CONTRASTING EFFECTS OF FLEXOR GROUP II AFFERENTS DURING FICTIVE LOCOMOTION

by

KATINKA STECINA

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
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MASTER OF SCIENCE

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ABSTRACT

1. This study examined the reflex actions of hindlimb flexor muscle nerves on fictive locomotion produced by stimulating the midbrain locomotor region in decerebrate, paralysed, adult cats. Electrical stimulation (typically 100- 200 ms trains of 10-20 shocks) of flexor nerves was delivered at particular times during the step cycle and the effects on motoneuron activity recorded in electroneurograms from selected hindlimb nerves were examined.

2. The effects of flexor nerve stimulation depended on the nerve being stimulated. Thus stimulation of the *tibialis anterior* (TA) nerve at 5 times threshold (5T) terminated ongoing flexor activity and reset the locomotor rhythm to extension. In the same preparation, similar stimulation of the *extensor digitorum longus* (EDL) nerve prolonged the flexion phase and enhanced the activity of hip knee and ankle flexor motoneurons. These contrasting actions were evoked consistently in different preparations. Stimulation of other flexor nerves, such as *iliopsoas* (Psoas), *sartorius* (Sart) and *peroneous longus* (PerL) at 5T strength evoked a prolongation and/or enhancement of the ongoing flexor activity that was similar to the effects of EDL stimulation. The results suggest that some flexor afferents can evoke flexion enhancement during fictive locomotion by modifying the activity of the spinal central generator circuitry.

3. Enhancement of ongoing flexor motoneuron activity by EDL nerve stimulation was not seen with less than 2T stimulation intensity. This suggests that recruitment of group I afferents alone is insufficient to perturb the step cycle. The clear effects on the step cycle as stimulus intensity was raised to 5T suggests that recruitment of group II afferents is

crucial to evoking flexion enhancement. However, the possibility that group I afferents may have subthreshold actions in these preparations requires further investigation.

4. When stimulation was delivered during extension, the effects of TA and EDL nerve stimulation were weak and variable. TA stimulation was seen to enhance extension, initiate a new extensor burst or reset the rhythm to flexion in different preparations. EDL stimulation could reset the step cycle to flexion or initiate a new flexor burst but never prolonged the ongoing extensor phase.

5. There was also some variability in the effects of 5T TA and EDL stimulation delivered during flexion. In a few step cycles, EDL evoked a resetting to extension and TA evoked a prolongation of the flexor phase. The averaged effects, however, were a resetting to extension from TA and an enhancement of flexion from EDL stimulation. Despite the rarity of these variations in the effects on the step cycle, they indicate that there are multiple spinal reflex pathways that can be recruited by flexor group II afferents during fictive locomotion.

6. The opposite effects of TA and EDL group II stimulation suggest that the reflex effects of flexor group II afferents differentiate into (at least) two classes during fictive locomotion; one promoting extension and another promoting flexion. Evidence that the enhancement of flexion is evoked by locomotor-dependent reflex pathways and not the flexion reflex system operating at rest is discussed.

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...all you are am I...

Thank you Dave for your genuine wisdom of life.

Sincerely yours,

P.S. Life is not over yet...

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LIST OF ABBREVIATIONS

CPG - central pattern generator
EDL - extensor digitorum longus
EFA - enhancement of flexor amplitude
ENG - electroneurogram
EPSP - excitatory postsynaptic potential
FRA - flexor reflex afferent
FDL - flexor digitorum longus
FHL - flexor hallucis longus
GS - gastrocnemius + soleus
LGS - lateral gastrocnemius + soleus
MG - medial gastrocnemius
MLR - mesencephalic locomotor region
MTP - metatarsophalangeal
PbSt - posterior biceps + semitendinosus
PerL - peroneous longus
PIP - proximal interphalangeal joint
Psoas - iliopsoas (iliacus + psoas major)
dPsoas - distal iliopsoas
pPsoas - proximal iliopsoas
Q - quadriceps
RF - rectus femoris
Sart - sartorius
lSart - lateral sartorius
mSart - medial sartorius
SmAB - semimembranosus + anterior biceps
SP - superficial peroneal
St - semitendinosus
T - threshold
TA - tibialis anterior

INTRODUCTION

I. Generation of locomotor behaviour

In the early 1900s, Sherrington described locomotor activity in animals with severed connections from the brain to the limbs and named it as 'reflex stepping' (Sherrington 1910). He suggested that locomotion resulted from the alternation of flexion and extension reflexes in the limbs. A contemporary colleague of Sherrington, Graham Brown, explained the generation of locomotor activity differently. Based on his observations that alternating contractions of pairs of flexor and extensor muscles were successfully evoked in acute spinal animals with cut dorsal roots and lacking rhythmic afferent input, Brown suggested that locomotion was not a result of reflexes evoked by afferent input but rather it was generated centrally by the spinal cord (reviewed in Gossard and Hultborn 1991). Brown proposed the spinal half-centre hypothesis to explain the neural circuitry generating locomotor-like motor behaviour. Flexor and extensor motoneurone pools were thought to be driven by a corresponding group of spinal interneurons referred to as 'half-centres'. Both half-centres were thought to receive common excitatory drive and to mutually inhibit each other to produce alternating activity of flexor and extensor muscles (reviewed in Gossard and Hultborn 1991). Since the time of Sherrington and Brown, the innate ability of the spinal cord to generate locomotor-like motor activity without sensory input was demonstrated in cats (Grillner and Zangger 1974), monkeys (Taub 1976), neonatal rats (Kudo and Yamada 1987) and mature mice (Jiang et al. 1999). Although Sherrington suggested that sensory input has a role in the generation of locomotion, most research has focused on finding the location

and understanding the operation of the CPG. A recent monograph by Orlovsky, Deliagina and Grillner (1999) summarizes our present knowledge about the organization and operation of the locomotor system in different species. In the 321 pages of this monograph there is virtually no mention of how sensory information affects the CPG. As will be discussed, it is now clear that much of the motoneurone activity that occurs during locomotion in the intact animal is the result of afferent feedback from muscle receptors. The emerging view is that while the CPG produces the basic pattern of flexor and extensor movements, this activity is reinforced and shaped on a step-by-step basis by proprioceptive feedback. It is only in the last decade or so that we gained insight into the profound and complex functional role of peripheral muscle sensory information in controlling the CPG during locomotor behaviour.

II. Muscle afferents

Muscle sensory information is conveyed by afferent fibres arising from receptors located in the muscles. Muscle afferent fibres were characterized according to conduction velocity by Lloyd (1943) into the following groups (Sheperd 1994): group Ia, group Ib, group II, III and IV. Group Ia afferents arise from primary spindle organs located in the intrafusal muscle fibres. These nerve fibres are large diameter, myelinated fibres with a conduction velocity of 70-120 m/sec in the cat. They carry information about both static and dynamic changes of muscle length (eg. Prochazka, Westerman and Ziccone 1977). Group Ib fibres are also large diameter, myelinated fibres with conduction velocities ranging from 65-115 m/sec. In many muscle nerves they have a slightly higher threshold for electrical activation than Ia afferents. They arise from the Golgi tendon organs located

in series with the tendons of the muscles. They convey information mainly about the force exerted by the muscle and are activated by even modest muscle contractions (eg. Prochazka and Wand 1980). Group II afferents arise mainly from secondary endings on the muscle spindles. They convey information on the static length of the muscle and have little dynamic sensitivity (Jack 1978). They consist of smaller diameter, myelinated fibres with conduction velocity ranging from 35-70 m/sec. Some of the muscle afferents in the group II range also originate from Paciniform corpuscles, joint receptors, muscular free nerve endings and extramuscular mechanoreceptors (Boyd & Davey 1968, Stacey 1969, Jack 1978). Group III fibres are relatively fast (5-30 m/sec), myelinated fibres and group IV fibres are unmyelinated, slow (0.5-2 m/sec) nociceptive fibres. Group III fibres carry temperature, crude touch and pricking pain sensation mostly from cutaneous receptors. Group IV fibres convey pain, itch, temperature and crude touch sensation also mainly from cutaneous origin.

During voluntary movements, primary spindle afferents fire at a rate that is proportional to muscle velocity and in slower motions to muscle stretch (Prochazka and Gorassini 1998b). In a recent study by Prochazka and Gorassini (1998a) it was shown that neither spindle primary nor secondary endings were completely silenced at any point in the step cycle. This study also showed that tendon organs in the cat *triceps surae* muscle nerve gave predictions of whole-muscle force during locomotion and became silent only when the muscle was inactive. Thus during motor activity both muscle length and tension receptors send proprioceptive input to the spinal cord. The reflex actions evoked by these receptors during locomotion are the subject of this thesis.

III. Reflexes evoked in resting preparations

In the absence of locomotor behaviour activation of group Ia afferent fibres arising from primary muscle spindles evokes excitation in homonymous and synergist muscles via mostly monosynaptic pathways in anaesthetized cat (Lloyd 1943, Eccles at al. 1957b). Group Ia fibres are considered as a positive force generating feedback system to synergist and homonymous muscles whereby muscle stretch results in reflex contraction to restore length. In addition, Ia afferents also evoke inhibition in antagonist (heteronymous) muscles via a disynaptic spinal reflex (see Jankowska and Roberts 1972) that has been termed 'reciprocal inhibition'. Group Ib afferents primarily evoke inhibition in motoneurones of synergistic and homonymous muscles (Laporte & Lloyd 1952, Eccles et al. 1957b) that has been often referred to as 'non-reciprocal' inhibition (reviewed in Jami 1992). Therefore, afferent fibres from the force sensitive Golgi tendon organs are considered to be a part of a negative feedback system in the absence of locomotor behaviour.

