated in the model such as Ia-mediated reciprocal inhibition of antagonists and reversal of inhibition to excitation from group Ib afferents during locomotion.

Simulations showed that the modeled circuitry generated stable locomotion of the hindlimbs and that kinematics closely resembled real cat stepping. However, EMG patterns differed somewhat with published data (Rossignol 1996; Yakovenko et

al. 2002). For example, modeled flexor (iliopsoas, semitendinosus, tibialis anterior) and extensor muscles (biceps femoris anterior, vastii, gastrocnemii) were inactive prior to swing and stance, respectively, which is not the case during real locomotion. Moreover, rectus femoris (hip flexor/knee extensor) should fire before and after stance but in the model it only fired from mid- to late-swing. Biceps femoris posterior (knee flexor/hip extensor) had a short burst of modeled activity prior to stance and swing, whereas in real locomotion this muscle fires before and after liftoff and/or foot contact. The model also showed that soleus (ankle extensor) fired during mid-swing then fell silent before foot contact before becoming active during stance, while in real locomotion soleus activity is characterized by activity commencing just before stance and continuing through this phase. Furthermore, vertical ground reaction forces predicted by the model were much higher than actual forces. The abnormal patterns of activity in several muscles of the hindlimbs might have contributed to the greater ground reaction forces. The model was also not tested under different circumstances such as incline or decline walking. Thus, although the model was successful in generating rhythmic locomotor activity and incorporated several reflex pathways known to operate during locomotion further studies are required to refine and test the validity of this approach for modeling central locomotor control in the cat.

Another important aspect of locomotor control, as discussed in section 1, is the interaction of the CPG with reflex pathways from limb receptors. Stretch reflexes are thought to contribute strongly to the force-generating capacity of muscles during the stance phase of locomotion (Yang et al. 1991; Bennett et al. 1996; Stein et al. 2000) even though muscles possess spring-like properties capable of accomplishing a similar function. As such, the role of stretch reflexes has been questioned (Prochazka et al. 2002) and the extent to which this sensory input contributes to weight-bearing during locomotion was recently addressed (Yakovenko et al. 2004). In this study, the model consisted of two hindlimbs attached to a horizontal torso supported at the front by a frictionless wheel. Hindlimb muscles were driven by a CPG and during activity of a given muscle, reflex feedback from group Ia and Ib afferents contributed to

contractile force at a latency of 35 ms, adding 30% to the CPG EMG of that muscle during the step cycle. Finite-state rules, as described above, were used to govern transitions from stance to swing and from swing to stance (Granat et al. 1993; Prochazka 1993). What the model demonstrated was that in instances where CPG output was sufficiently high to generate a stable locomotor pattern the addition of reflex feedback from group I afferents did not improve the quality of locomotion. However, when CPG output and reflex feedback both contributed to locomotion the sudden loss of group I reflexes then caused the model to collapse almost instantly (see Figure 3). Thus, if CPG output is inadequate for the demands of the task then reflex contribution becomes critical but if CPG output is sufficient then reflexes only participate minimally. The authors further added that stretch reflex latency is not too long to assist in load compensation and augment central drive during stance.

What is unclear at present is whether CPG output is sufficient during normal locomotion in cats. In other words, do stretch reflexes normally supplement CPG output for weight bearing during locomotion? It is probable that stretch reflexes complement CPG output during simple locomotion thus allowing the system a greater range through which it can operate. Moreover, we can speculate that under certain circumstances the output from the CPG is by itself incapable of meeting the task requirements and that group I feedback from limb proprioceptors then becomes critical, if not essential. It would be interesting to test these hypotheses by simulating locomotion during more difficult tasks such as incline or decline walking using the model. Decline stepping has already been shown to be severely impaired in cats with sensory denervations of triceps surae muscles (Abelew et al. 2000). The loss of sensory information from ankle extensors in these cats did not produce visible deficits in level or incline walking but considerable disruption of interjoint coordination was seen during decline walking where muscles undergo active lengthening and group I feedback is normally elevated. Therefore, it would seem that CPG output is not necessarily sufficient for all locomotor tasks and that group I feedback normally complements central drive for the execution of flawless locomotion.