Since there appeared to be a positive (Ia) and a negative (Ib) feedback system for coordinating muscle activity by afferent input, it was surprising when experiments revealed that similar reflexes could be evoked by both Ia and Ib afferents. The similar action of Ia and Ib fibres resulted in the use of the term "group I-evoked" reflexes to describe common reflex actions. Group I reflexes (i.e. evoked by both Ia and Ib fibres) have been described for inhibition evoked in homonymous and close synergist motoneurones (Fetz et al. 1979) and for inhibition and excitation of heterogeneous motoneurones (Jankowska et al. 1981a, b). These common (Ia and Ib) reflex actions are

the results of convergence of spindle and tendon organ afferents onto common interneurons (reviewed in Jankowska 1992). The non-reciprocal reflex system serves as a good example of multi sensory convergence where reflexes can be evoked by activation of a variety of afferent sources (Jami 1992). It is also a system in which feedback control of the limbs is based on proprioceptive information collected from the whole limb.

Group II and III muscle afferents along with joint and cutaneous afferents are often referred to as flexor reflex afferents (FRA) (see references in McCrea 1992). This collective name, FRA, reflects the common reflexes evoked by these different types of sensory fibres in certain preparations. Thus in low-spinal, anaesthetized cats, activation of any of the FRA results in a flexor reflex pattern with a short latency (mostly disynaptic) excitation in flexor and (mostly trisynaptic) inhibition in extensor motoneurones of the ipsilateral limb (Eccles and Lundberg 1959). The functional uniformity of the FRA system is most likely due to the widespread convergence from several different afferent groups onto common sets of spinal interneurons (McCrea 1992). Experimental results support the convergence of cutaneous, joint and high threshold (group III) muscle afferents on common interneurons (reviewed in Baldissera et al. 1981). As part of the FRA system, electrical stimulation of group II fibres evokes polysynaptic reflex actions resulting in the contraction of ipsilateral flexor and contralateral extensor muscles in low spinal cats (reviewed in Baldissera et al. 1981 and McCrea et al. 1992). Both excitation and inhibition are evoked in some motoneurones following FRA stimulation. This suggests the presence of parallel spinal pathways with interposed interneurons that can receive input from multiple sites (for references see McCrea 1992). Thus muscle

activation evoked by FRA can only be predicted through understanding the control of interneurons mediating FRA actions. However, only a few interneurons with monosynaptic group II afferents were found to be part of such convergent FRA pathways (Lundberg 1987a, Edgley & Jankowska 1987b). Since many group II interneurons examined by Lundberg and colleagues (1987a) seemed to have rather specific input from selected group II afferents, it was hypothesized that excitatory group II interneurons might be used by the higher motor centres in the brain to mediate and control motor commands (Lundberg et al. 1987b). This hypothesis stresses the "fractionalization" of the FRA and specifically the group II input during real movements. This idea is strongly supported by experimental evidence presented in this thesis.

Cutaneous reflexes are perhaps the most complicated and least understood reflexes. Their studies began with the flexor reflex evoked from skin stimulation but later it became evident that cutaneous fibres can elicit other reflex responses as well (reviewed in Baldissera et al. 1981 and McCrea 1992). For example, light pressure on the plantar surface of the paw evokes the extensor trust instead of the flexor reflex. Cutaneous reflexes often produce a detailed pattern of excitation and inhibition that is particularly well described in motoneurones of foot and ankle muscles (LaBella et al. 1989, Leahy and Durkovic 1991).

IV. Reflexes evoked during locomotion

IV-1 Group Ia reflex modulation

Most of the reflexes described in non-locomoting preparations are modulated during locomotion. Evidence for modulation of the group Ia monosynaptic reflex during

locomotion comes from studies in decerebrate cats (Bennett et al. 1996) and humans (Capaday and Stein 1987, Yang and Whelan 1993). There is a reduction in the reflex amplitude underlined by the reduction of the monosynaptic excitatory post synaptic potentials (EPSPs) evoked in homonymous motoneurones during locomotion possibly due to presynaptic inhibition of Ia afferent terminals (Gosgnach et al. 2000). This is an example of changing reflex gain during locomotion.

IV-2 General effects of afferent input

Sensory input has been shown to influence locomotor behaviour by affecting the rhythm generator (Duysens and Pearson 1976, Grillner and Rossignol 1978). Theoretically there are several ways in which afferents could alter the step cycle. The first is by changing the relative duration of the flexor and extensor burst without altering the cycle period. The second is by changing the amplitude of flexor or extensor activity without altering the duration. The third is to force an adjustment of the stepping cycle. This is entrainment whereby periodic afferent input results in a period of a matching efferent activity. The fourth is the temporary adjustment of the cycle period (eg. a shortening of the ongoing phase and a premature initiation of subsequent phasic activity). This is resetting. In theory, afferent input could evoke more than one of the above actions.

IV-3 Extension enhancement from extensor group I afferents

Activation of extensor afferents signaling loading of weight-bearing muscles during flexion has been shown to reset the locomotor rhythm. Extensor afferent stimulation resets to extension (i.e. terminates ongoing flexion and initiates extension) during fictive locomotion in decerebrate (Guertin et al. 1995) and in spinal, DOPA-

treated (Conway et al. 1987) cats. Extensor afferents are also able to entrain the locomotor rhythm in different preparations (Conway et al. 1987, Pearson et al. 1992). This suggests that extensor group I afferents can control the bursting frequency of the pattern generating circuitry.

The stimulation of extensor afferents during extension results in extension enhancement that is defined as the increased amplitude and/or prolongation of the ongoing extensor activity. First it was suggested that only group Ib afferents evoked the enhancement of extensor activity (Conway et al. 1987) but later it became evident that group Ia activation was also contributing to the step cycle modulation (Guertin et al. 1995). Extensor afferents evoke extensor enhancement during treadmill locomotion in conscious and decerebrate animals (Whelan and Pearson 1997) as well as during fictive locomotion induced by DOPA in spinal cats (Gossard et al.1994) and induced by MLR-stimulation (Guertin et al. 1995). The effects of extensor group I fibres during locomotion are in contrast with their effects evoked at rest (Gossard et al.1994). The non-reciprocal group I inhibition described in non-locomoting preparations is replaced by disynaptic excitation in extensor muscles across all hindlimb joints during fictive locomotion (McCrea et al. 1995, Angel et al. 1996).

The "extension enhancement" evoked by extensor group I afferents seems to be a consistent and invariable effect independent of the preparation. The proposed functional significance of these extensor afferent actions is the increase of support in response to increased load and prolongation of stance phase as long as the limb is loaded (Duysens and Pearson 1980). In a recent study Hiebert et al. (1999) have shown that in cats 30-70%

of ankle extensor activity is generated by sensory feedback during treadmill locomotion. Group I afferents from extensor muscles seem to have similar functions in humans (Stephens and Yang 1996; 1999, Sinkjaer et al. 2000). The powerful actions of sensory information from extensor afferents are being used to assist the restoration of weight-bearing (i.e. extension enhancement) and stepping following spinal cord injury in humans (Harkema et al. 1997).

IV-4 Flexor group I afferents

Can flexor afferents influence the pattern generator like extensor afferents do? The answer to this question is not known because there is less information available on the reflexes evoked by group I flexor afferents than those evoked by extensor group I fibres during locomotion. Stimulation of flexor group I and group II afferents has been examined in decerebrate cats during treadmill (Hiebert et al. 1996) and fictive locomotion (Perreault et al.1995). Group I afferents from an ankle flexor muscle, *extensor digitorum longus* (EDL), were found to be effective in resetting the locomotor rhythm to flexion when stimulated during the extension phase (Hiebert et al. 1996). When stimulated during flexion, EDL group I afferents prolonged the flexor phase in decerebrate cats walking on a treadmill (Hiebert et al. 1996). In the fictive locomotor preparations the electrical stimulation of group I afferents from a hip flexor, *sartorius* (Sart), increased flexor activity in other muscle nerves but only during the flexion phase (Perreault et al. 1995).

IV-5 Flexor reflex afferents (FRA)

As mentioned earlier, FRA fibre stimulation evokes the flexion reflex under

resting conditions in low-spinal anaesthetised cats (Eccles and Lundberg 1959). The flexion reflex consists of ipsilateral flexion and crossed extension as first described by Sherrington (1910). FRA reflexes are preparation dependent (Eccles & Lundberg 1959) and they may be reorganized during locomotion (for references see McCrea 1992). Jankowska et al. (1967) described long-latency, long lasting discharges in ipsilateral flexor and contralateral extensor motoneurones following FRA stimulation in unanesthetized, DOPA-treated, spinal cats. Combined stimulation of ipsi- and contralateral FRA resulted in the activation of flexor and extensor motoneurones in a reciprocal manner similarly to locomotor activity suggesting that FRA reflexes are involved in the generation of locomotion. Several experimental observation supported the hypothesis, that FRA play an active role in stepping (see references in Lundberg 1979). From a functional point of view it is understandable that evoking a flexion reflex during locomotion may not be desired and the FRA reflex pathways have to be gated during movements (Lundberg 1987b, Perreault et al. 1999).

Stimulation of the FRA in spinal cats during DOPA-induced fictive locomotion was examined in different phases of locomotion (Schomburg et al. 1998). In this study electrical stimulation of mixed flexor muscle nerves, joint and cutaneous nerves reset the rhythm to flexion when stimulated during extension and prolonged flexion when stimulated during the flexor phase. These actions were claimed to have a flexor reflex pattern and therefore attributed to FRA reflexes. The problems with this study arising from the mixed nature of the tested muscle afferents are discussed later (see Discussion).

Extensor group II afferents did not increase the effects evoked by group I fibres

when the stimulus strength was increased to 5 times threshold. The powerful actions of extensor group I fibre activation make it difficult to assess additional contributions from extensor group II afferents (Guertin at al. 1995). Stimulation of flexor group II afferents in the EDL nerve (Hiebert et al. 1996) terminated ongoing extension and reset to flexion in decerebrate cats during treadmill locomotion. Contrary to this, the electrical activation of group II afferents in other hip and ankle flexors during flexion reported by Perreault et al. (1995) reset the step cycle to extension in decerebrate cats during fictive locomotion. Based on these findings it seemed that the effects of flexor group II afferents were preparation-dependent. However, there is reported evidence showing that group II fibres of EDL (a TA synergist flexor) evoke the prolongation of the ongoing flexor phase when stimulated during flexion in fictive locomoting cats (McCrea 1998). The comparison of reflex actions evoked by group II afferents from TA and EDL is particularly intriguing. Both of these muscles are ankle flexors located side-by-side along the tibia. As will be discussed, their activities during locomotion are similar but EDL can display variations.

IV-6 Cutaneous reflexes

Discussing in detail the modulation of cutaneous reflexes during locomotion is beyond the scope of this study (see Rossignol 1996 for review). However, one cutaneous reflex during locomotion will be described here. Stimulation of the *superficial peroneal* (SP) nerve (which innervates the dorsum of the paw) evokes a reflex called the "stumbling corrective response" during locomotion in intact and in spinal cats (Forssberg 1979) as well as in humans (Zehr et al. 1997). In the cat, this reaction involves the following pattern of muscle activation: a brief increase and then cessation of ongoing

ankle flexion, initiation of ankle but not hip or knee extension and then initiation of knee flexor activity (Forssberg 1979). This pattern of hindlimb activity allows the animal to avoid tripping and to step over the obstacle that was stimulating the dorsal paw surface. Recently, it has been shown by Quevedo, McCrea, Stecina and Gosgnach (in preparation) that the stumbling corrective response is hard-wired in the spinal cord and it can be evoked by SP stimulation alone in the absence of rhythmic peripheral sensory input during fictive locomotion in decerebrate cats.

V. Relevant anatomy

The present investigation will focus on the reflexes evoked by TA, EDL and other flexor afferents in decerebrate cats during fictive locomotion. The knowledge about the anatomy of the flexor muscles in which afferent actions were tested is necessary for understanding the results in functional terms. All the anatomical descriptions were taken from Text-Atlas of Cat Anatomy by J. Crouch (1969). Figure 1A illustrates the anatomical organization of TA and EDL muscles. Both of these muscles are often considered as ankle flexors, although, there are clear differences in their function. EDL originates from the femur and inserts through 4 tendons on the base of the distal phalanx. TA originates from the shaft of the tibia, fibula and from the intervening interosseous ligament and inserts into the lateral surface of the first metatarsal. Thus TA mainly acts across one joint (ankle) while EDL acts across several joints (knee, ankle and phalanges). EDL has a minor while TA has a major contribution to adduction (Nichols 1994). In real locomotion, EDL activity starts after TA (Trank et al. 1996, Abraham & Loeb 1985) and this pattern holds also during fictive locomotion (McCrea et al. 1998). EDL activity

usually continues during extension while TA is active only during the swing phase. The change in EDL muscle length during locomotion is smaller than that of TA (Goslow et al. 1977). In the transition from stance to swing, EDL muscle length increases as ankle angle increases and as the metatarsophalangeal joint (MTP) and proximal interphalangeal joint (PIP) decreases (Kuhtz-Buschbeck et al. 1994). TA also lengthens as the ankle angle increases but it is less affected by the changes of the MTP and PIP angles than EDL.

The hip flexor and rotator *iliopsoas* muscle has a complex origin since it consists of two muscles (*iliacus* and *psoas major*). The *psoas* portion has 10 heads: 5 arising from the cranial tendons of the *psoas minor* muscle, the sixth from the transverse process of the fifth lumbar vertebrae and four from the centra of the last four lumbar vertebrae. The *iliacus* portion originates from the ventral portion of the ilium. All parts of the muscle insert into the apex of the lesser trochanter of the femur through a common tendon. Nerve branches innervating the *iliopsoas* muscle are usually found in two groups: a more distal (dPsoas) and a more proximal (pPsoas) group (Aggelopoulos et al. 1996). The latter (pPsoas) consists of two to four short (5-10 mm) branches emerging from the 4^{th} and 5^{th} lumbar spinal nerves before merging with the L6 spinal root. These branches terminate in the *iliopsoas* (Psoas major) muscle and because of their location they are more difficult to dissect than the branches of the distal group. There are also two to four nerve branches found in dPsoas emerging from the femoral nerve trunk just distal to the site of separation from the obturator and from the connecting branch of L6/L7 spinal nerves. This site is about 30 mm proximal to where the femoral nerve emerges from the inguinal ligament (Aggelopoulos et al. 1996). The distal group of the nerves innervating the *iliopsoas*

muscle (dPsoas) was successfully stimulated and recorded from in three experiments.

The hip flexor, sartorius (Sart) muscle consist of two portions, the lateral (lSart) and the medial (mSart) (Sherrington 1910, Eccles & Lundberg 1958). The lateral branch is inserted on the patella and the smaller, medial part is inserted on the tibia. In the cat these two muscle bands are conjoined and innervated by separate nerves as shown by the illustration in Fig 1B. Sometimes, the medial portion receives its innervation not directly from the femoral but from the saphenous nerve. Due to the different insertion sites, lSart acts as a hip flexor and a knee extensor muscle while mSart flexes both hip and knee. According to Sherrington (1910), the knee flexion in the medial strip is stronger than the knee-extensor function of the lateral band. The activity of lSart was found to span both flexion and extension phases while mSart was active only during the flexion phase in cats during real locomotion (Hoffer et al. 1987, Pratt & Loeb 1991, summarized in Gordon et al. 1991).

VI. Objectives

The main goal of this study was to gain more information about the effects of flexor afferents on the spinal rhythm generator circuitry. It was necessary to extend our observations not only on the effects of TA and EDL but also on hip flexor nerve stimulation as well. Another goal was to determine whether flexion enhancement could be evoked by flexor afferents during MLR-induced fictive locomotion similarly to extension enhancement evoked by extensor afferents. The results confirm the contrasting effects of TA and EDL group II afferents during flexion as reported previously. Electrical stimulation of TA resets the cycle to extension terminating ongoing flexion while EDL

prolongs and/or enhances ongoing flexion (only at group II and not at group I strength). Additionally, this study shows that not only EDL but other hip and ankle flexors can evoke flexion enhancement during MLR-evoked fictive locomotion. Another important finding in this study was the occasional variability of the effects evoked by flexor nerve stimulation on a step-by-step basis.

METHODS

Preparation

Data presented in this paper were collected from 16 cats (2- 4 kg), eight of which were also used in the study of disynaptic group I EPSPs in hindlimb motoneurones (Quevedo et. al. 2000) and of the stumbling corrective response (Quevedo et al. in preparation).

The animals were anaesthetized with a halothane (1-2%), nitrous oxide (70%) and oxygen (30%) mixture for surgery. Blood pressure was monitored from the carotid artery and to administer drugs and fluids, two veins were cannulated. Atropine (0.05mg/kg s.c.) and dexamethasone (2mg/kg i.v.) were given at the beginning of the surgery and a 5% glucose and bicarbonate solution was delivered intravenously throughout the experiment at a rate of 5ml/hr. Supplemental saline and dextran infusions were given as required to maintain blood pressure. Tracheotomy was performed for the use of artificial ventilation later on the course of the experiment. The level of anaesthesia was assessed by monitoring arterial blood pressure, the lack of withdrawal reflexes and muscle tone.

Peripheral muscle nerves were dissected and cut in preparation for electrical stimulation and monitoring locomotion. The dissected nerves in the left leg included the *semimembranosus and anterior biceps* (taken together as SmAB), *posterior biceps* taken together with *semitendinosus* (PbSt), *lateral gastrocnemius-soleus* (LGS), *medial gastrocnemius* (MG) or sometimes LGS and MG taken together as the *gastrocnemius-soleus* (GS), *tibialis anterior* (TA), *peroneous longus* (PerL), *extensor digitorium longus* (EDL), *flexor digitorium longus* (FDL) and *flexor hallucis longus* (FHL). The ventrally

located *sartorius* (Sart), *rectus femoris* (RF) (sometimes together with the *vasti* as Q, *quadriceps*) nerves were placed in a triple cuff electrode for stimulation and recording. The lateral (lSart) and the medial (mSart) portions of Sart were mounted separately in one experiment. The distal branch of the nerves innervating the *iliopsoas* (*Psoas major*) muscle (dPsoas) was put in a single bipolar cuff electrode for stimulation and recording in three experiments. In some cases the AB or SmAB nerves in the right hindlimb were dissected in order to monitor contralateral ENG activity although it is not reported on in this thesis. Other femoral, sciatic and obturator nerve branches, as well as tendons around the hip, were cut bilaterally.