Models of fictive locomotion

To highlight the complexity of interneuronal connections and neuronal properties involved in CPG circuitry and rhythm generation, respectively, recent studies (McCrea et al. 2004; Rybak and McCrea 2005) designed a bipartite model comprising a half-center rhythm generator (RG) and a pattern formation (PF) network to simulate the motoneuronal activity recorded during fictive locomotion in decerebrate cats. Whereas, the RG provided the basic locomotor rhythm by delineating the duration of flexor and extensor bursts, the PF distributed and coordinated motoneuron activity via numerous excitatory and inhibitory interneuronal connections, thus shaping the final output to motoneurons. Through these various interactions, and by receiving input from the RG, only some neuronal populations in the PF were active during specific phases of the step cycle. The two-level CPG organization enabled sensory inputs to directly access either the RG or PF and thus account for observed physiological phenomena such as deletions or resetting of the locomotor rhythm (Lafreniere-Roula and McCrea 2005). Motoneurons were modeled according to a previous study (Booth et al. 1997) and properties such as persistent sodium currents were incorporated to endow excitatory neurons of the RG with rhythmogenic properties as has been modeled for respiratory CPGs (Butera, Jr. et al. 1999; Rybak et al. 2003). However, whether such endogenous rhythmogenic properties are present in locomotor CPG neurons is currently unknown.

A tonic MLR-like excitatory descending drive initiated locomotion via distributed connections to excitatory neurons of the RG and PF, which were both separated into flexor and extensor half-centers with mutual excitatory and inhibitory connections. The alternating activity of flexors and extensors respectively defined the flexion and extension phases of the locomotor cycle in the absence of sensory input. Whereas the persistent sodium current mediated burst onset the termination was mostly governed by reciprocal inhibition between half-centers. It was shown that the modeled motoneuron discharge rate was similar to that recorded previously during fictive locomotion in the cat (Brownstone et al. 1992). Separating rhythm generation from pattern formation allowed the model to faithfully reproduce deletions

with a phase shift (resetting) or without. In one example of a resetting deletion, increasing and sustaining the MLR drive to the extensor RG severely reduced activity in the flexor RG, which led to a deletion of flexor motoneuron activity. Once the MLR drive was discontinued the flexor rhythm reappeared but was phase shifted in time compared to before the stimulation. In contrast, altering the excitability at the PF level can produce a deletion without phase shifting the post-deletion locomotor rhythm since the activity at the RG level remains unchanged. Furthermore, although a bipartite (two layers) CPG accounts for phenomena such as deletions with or without resetting it is a contentious issue for several reasons since other elements in the pattern generating circuitry (forelimb, contralateral limb, brain stem neurons) could also keep track of the timing.

In another study Rybak and colleagues (McCrea et al. 2004) investigated the effects of stimulating various sensory afferents on CPG function by adding several interneuronal populations, such as Ia and Ib inhibitory interneurons, Renshaw cells, and interneurons intercalated in cutaneous reflex pathways, to their fictive locomotion model. During locomotion the spinal circuitry was modified and the reflexes reorganized. For example, the non-reciprocal inhibition from extensor group I afferents was suppressed by the MLR descending drive and instead disynaptic excitation of homonymous motoneurons by group I afferents emerged (see Figure 1), as has been described in decerebrate cats (Gossard et al. 1994; McCrea et al. 1995b). The model also incorporated presynaptic inhibition of group Ia afferent inputs during locomotion. Stimulating afferent activity with these modeled connections was in agreement with previously published data (Guertin et al. 1995). For example, stimulating the extensor group I afferents during the flexion phase terminated this phase and initiated extension but a few cycles later the rhythmic activity occurred at its appropriate place, as if no stimulation had occurred (see (Guertin et al. 1995) for corresponding physiological data). Due to the stronger synaptic strength afforded to the PF compared to the RG the modeled afferent connections can reset PF activity without influencing the basic locomotor rhythm produced by the RG. If extensor group I afferents were stimulated during the extension phase at intensity where only

the PF was acted upon this enhanced and prolonged the activity of extensor motoneurons. The following flexion phase was delayed and shortened to maintain the timing of rhythmic activity since RG was unaffected. However, if the stimulation was strong enough to influence RG the extension phase was enhanced and prolonged and a reset in the locomotor rhythm occurred. Cutaneous afferents from the tibial nerve were modeled with disynaptic excitatory connections to RG and PF with equal strengths. Thus, stimulating cutaneous afferents during modeled fictive locomotion always reset the rhythm to a new state since the RG was always influenced. The model also incorporated competing influences from group I and II afferents but will not be described here.