The animals were moved to a rigid frame after a laminectomy (L4-L7). Following craniotomy, with a mechanical precollicular-postmammillary decerebration of both hemispheres and all tissue rostral to the transection were removed. The anaesthetic was discontinued and the cat was paralysed with gallamine triethiodide (Flaxedil, 2-3 mg $kg^{-1}h^{-1}$) and artificially ventilated. Radiant heat was used to warm the animal and the mineral oil pools covering the exposed spinal cord and the peripheral nerves. A lethal injection of barbiturate anaesthetic was administered at the end of the experiment. All surgical and experimental protocols were in compliance with the guidelines set out by the Canadian Council for Animal Care and the University of Manitoba.

Stimulation, Recording and Data Analysis

The mesencephalic locomotor region (MLR) 1-2mm posterior to the border between the inferior and superior colliculi, 4 mm lateral to the midbrain and 3-6 mm below the surface of the colicculi (Shik et al. 1966) was stimulated by using a monopolar

tungsten electrode (varnish-insulated, exposed tip diameter 80-100 μm) and continuously applying rectangular pulses (0.5 ms duration) at15-30 Hz. Unilateral (either left or right side) MLR stimulation was often sufficient to evoke locomotion but sometimes bilateral stimulation was required. The location of the stimulating electrodes and the stimulus parameters were optimized for each experiment. The alternating rhythmic activity of flexor and extensor ENGs was used as the criteria for fictive locomotion. Data were usually collected on control and stimulated steps over a 120 s long bout of locomotor activity.

Dissected nerves (except for the ventral nerves placed in cuff electrodes) were mounted on bipolar silver/silver-chloride hook electrodes suspended in the mineral oil pool. Although in Fig 2.A we illustrate an example of non-rectified, raw ENG recordings (5000-50000 gain amplification) taken at a 2000 Hz sampling rate, most of the collected data were filtered (3dB, high pass 30 Hz, low pass 3 kHz), rectified and integrated (envelope follower with a time constant of 100 ms) and digitized at a 500 Hz sampling rate as shown in Figure 2B.

A monopolar electrode was placed on the dorsal surface of the spinal cord (cord dorsum electrode) to record incoming afferent valleys after peripheral nerve stimulation. Threshold current (T) was defined as the smallest current producing a detectable extracellular compound action potential volley at the cord dorsum recording electrode. The onset of rectified-integrated activity in selected flexor or extensor nerves was used to trigger the onset of a stimulus train to peripheral nerves. Cycle phase-triggered electrical stimulation of peripheral nerves consisted of 10-35 shocks at 200 or 300 Hz. Marker

pulses generated during data collection indicated either the control steps without stimulation or the shocks to peripheral nerves. These pulses were used for subsequent averaging of ENG data. Data capture and analysis was performed using software developed within the Winnipeg Spinal Cord Research Centre (a Pentium PC running QNX for data capture and QNX or Linux for analysis).

The ENG of control and stimulated steps were averaged with a time window usually spanning 500 ms before and 700 ms after the onset of the stimulus. Changes in ENG amplitude were evaluated after overlaying the averages from the control and the stimulated steps. Cycle period was defined as the time interval between the onset of consecutive bursts of activity in selected nerves. The values for each cycle period during an experimental run were calculated from the stored data after visually marking the cycle onset and termination of the selected ENG with cursors. The values for the control and the stimulated steps were separately averaged and compared with a t-test: Two Samples Assuming Equal Variances (α=0.05) using Microsoft Excel 97 SR-1l. Each tested nerve was counted effective if there was at least one run during an experiment in which the stimulation of the peripheral nerve evoked a significant change of the cycle period in the selected ENG. All means are reported with ● standard deviation.

RESULTS

The effects of electrical stimulation of flexor muscle nerve afferents were investigated in sixteen decerebrate, paralysed cats during MLR-evoked fictive locomotion. The majority of results were obtained using 15-35 pulse (usually 200Hz) stimulus trains delivered during the flexion phase of fictive locomotion and using 5T stimulus intensity. The main finding was that this stimulation resulted in two distinct and contrasting actions on the step cycle. As will be shown, stimulation of some flexor nerves terminated the ongoing flexion phase and initiated extension while other nerves prolonged the flexion phase and delayed the onset of subsequent extensor activity.

Differences in the activity patterns of flexor muscles during fictive locomotion

Figure 2, illustrates the electrical recordings (ENGs) of selected peripheral nerves from a 120 s long bout of fictive locomotion evoked by continuous stimulation of the MLR (15-30 Hz). Panel 2A, displays 2 s of activity in 7 selected nerves. These recordings are non-rectified and non-integrated ENGs, unlike the records shown in panel 2B and 2C that were rectified and integrated before being stored on a computer. The data presented in panels 2A and 2B are from the same experiment collected no more than 15 minutes apart. During this experiment we compared the onset of ENG activity using the raw and the integrated records. There was no apparent difference in the onset of the bursting (waveform) activity recorded with or without integration but occasionally the apparent termination of the bursting activity in the integrated data was somewhat prolonged compared to the non-integrated waveforms. Since the integration (or envelope follower) involves the discharge of a capacitor, the time to discharge will depend on the amount of

ENG activity. The integration of the data has little effects on the estimates of the onset of peripheral nerve activity. Thus measurements of cycle period (onset to termination) would not be compromised by the integration.

As reported earlier (McCrea et al. 1998), the activities of TA and EDL are not identical during fictive locomotion. The two vertical dashed lines in panels 2A and 2B mark a flexor burst in TA. Note that the onset and termination of EDL activity occurs later than that of TA. The same tendency is also obvious in the integrated data shown in panel 2B. The active period of TA and EDL nerves can be compared best on the averaged data shown in panel 2C. The averaged data shown in panel 2C are from the same trial as panel B. There were 31 steps averaged in each of the 11 nerves illustrated during a 20 s bout of fictive locomotion. The averaged ENG activity is displayed in 2 consecutive cycles with the vertical lines marking the beginning of flexion and extension. The onset of flexion is lined up with the onset of the TA ENG because the step cycle averaging was based on TA activity. The average duration of the step cycle was 609 ms and extension composed only 25% of the step cycle. Note that the activity of EDL starts later than TA and it does not terminate until well into the extension phase.

Based on the anatomy of the two muscles innervated by Sart branches (see Introduction), a difference in their activity during fictive locomotion is expected. Panel C of figure 2 shows that the activity of lSart spans both flexion and extension phases. The activity in lSart starts during mid-flexion and last into late-flexion. Then it is briefly inhibited before it peaks again in late stance and early swing. The activity in mSart begins slightly before ankle flexor activity and it terminates as hip extensor activity is initiated.

The pattern of activation of the two Sart branches during fictive locomotion as described in this study is identical to their activity during normal walking as reported previously (Hoffer et al.1987, Pratt & Loeb 1991, summarized in Gordon et al. 1991).

TA and EDL group II afferents evoke opposite effects on the locomotor cycle

This study was primarily designed to extend earlier work from our laboratory on the effects of flexor nerve stimulation during locomotion (Perreault et al. 1995). In that study it was shown that stimulation of the nerves to TA , PbSt and Sart muscles at group II strength during flexion resulted in termination of the ongoing flexor phase and initiation of extensor activity (i.e. reset to extension). Fig 3A shows these effects evoked by TA stimulation. Panel A shows 3 s (taken from a 120 s long data set) of rhythmic alternating activity in rectified, integrated ENGs from four selected ipsilateral hindlimb nerves during MLR-evoked fictive locomotion. There were 86 fictive step-cycles during 40 s (from the 120s run) in which the Sart nerve activity (a hip flexor) was used to trigger stimuli (33 shocks at 200 Hz) to either the TA or EDL nerves about 100 ms after the onset of Sart ENG activity. Vertical lines indicate the duration of the stimulus train. TA or EDL stimulation was delivered every third step cycle giving 14 and 15 stimulus presentations to these nerves respectively.

Stimulation of the TA nerve shortened the duration of flexor motoneurone activity (Sart) and subsequently initiated premature extensor activity (SmAB and GS nerves). Compare the control cycle duration (○) with the cycle duration following TA stimulation (■). The latency from the onset of TA stimulation to the onset of extensor activity in the SmAB nerve is 136 ms. The initiation of activity in GS appears to be also on the order of

100 ms but could not be precisely determined because of stimulus artifact. Figure 3 shows, as reported previously (Perreault et al. 1995), that 5T electrical stimulation of the TA nerve during flexion resets the fictive locomotor rhythm to extension.

The EDL nerve was stimulated alternately with the TA nerve in the same trial. Fig. 3A illustrates that the effects of EDL nerve stimulation are quite different from those of TA stimulation. EDL stimulation resulted in a prolongation of flexor activity (Sart and PerL nerves) and a delay in the onset of extensor nerve activity (▲). Note the increase in the step cycle duration produced by EDL stimulation and the decrease followed by TA stimulation. Thus 5T EDL stimulation during flexion evoked effects on the step cycle essentially opposite to those produced by TA stimulation. The large increase in the amplitude of PerL ENG at the onset and only during the stimulus train is likely a result of monosynaptic excitation of PerL motoneurones from EDL but not from TA afferents (Eccles *et al.* 1957a).