Although at present time the model only includes activity from one pair of antagonist motor pools it does show that modeled neuronal properties and connections can generate a basic locomotor rhythm of alternating flexor and extensor activity and that sensory input can greatly influence CPG output. The coming years should shed more light as to the general organization of the CPG, the neuronal properties of its constituent elements and how sensorimotor information interactions occur.

Human locomotion

Motion of the entire body during human walking has often been simplified as two coupled pendula with the stance leg acting like an inverted pendulum moving about the stance foot and the swinging limb behaving as a regular pendulum moving at the hip (Taga 1995a; Kuo et al. 2005). Using this analogy several models of varying complexity have been formulated to illustrate some aspects of the kinetic and kinematic patterns of human walking but relatively few attempts to combine neural control mechanisms such as CPGs and/or sensory feedback from the moving limbs have been made (Kuo et al. 2005; Pandy 2001; Pandy 2003; Zajac et al. 2003b; Zajac et al. 2002; Zajac 2002). This undoubtedly stems from the fact that, contrary to cat locomotion, many more assumptions with regards to neurophysiological mechanisms

must be made and little is known as to the sensorimotor interactions that take place during bipedal human walking.

Despite these limitations and potential pitfalls a few models have been devised using CPGs capable of integrating sensory input (Taga 1995a; Ogihara and Yamazaki 2001; Paul et al. 2005; McFadyen et al. 1994). In a series of papers Taga (Taga 1995a; Taga et al. 1991; Taga 1995b; Taga 1998) investigated the interaction of CPGs with the musculo-skeletal system to produce human locomotion and tested the model under various conditions. The model comprised seven neural oscillators, each controlling a single joint, which were regulated by tonic descending input. Each neural oscillator consisted of two paired tonically active neurons with recurrent and reciprocal inhibitory connections. This half-center model (Brown 1914) whereby a flexor neuron inhibits its contralateral flexor homologue and its ipsilateral extensor antagonist, and vice-versa, generated the basic step cycle. The timing of muscle activation by the neural oscillators was determined by specific events within the step cycle and by inhibitory and/or excitatory connections. The step cycle was divided into six separate states and sensory signals relating the current state of the biomechanical system were sent to the ensemble of neural oscillators (e.g. CPG). The "global angle", which is the orientation of the vector from the center of pressure to the center of gravity in an earth-fixed frame of reference, was computed and informed the CPG about the current state of the system, which then filtered this input according to the phase of the step cycle, thus allowing unwanted information to be ignored in phases where it could produce instabilities (e.g. phase-dependent modulation). Moreover, the model adapted well to different conditions including mechanical perturbations, loading, and terrain alterations (Taga 1995b). However, although the model generated stable walking under varying conditions, it did so within a limited range and without recalibrating the system (e.g. new steady-states were produced).

Simulations showed that the model was capable of sensorimotor interactions such as changing walking speed in response to modifying the tonic activity to each neural oscillator and via entrainment of the walking rhythm by imposing signals to hip oscillators. However, unlike the MLR in cats (Shik et al. 1966b), whereby

velocity increases linearly with stimulation intensity, walking speed as well as stride cycle and stride length 'jumped' considerably once tonic activation reached a certain level, despite no corresponding changes in neural activity, muscle torques, and segment displacements. Moreover, entrainment of the walking rhythm could be induced by imposing signals on the hip oscillators but only within a very narrow range above or below the normal rhythm. Thus although the model's CPG integrated sensory information to modify its output its modus operandi was somewhat limited. This model, however, provided an adequate framework for incorporating more complex and realistic neural control mechanisms. Indeed, in a follow up study, a discrete movement generator (DMG), which mimics descending commands from the motor cortex, was implemented to enable the model to avoid obstacles (Taga 1998). The DMG modulated gait by changing the timing and amplitude of specific muscles in response to 'visual' information and the current state of the CPG; a similar system is thought to operate in cats (Drew 1988; Drew et al. 1996).

As stated by the author this model generated stable walking with "characteristics of human gait" although there were some notable anomalies such as a high foot clearance during swing and large ground reaction forces at foot contact. A serious limitation of this approach was the lack of validation against published empirical data of human walking. Thus, we know relatively little as to the similitude of the model's kinetic and kinematic patterns compared to real human walking. Moreover, the model did not attempt to incorporate realistic muscle actuators or limb proprioceptors, which undoubtedly would enhance the overall functioning of the system. This is critical since a given motoneuronal input to a muscle will generate different torques at various joint angles and walking speeds due to the force-length and force-velocity relationships. However, the model did produce a stable bipedal gait and the addition of more components using principles derived from animals could prove useful in testing hypotheses of sensorimotor interactions that occur during human locomotion.

In a more recent study, a model of human locomotion incorporated a CPG with Ia, Ib, and cutaneous reflex pathways (Paul et al. 2005). Similar to Taga's

model (Taga 1995a) the CPG consisted of mutually inhibitory half-center models each controlling one of six joints (hip, knee, and ankle bilaterally), which were each actuated by a flexor and an extensor. Recurrent inhibition produced the rhythmic activity by delineating the amount of time each half-center remained active. The stretch reflex pathway was modeled as a primary muscle spindle with static and dynamic components, but without corresponding fusimotor drive. Reciprocal inhibition between flexor and extensor antagonists mediated by Ia inhibitory interneurons was also incorporated. The Ib pathway from GTOs was modeled using two separate Ib interneurons, which included an inhibitory and an excitatory connection to motoneurons. This was to highlight the shift from inhibition to excitation that occurs in the Ib pathway during locomotion (Gossard et al. 1994). The inhibitory pathway was active during locomotion only when force exceeded high values. The excitatory Ib interneuron also received input from pressure-sensitive cutaneous receptors from the sole of the foot thus exciting ipsilateral extensors during the stance phase of that. This neural circuitry actuated flexors and extensors at each joint, which were modeled as described previously (McMahon 1984; Hill 1938) accounting for force-velocity and force-length relationships.

The model could generate stable bipedal walking and was devised as a means to test the relative importance of various sensory pathways in the generation of gait. As such, the gain of the different neural components (e.g. CPG and reflex pathways) could be modulated to test their relative contribution during locomotion. It was shown that completely removing one of the sensory modalities caused the model to collapse during walking thus indicating that each is critical for normal locomotion. On the other hand, increasing the gain of some these modalities introduced abnormalities in gait. For example, augmenting the gain of muscle spindles caused the muscle to collapse during stance due to strong activation of stretched knee flexors during this phase whereas increasing the gain of cutaneous receptors deteriorated gait stability, even though speed could be maintained more easily. Increasing the gain of GTOs did not influence locomotion.

Therefore, although the accuracy of reflex pathways was questionable the model did suggest that sensory pathways are critical for the generation of locomotion. What is curiously omitted in this model is phase-dependency whereby some inputs are filtered out in specific phases where they would generate unwanted behaviors. Phase-dependent modulation of sensory input would have prevented the model from collapsing during stance due to excessive activation of knee flexors by canceling out this action during this phase. Moreover, contrary to Taga's model, the pure feed-forward nature of this system prevents sensory feedback from entraining or modulating the locomotor rhythm because reflex pathways did not have direct connections with the CPG, which is not the case in biological systems. As we saw earlier phase transitions critically depend on sensory feedback from limb proprioceptors and this model does not include these important connections.

To summarize, models of human locomotion currently available, although they necessitate some refinement, provide a viable means for testing hypotheses such as the role of sensory feedback in generating a stable bipedal gait. Incorporating accurate sensorimotor interactions from other mammals such as the cat into simulations of human locomotion will undoubtedly ameliorate the robustness and appropriateness of these models. Furthermore, data derived experimentally and tested via computer simulations should elucidate and confirm the multitude of sensorimotor interactions occurring during bipedal human locomotion.