Figure 3B shows the effects of TA and EDL stimulation on the timing of the locomotor step cycle for all 86 steps during the run partly shown in panel 3A. Cycle period, measured as the interval between the onset of consecutive bursts of activity in the SmAB nerve, is plotted with open circles indicating steps without peripheral nerve stimulation (control, see Fig. 3A). The average control cycle period (solid line) was 441 ±16 ms (n=57). The standard deviation is shown in panel 3B by the dashed lines. Cycle periods for steps in which the TA nerve was stimulated are represented by squares (n=14) and periods with EDL stimulation by triangles (n=15). As shown, each trial of TA stimulation shortened the SmAB cycle period; i.e. terminated flexion and initiated

extension. The average cycle period following TA stimulation was 400 ● 14ms and significantly smaller than control ($p<0.001$, t test). During the same run, the cycle period increased each time when the EDL nerve was stimulated (mean 568.5 ± 8 ms) and became significantly longer than control ($p<0.001$). Thus the opposite effects of TA and EDL are clearly reflected in the cycle period changes.

Figure 4 illustrates the results of TA and EDL stimulation in another experiment. It shows a 1200 ms period of averaged ENG activity recorded from 10 peripheral nerves in panels 4A and 4B and 7 nerves in panel 4C. Dashed lines in each panel represent the averaged ENGs during control steps with no peripheral nerve stimulation and the solid lines show the averaged ENGs during nerve stimulation. The averages in 4A and 4B were collected within 2 minutes of each other. In Fig. 4, the onset of activity in a flexor nerve (EDL in A, TA in B and Sart in C) was used to trigger the stimulation of TA and EDL nerves respectively in every fourth step. The onset and the period of peripheral nerve stimulation is marked by the vertical dashed lines. In the example shown in Fig. 4A, there are 44 steps in the control average and 6 in the average during TA nerve stimulation. In 4B averages consists of 53 control and 7 stimulated (EDL) steps; and in Fig. 4C 57 control and 14 stimulated step cycles.

As in Fig. 3, stimulation of the TA nerve during flexion (Fig 4A) advanced the onset of hip and ankle extensor ENG activity (Q, SmAB and GS) and terminated mSart, Psoas and EDL activity. There was a brief excitation with a short latency in lSart, PbSt and EDL (6.8, 12 and 5.1 ms latency respectively). The excitation in the extensors occurred with a latency of 36 ms in Q, 80 ms in FDL, 87 ms in FHL and 91 ms in GS.

The latencies reported here are defined as the time between the first shock in the stimulus train and the initiation of excitatory effects in individual ENGs. The stimulus train consisted of 15 shocks at 200 Hz, lasting for 70 ms during both TA and EDL stimulation (Fig. 4A and B).

There were 11 animals from the 16 total in which both TA and EDL nerve stimulation was tested simultaneously. In 8/11 the effects evoked by TA and EDL afferents were clearly different as illustrated in Fig 3 and 4. Note that in Fig 4B there was a significant prolongation of the ongoing flexor phase by EDL stimulation in the same experiment in which TA reset to extension. The ongoing flexor activity in lSart, mSart, Psoas and TA ENGs was enhanced and prolonged while the onset of the next extensor phase (see GS, Q and SmAB nerves) was delayed by about 100 ms. There was a brief inhibition of the hip flexors with a latency of 21 ms in mSart and 32 ms in Psoas followed by a longer latency (95 ms in both mSart and Psoas) excitation. As in Fig 3A, the short latency (5 ms) excitation in the TA nerve was most likely due to monosynaptic excitation from EDL (Eccles *et al.* 1957a) and it was followed by a longer latency (112 ms) excitation. In lSart, PbSt and FDL the excitation had a latency of 4, 11 and 18 ms respectively followed by a longer latency excitation in lSart (93 ms) and in FDL (91 ms). Note the prominent increase in flexor activity after the end of EDL nerve stimulation (see panel 4B) and the long latency excitation evoked in extensors after the termination of TA nerve stimulation (see panel 4A). Thus the predominant effects of either EDL or TA stimulation (5T) are not simply stimulus-locked reflexes.

In a different experiment illustrated in Fig 4C, the flexor enhancement evoked by

EDL was not accompanied by significant effects on the step cycle duration. There was an increase in the Sart and the TA activity with EDL stimulation but unlike in Fig 4B, there was no delay in the onset of the subsequent extensor bursts. The stimulus (25 shocks at 200 Hz) did not evoke short latency inhibition in flexors. The hip flexor Sart and the ankle flexor TA received only short latency excitation (2 and 5 ms respectively) followed by a longer latency (94 ms) facilitation. There was excitation also in St and FDL with a latency on the order of 25 ms. It is important to note the increased amplitude of the flexor ENGs as a result of EDL nerve stimulation. In this example, the sensory information from EDL group II afferents clearly influences the excitation of flexor motoneurones without having an effect on the locomotor "clock". As mentioned before, the short latency excitatory effects on EDL on TA may be due to monosynaptic connections (Eccles et al.1957a) but the longer latency effects and the excitation in other ankle and hip flexors must be mediated by interneurons excited by afferents in the EDL nerve. The changes in the activities of hip and ankle flexors present strong evidence that afferents in EDL and TA nerves contact elements of the central locomotor circuitry that in turn act to either prolong flexion or reset the locomotor cycle to extension.

Table 1 summarizes the effects of flexor nerve stimulation (5T) delivered during flexion. In 11/12 cats TA nerve stimulation significantly shortened the cycle period and reset the fictive locomotor cycle to extension at least in one run during the experiment. In one experiment it had no effect on the step cycle. Stimulation of the EDL nerve prolonged the flexor phase and enhanced flexor ENG activity in 9/13 experiments. In 4 experiments EDL stimulation did not have significant effects on the duration of flexor

activity (e.g. 4C). However, in 2 of these 4 experiments EDL stimulation enhanced the amplitude of other flexor ENGs.

Effects of flexor nerve stimulation during the extensor phase of locomotion

The effects of TA and EDL stimulation at 5T were also tested during the extension phase of fictive locomotion. TA and EDL stimulation had no significant effect on the cycle period in 3/6 and 5/7 animals respectively. Stimulation of TA reset to flexion in one experiment and in another it inhibited the ongoing extension but only slightly advanced the next flexor phase. Oddly, stimulation of TA appeared to evoke a new extensor burst without an intervening flexor burst in one experiment. This is illustrated in Fig 5. In this experiment alternating TA and EDL stimulation (33 shocks at 200 Hz) was triggered by the activity in the SmAB nerve every third step (see vertical lines). Following TA stimulation, the ongoing extension was terminated and a new extensor burst was evoked in hip and ankle extensors. Note the lack of a flexor burst in Sart, PB and St following the termination of extension. Ankle flexors were not recorded in this run. EDL stimulation during extension shortened the ongoing extensor phase and advanced subsequent flexor activity (Fig. 5) but not significantly ($p=0.25$). In two other experiments (not illustrated) stimulation of EDL during extension reset to flexion with significant modulation of the cycle periods. EDL 5T stimulation never seemed to prolong ongoing extension.

Effects of other flexor nerves

Along with TA and EDL, the effects evoked by other flexor nerves were also examined during both flexion and extension. Figure 6 illustrates the effects of Psoas, Sart

and PerLong nerve stimulation at 5 times threshold. In panel 6A, the cycle period of the EDL nerve is shown for 38 steps recorded during this run. Similarly to Fig 3B, points indicated by open circles are cycle periods for steps without peripheral nerve stimulation (mean = 779 ±51 ms, n=27). EDL cycle periods during steps in which either the Psoas (filled squares) or the Sart (filled triangles) nerve was stimulated always had longer duration than those in which neither nerve was stimulated. Following Psoas stimulation, the average cycle period was 959 ±31 ms (n=5) and after Sart stimulation it was 993 ±35 ms (n=6). As illustrated in this figure, the stimulation of both Psoas and Sart resulted in the significant lengthening of the EDL cycle period ($p<0.001$, t test). Overall, Psoas stimulation showed the same effects as illustrated here in 3/3 experiments while Sart prolonged flexion in 3/4 experiments and did not have significant effects in one case. Stimulation of Sart (5T) during flexion was shown to reset the fictive locomotor rhythm to extension in 5/8 experiments by Perreault et al. (1995) but no such effects were shown in these experiments. However, Sart stimulation at 2T was also described by Perreault et al (1995) and it was shown to prolong flexion similarly to the effects reported in this study. The differences encountered in the two studies regarding Sart effects are addressed in the Discussion.

Panel B of Fig 6 shows the effects of PerL nerve stimulation at 5 times threshold during flexion. The ENG activity in six peripheral nerves is shown during a 7 s period of fictive locomotion. Stimulation of PerL nerve (25 shocks at 200 Hz) was delivered every sixth step as shown by the vertical dashed lines. The stimulus resulted in the enhancement of ENG amplitude in flexor nerves. Note the increased burst in Sart, TA and EDL during

the stimulated steps (arrows in Fig 6B).There was a second peak of activity elicited in EDL after the stimulus train with a long latency (100 ms) excitation. PerL stimulation had no effect on the length of the flexor bursts nor did it delay the onset of the following extensor activity. However, in 3/4 experiments it resulted in the facilitation of other flexor ENGs as illustrated in Fig. 6B.

The effects of Psoas, Sart and PerL stimulation during extension were examined only in a few experiments. Psoas stimulation (5T) reset to flexion in 2/3 experiments, Sart prolonged extension in 1/2 experiment similar to the results reported in Perreault et al. (1995). Stimulation of PerL (5T) delayed the onset of the next flexor burst in 1/3 experiments and in two it had no effects.

Effects of 2T vs. 5T stimulation of EDL

The effects of EDL stimulation at various strengths are illustrated in Fig 7. As in Fig 4, the averages of control and stimulated steps are overlayed. The activity of a hip flexor (Sart), a hip extensor (SmAB), an ankle flexor (PerL) and an ankle extensor (LGS) is displayed. Panels A and B are from the same run while C and D are from different trials collected within a few minutes. The vertical lines represent the onset and the duration of the stimulus train (25 shocks at 200 Hz) delivered shortly after the onset of flexor activity in the PerL nerve. The number of control steps averaged is 27 in A and B, 28 in C and 37 in D while the number of averaged stimulated steps is 7, 6, 7 and 8 in A, B, C and D accordingly. Even the lowest tested stimulus (1.2 T) showed the amplitude facilitation of the PerL ENG (see panel A); most likely representing the monosynaptic excitation from EDL group I afferents (Eccles et al. 1957a). There was no effect on the

cycle duration at 1.2 or at 1.6T stimulation (Fig. 7A and B). At 2T there was a small prolongation of the PerL burst and a slight delay of the extensor activity in GS (Fig. 7C). At 5T the enhancement of ENG amplitude in PerL outlasted the duration of the stimulus train (see Fig. 7D). The amplitude enhancement of the hip flexor Sart was more prominent with 5T stimulation of EDL than with 2T and the delay of the subsequent extensor burst in LGS and SmAB was increased by the higher strength stimulation as well.

In the next figure (8B), stimulation of EDL at 2T clearly evoked a prolongation of flexion similar to that evoked by 5T stimulation. Significant prolongation of the flexor ENGs was evoked by 2T EDL stimuli in a few experiments (3/5). Our interpretation is that significant effects of EDL stimulation on the step cycle require contribution from group II muscle afferents. A similar conclusion was reached previously for the effects of TA nerve stimulation (Perreault et al. 1995). From the other flexor nerves tested at 2T, PerL stimulation in 0/2 experiments, Psoas stimulation in 1/2 and Sart stimulation in 2/2 cases evoked significant prolongation of ongoing flexor activity. These results suggest that in the fictive locomotor preparations, group I afferents from EDL, Psoas and Sart nerves may contribute to flexion enhancement when stimulated during the flexion phase. In the case of TA and EDL nerve stimulation there is a clear contribution from group II afferents to the effects evoked on the step cycle.

Variability of TA and EDL effects

From the data presented in figures 3, 4 and 6 it seems that the effects of flexor nerve stimulation at 5T differentiate into 2 classes when delivered during the flexor phase

of fictive locomotion: the resetting to extension evoked by TA, PbSt and Sart (Perreault et al. 1995) and the enhancement and/or prolongation of flexion evoked by EDL, Psoas, PerL and occasionally Sart nerves as reported in this study. The analyses presented so far reveal the "average" effect of nerve stimulation. However, there can be considerable variation in effects when each presentation is examined on a step-by-step basis. In 3/11 experiments the effects of TA and in 2/12 the effects of EDL nerve stimulation showed inconsistency. In figure 8 we illustrate some of our observations when TA and EDL stimulation during flexion did not produce the more characteristic effect on the step cycle. Panel 8A shows a 9.5 s period from a 2 min long data set when the stimulation of the TA nerve at 2 and 5 times threshold (25 shocks at 200 Hz) was alternated during the flexor phase of MLR-evoked fictive locomotion. This figure illustrates the ENG activity of a hip and an ankle extensor (SmAB and GS) along with two ankle flexors (EDL and PerL). The vertical dashed lines mark the onset and the termination of the peripheral nerve stimulation. After the first stimulus (TA 5T) there was a new burst elicited in the two extensors (see arrows) along with the shortening of the ongoing flexor bursts in both EDL and PerL. The following episode of TA 5T stimulation is shown by the third pair of vertical lines. This time there was no premature burst evoked in the extensors but the activity of the ongoing flexors was prolonged in EDL and PerL (arrows). In this trial, TA reset to extension 4 times and prolonged flexion 10 times. Despite having more steps in which TA stimulation prolonged flexion the average cycle period with TA stimulation was shorter by 40 ms but this was not significantly different from the control steps ($p>0.06$, t-test). In this experiment, other runs displayed consistent effects of TA nerve

stimulation (5T) to significantly shorten the cycle period. There were two other experiments in which TA 5T stimulation showed variable effects. In one of these experiments (not illustrated) stimulation of TA 5T during mid-flexion evoked resetting to extension as expected. Later in the same trail, the delivery time of the stimulus train was shifted so that TA was stimulated very early in the flexion phase. Stimulation at the beginning of the flexion phase did not evoke resetting to extension but instead prolonged the ongoing flexion. The experiment illustrated in Fig. 8A brought to our attention the fact that spontaneous changes in the effects of peripheral nerve stimulation can occur in fictive-locomotor preparations on a step-by-step basis.

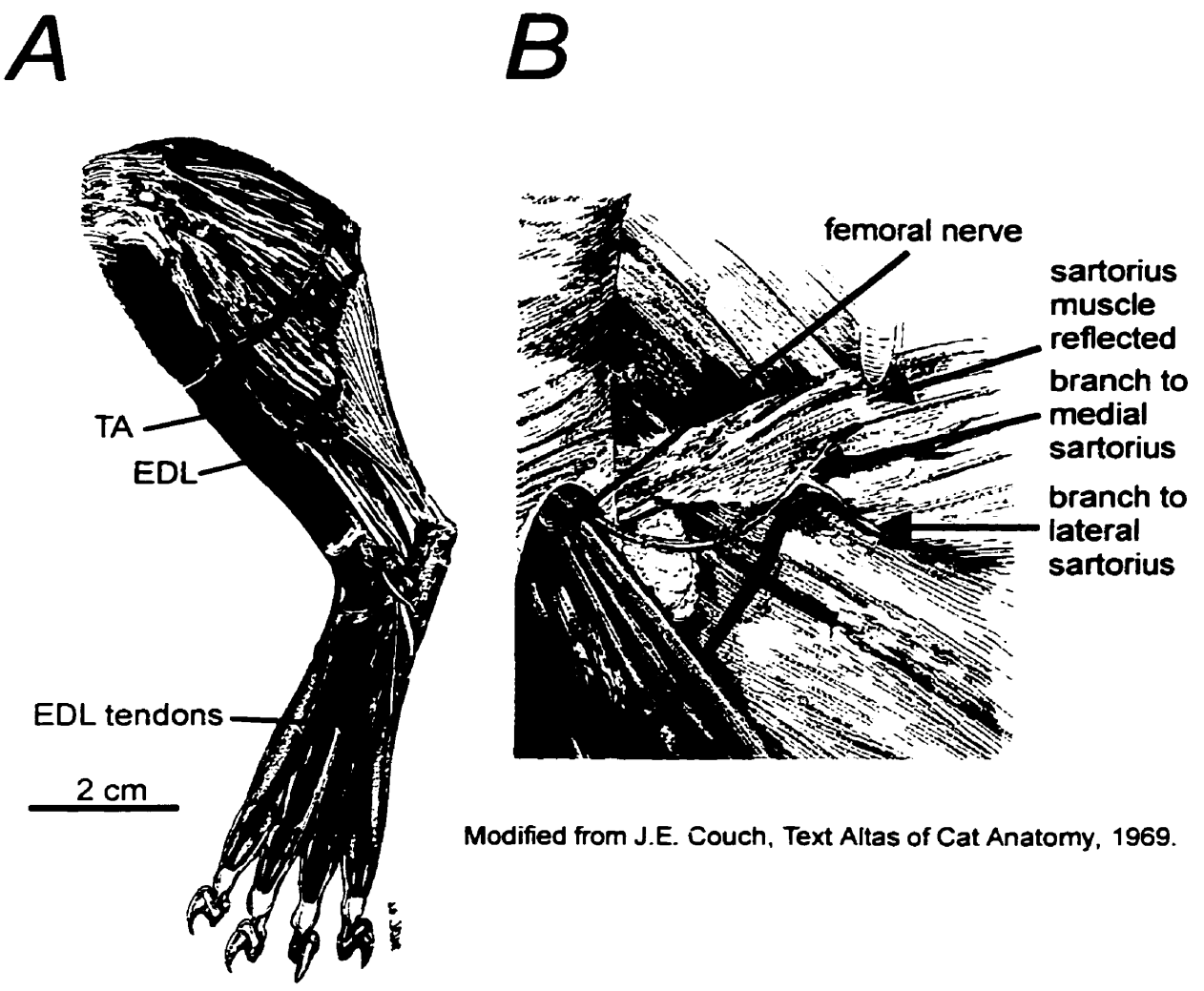
Figure 8B is a 10.5 s period from a 2 min run showing the ENG activity of a hip flexor (Psoas) and a hip extensor (SmAB). The vertical dashed lines indicate the onset of EDL nerve stimulation alternating at 2T and 5T (15 shocks at 200 Hz). These recordings are from the same experiment described in Fig 4B showing prolongation of flexion as the averaged effect of EDL stimulation. The segment used in Fig. 8, however, illustrates that the stimulation of EDL (5T) can evoke a resetting to extension on occasion. Note the arrow pointing to the 'premature' extensor burst in SmAB and the cessation of the ongoing flexor burst in Psoas after the first 5T stimulus. The following 5T stimulus train failed to evoke an extensor burst and it prolonged the ongoing flexor activity in Psoas as noted by the arrow. During this trial there were two occasions when EDL reset the rhythm to extension and the other eleven stimulated steps it prolonged the flexor phase. The averaged effect of EDL in this run was prolongation of the flexor phase by 32 ms but that was not a significant increase ($p=0.14$, t-test). In one other experiment EDL

stimulation resulted in a resetting to extension in 4 consecutive steps. Thus EDL stimulation also showed spontaneous variability in its effects on the step cycle. The results showing that both TA and EDL can evoke differential effects during fictive locomotion, further promote the idea about the existence of parallel group II pathways that can be selectively activated during locomotion (see Discussion).