Conclusions

The general aim of this review was to suggest that for the benefit of experimental and theoretical neuroscientists it would be important to take into consideration as much as possible many of the available biological information to increase the realism of the models. As is the case for network simulations, which incorporate several of the membrane properties of individual cells, locomotor networks must incorporate sensory feedback as well as governing rules (i.e. state- and phase-dependent modulation) as key operational elements. Again, this is not necessarily to make better walking robots but to design biologically relevant models

that will help clarify the theoretical framework for our understanding of locomotor mechanisms.

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Figures

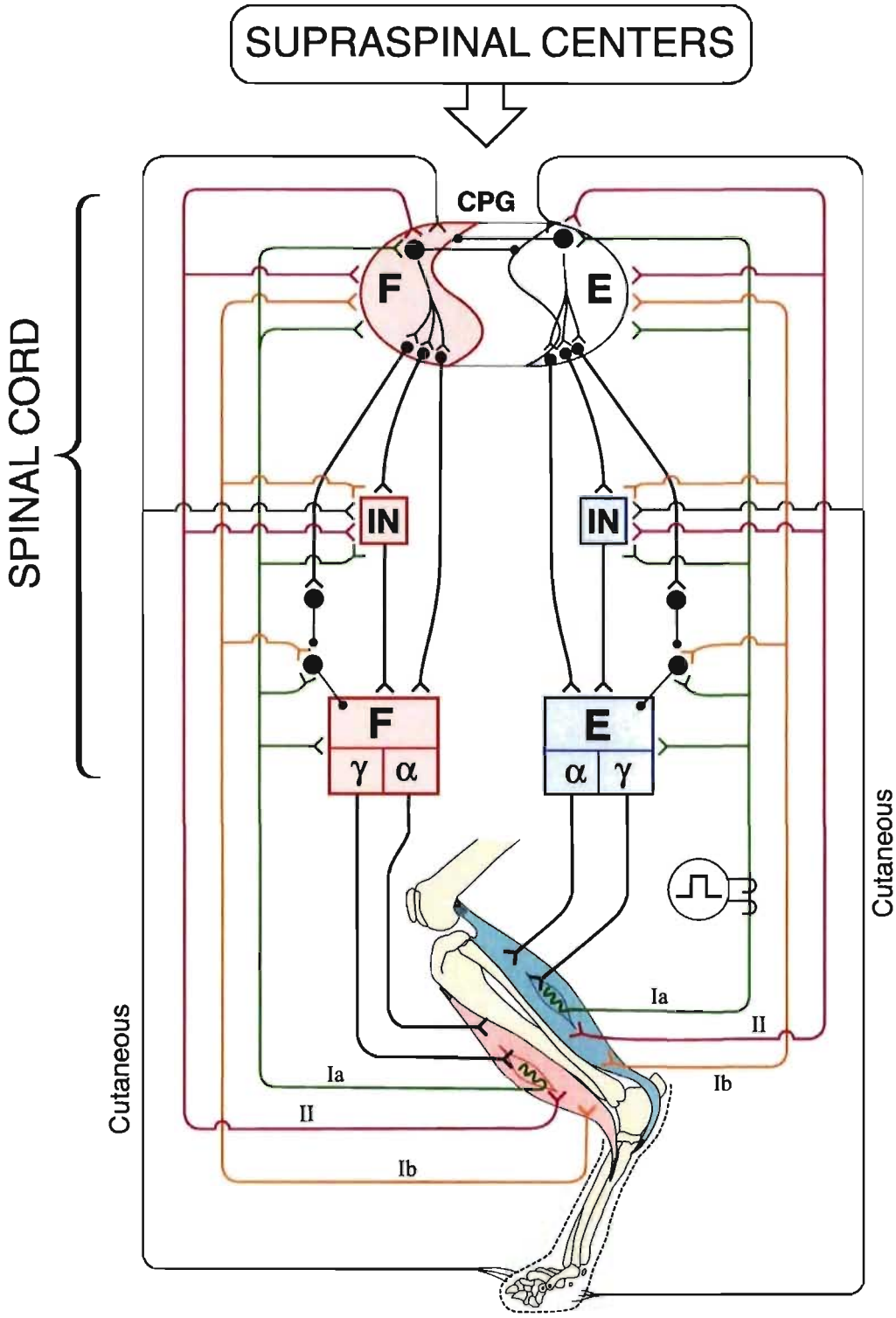
SUPRASPINAL CENTERS
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Figure 1 – General scheme of sensory pathways from the cat hindlimb on spinal locomotor control.