Figure 1. Anatomy of cat flexor muscles (TA, EDL, Sart)

A. Origin and insertion of *tibialis anterior* (TA) and *extensor digitorion longus* (EDL) muscles. Based on the anatomy as illustrated, the actions of TA influence mainly the ankle joint while EDL exerts actions on the knee, ankle, metatarsophalangeal and proximal interphalangeal joints.

B. Division of *sartorious* (Sart) muscle to a lateral and a medial branch. The two branches are separately innervated by the lSart and the mSart nerves.



Modified from J.E. Couch, Text Altas of Cat Anatomy, 1969.

Figure 1. Anatomy of cat flexor muscles (left hindlimb)

Figure 2. Activity of peripheral nerves during fictive locomotion

A. Continious mesencephalic locomotor region (MLR) stimulation (10-30 Hz) was delivered to evoke fictive locomotion. Non-integrated electroneurogram (ENG) recordings over a 2 s period from flexor (lSart, Psoas, TA, EDL) and extensor (SmAB, GS, FHL) hindlimb nerves taken from a 120 s long bout of fictive locomotion. Locomotor activity is represented by alternating activity of flexor and extensor ENGs. Vertical dashed lines label the onset and the termination of activity recorded from the TA nerve. Note that bursting activity in the TA nerve does not overlap completely with EDL activity.

B. Integrated and rectified ENG recordings from flexor (mSart, lSart, Psoas, TA, EDL) and extensor (Q, SmAB, GS, FHL) nerves in the same experiment as in A but from a different trial. The vertical dashed lines mark activity in TA nerve. Note that there is also an apparent difference in the activity of TA and EDL in the integrated data.

C. Averaged integrated and rectified ENGs for flexor and extensor nerves during a 20 ms run from the same trial as panel B. There were 31 steps averaged and the average is displayed twice as 2 consecutive cycle. Averaging was based on the cycles in the TA ENG. The average duration of TA activity (flexion) was 609 ms. The average ratio of flexion and extension was 75% and 25% respectively. The vertical lines mark the onset of the flexor phase. The differences in the active periods of TA and EDL are also seen in these averaged results. Also note that mSart is only active during the flexion phase while lSart activity spans both phases.

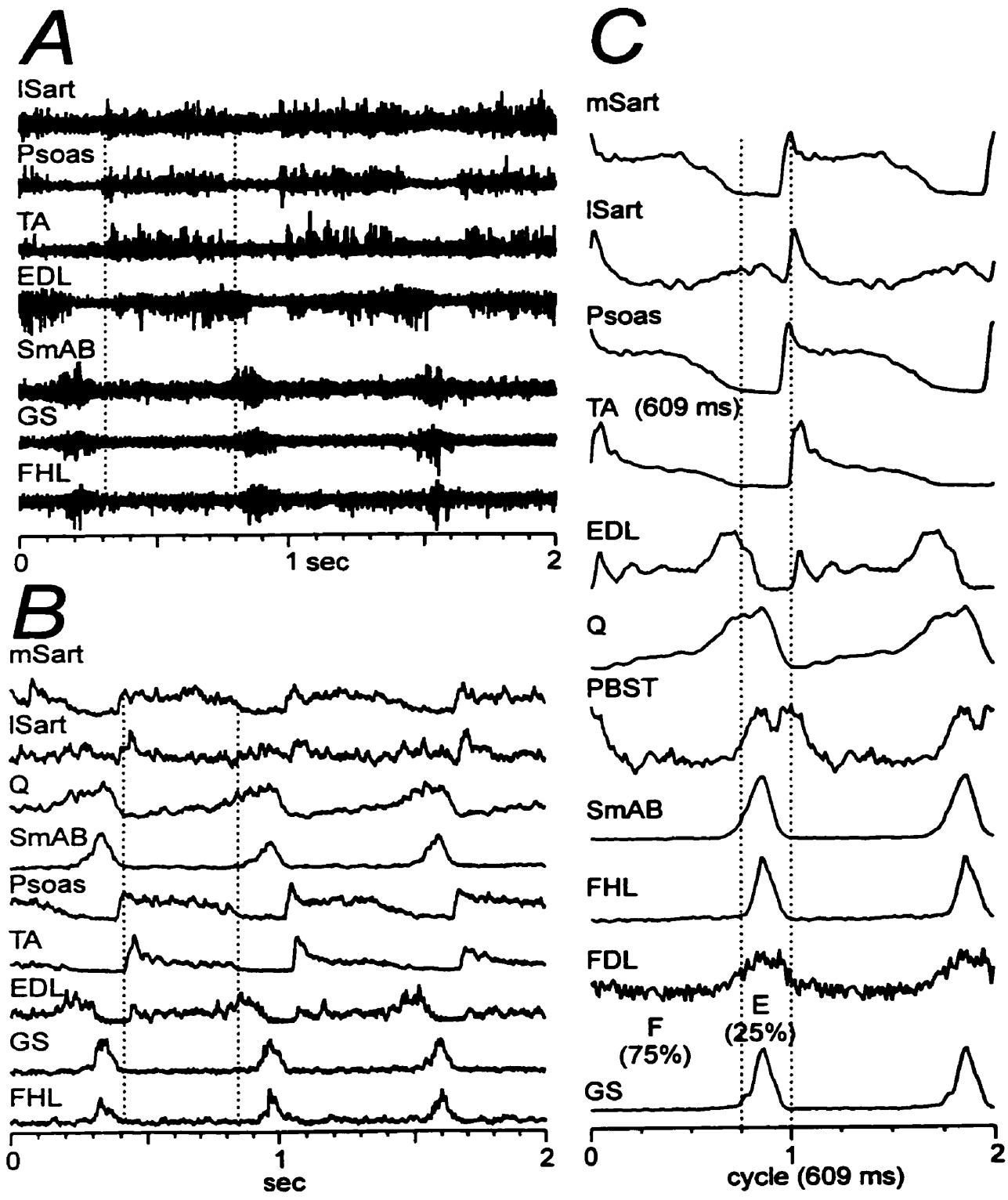


Figure 2. Activity of peripheral nerves during fictive locomotion.

Figure 3. Different effects evoked by electrical stimulation of TA and EDL nerves

A. Integrated and rectified ENGs recorded during MLR stimulation with alternating activity in two extensor (SmAB, GS) and flexor nerves (Sart, PerL) over a 3 s period from a 120 s bout of fictive locomotion. The dashed vertical lines mark the duration of the peripheral nerve stimulation (33 shocks at 200 Hz) at 5T strength alternately delivered to TA or EDL nerves every third step. The stimulus train was triggered during flexion from Sart activity. Stimulation of TA shortened the ongoing flexor phase in Sart and PerL and evoked a premature extensor burst in SmAB and GS (i.e. reset to extension). Stimulation of EDL enhanced the ENG amplitude in Sart and PerL as well as prolonged the flexor burst duration while delaying the onset of the following extensor burst in SmAB and GS (i.e. produced a flexion enhancement). The horizontal solid lines indicate the cycle period of the SmAB nerve during control steps with no stimulation to peripheral nerves (○), steps with TA stimulation (■), and steps with EDL stimulation (▲).

B. The cycle period of the SmAB nerve on the y-axis is shown in 86 recorded steps (x-axis) under three different conditions: control, TA and EDL stimulation. Data over a 40 s period were taken from the same trial as in panel A. Mean SmAB cycle period (441 ±16.4 ms)) during control conditions is shown by the solid line. The dashed lines indicate the standard deviation of the mean (n=57). SmAB cycle period was reduced in each step with TA stimulation (mean = 400 ±13.7 ms, n=14). EDL stimulation in each case increased the duration of the cycle period (mean = 569 ± 8.3 ms, n=15). The cycle period values during control were compared to the values during nerve stimulation using a paired t-test. The changes following peripheral stimulation were significant in both direction ($p>0.001$, t-test).