This scheme is subdivided into three parts. The supraspinal level includes various descending pathways from the telencephalon and brain stem involved in activating, stopping, or modulating characteristics of the spinal locomotor CPG as well as the excitability of transmission in reflex pathways at motoneuronal or pre-motoroneuronal (presynaptic and/or interneuronal) levels. The large arrow from the upraspinal level includes all these functions and is not discussed further.

The spinal cord level includes the CPG illustrated as reciprocally inhibited flexor (F) and extensor (E) networks. These two antagonist phases of the CPG are separated to indicate that each part may exert a function on other spinal mechanisms (represented by 3 output neurons emerging from each part of the CPG) as well as interact between each other (inhibitory connections between F and E). Interneurons (IN) for F (pink) and E (blue) receive input from several CPG and spinal afferents and project to F and E motoneurons, respectively. Other more specific inhibitory interneurons (in black) represent disynaptic inhibitory pathways (such as Ib inhibitory interneurons), which can also be inhibited by other interneurons in certain tasks such as locomotion. Motoneuron pools include both α- and γ-motoneurons projecting to extrafusal and intrafusal muscle fibers, respectively.

In the periphery, an ankle flexor (pink) and extensor (blue) muscle are shown each with a muscle spindle. Group Ia and II axons respectively represent sensory fibers from primary and secondary muscle spindles and signal rate and amount of muscle stretch. The stimulation symbol on the Ia fiber from the extensor illustrates direct stimulation of Ia afferents as performed during H-reflex studies. Ib fibers originated from GTOs, which measure the force output of the muscle. Cutaneous afferents from the paw are included and relay information relating touch, pressure, and vibration. Connectivity of the various afferents is partial and largely based on established work and is reviewed extensively elsewhere (Rossignol et al. 2006).

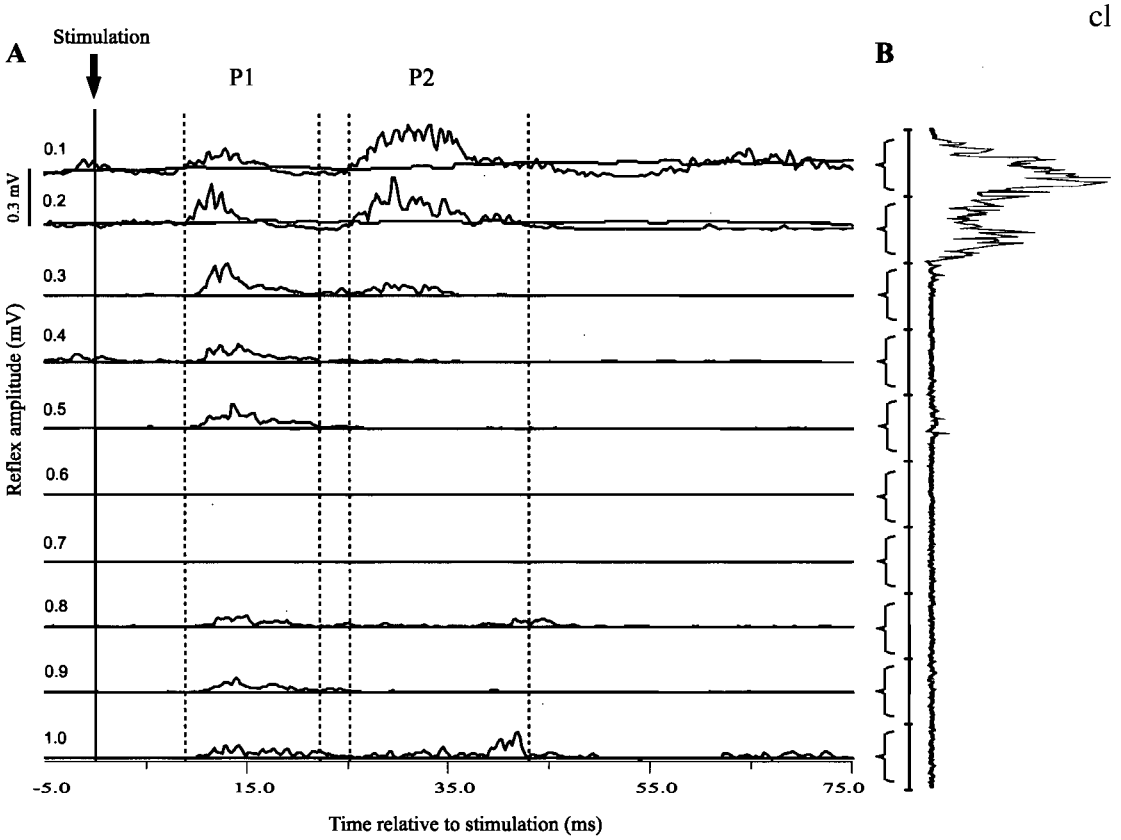

Figure 2 – Phase-dependent modulation of cutaneous reflexes during locomotion.

A. Stimulation of the tibial nerve innervating the plantar surface of the foot and reflexes recorded in the semitendinosus (St) during different phases of the step cycle. During a recording session about 120 stimulations are given at varying latencies in relation to the onset of activity of a given muscle to evoke responses during different phases of the cycle. The locomotor cycle is then divided into 10 phases and approximately 10 responses are grouped and averaged in each phase according to the time they were evoked in the step cycle. In St we see that both P1 (~ 10 ms) and P2 (~ 25 ms) are modulated throughout the step cycle varying in magnitude. Responses are typically higher when the muscle is active (i.e. first two traces) but can also be evoked despite quiescence in the muscle (i.e. bottom three traces). The activity of St during the step cycle is given in B.

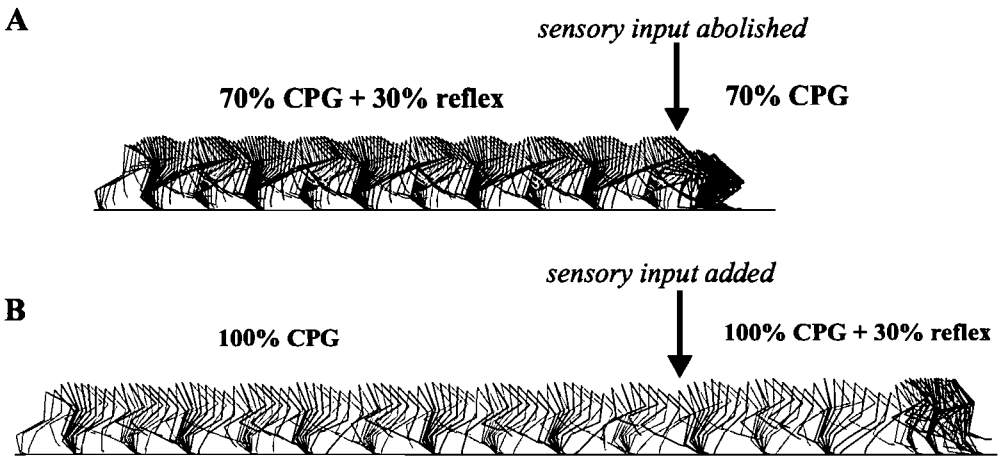

Figure 3 – Contribution of stretch reflexes to simulated hindlimb locomotion in the cat.

Simulation of hindlimb walking with muscle activity being generated by combined inputs of 70% CPG and 30% group I reflexes (A) or solely by the CPG (B). A) If motor output during walking is generated by a combination of CPG and sensory input to motoneurons than the abolishment of sensory input causes the model to immediately collapse. B) If however, the motor output is being supplied entirely by the CPG than the addition of sensory input does not cause the muscle to collapse but some anomalies in locomotion do occur a few step cycles later (reprinted and modified with permission).