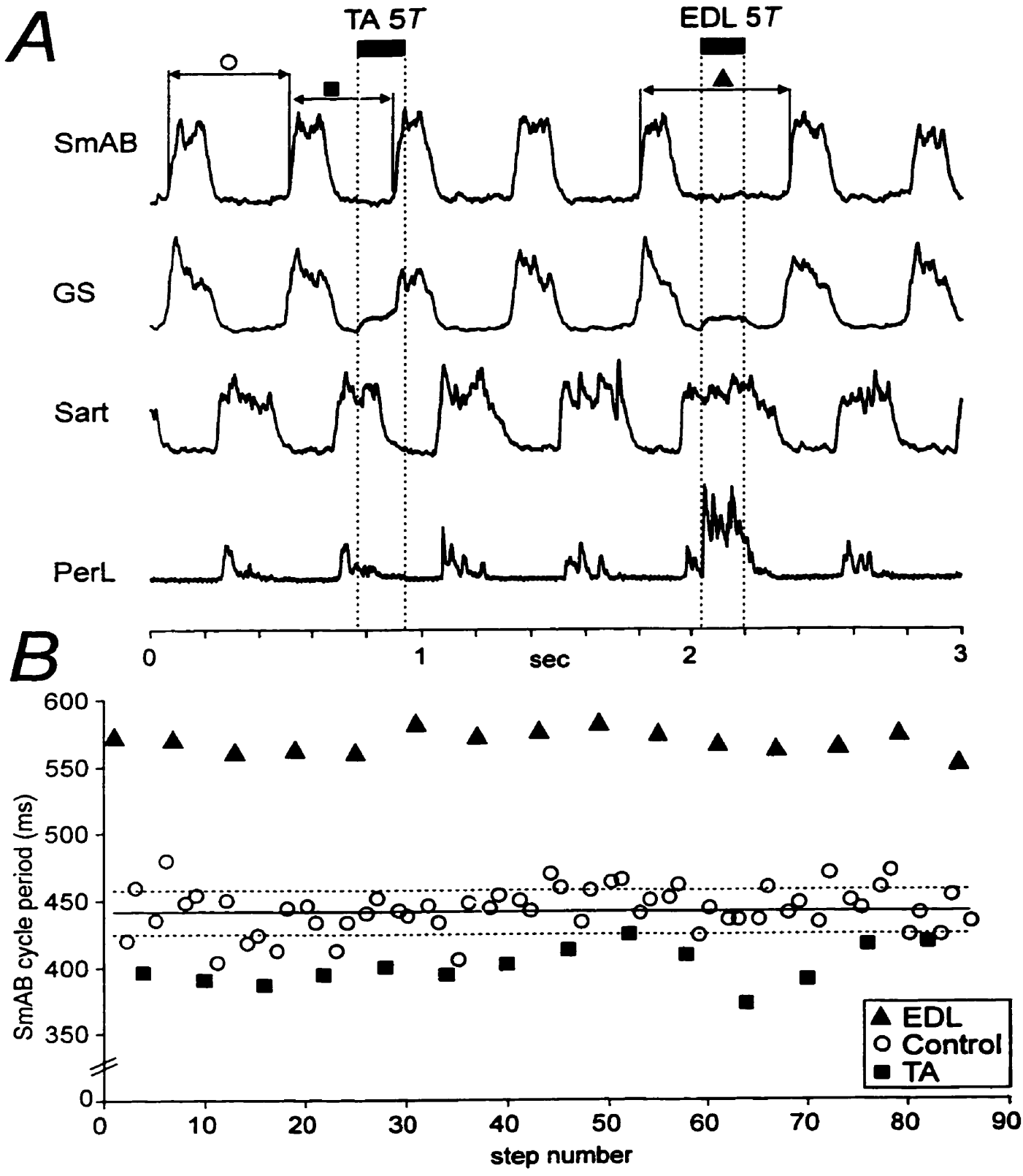


Figure 3. Different effects evoked by electrical stimulation of TA and EDL nerves.

Figure 4. Averaged effects of TA and EDL nerve stimulation showing contrasting actions

Averaged integrated and rectified ENG data collected during fictive locomotion are shown in each panel. The averages were constructed based on the stimulus markers indicating steps during control (no stimulation), TA and EDL stimulation. ENG amplitude during a period of 500 ms before and 700 ms after the stimulus onset was averaged in each nerve. The solid lines represent the ENG averages following peripheral nerve stimulation and the dashed lines show the averages from control steps. The onset and duration of the stimulus train is marked by the vertical dashed lines. Data in panel A and B are from the same experiment while C is from a different one.

A. Electrical stimulation of TA (15 shocks at 200 Hz) at 5T is triggered by EDL activity. TA nerve stimulation evoked inhibition in other flexors (mSart, Psoas, TA) and evoked a premature extensor burst (Q, SmAB, GS, FHL, FDL). The shortest latency excitation was evoked in Q (36 ms) and the longest in GS (91 ms). Latency was measured from the onset of the stimulus train to the point of increase in ENG activity.

B. Stimulation of EDL at 5T was triggered by activity in the TA nerve. EDL stimulation evoked an enhancement of the amplitude of activity in other flexors (lSart, mSart, Psoas, TA and FDL) and delayed the onset of subsequent extensor activity (Q, SmAB, GS, FHL). The prominent increase in the activity of other flexor nerves was evoked after the termination of the stimulus train.

C. Stimulation of EDL (25 shocks at 200 Hz) at 5T triggered by Sart activity in a different experiment resulted in the amplitude enhancement of other flexor ENGs without changing the duration of flexor activity or the onset of extensor discharges.

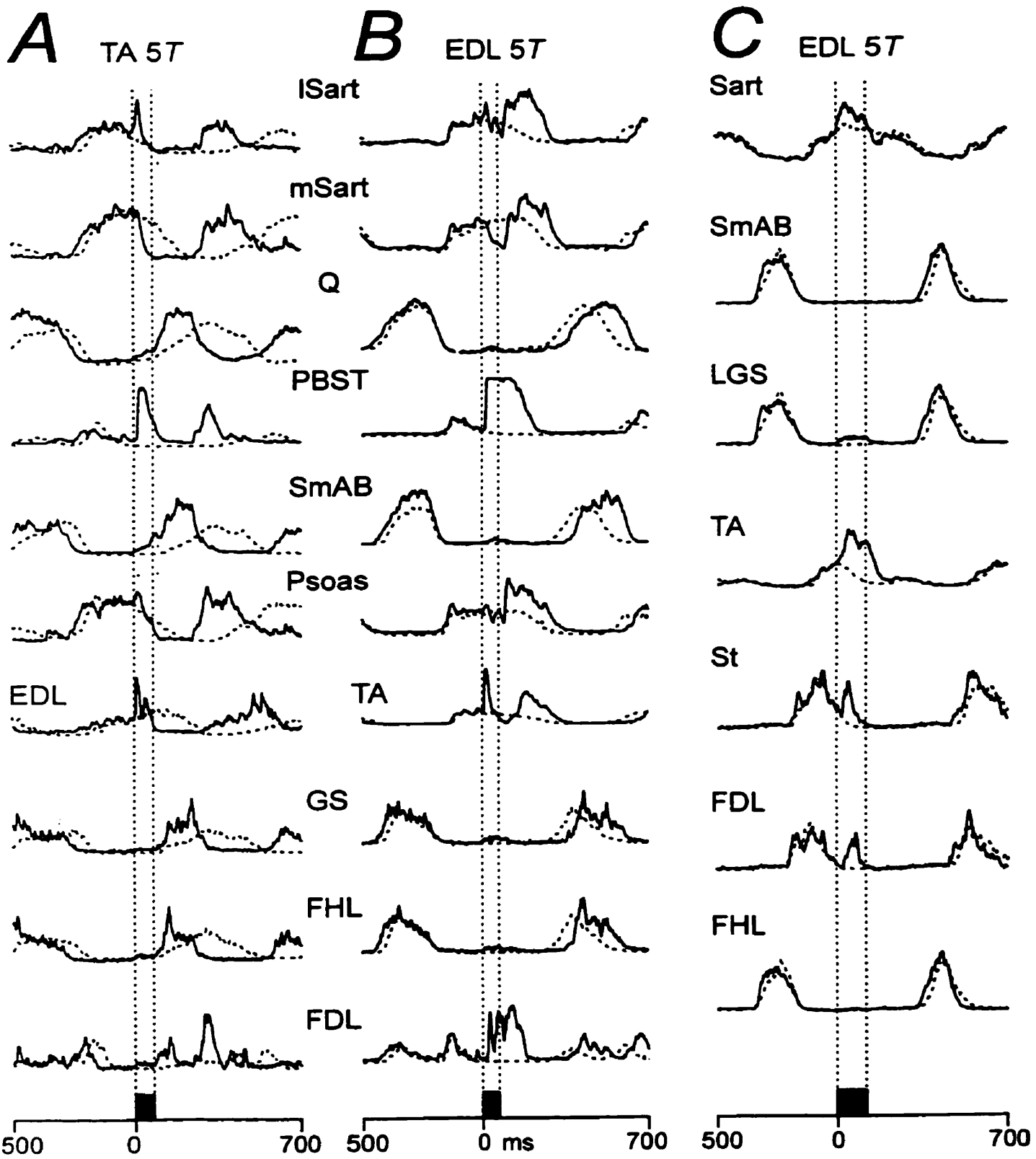


Figure 4. Averaged effects of TA and EDL stimulation showing contrasting actions.

Figure 5. TA and EDL effects during extension

Rectified and integrated ENGs over an 11 s period from flexor (lSart, PB and St) and extensors (SmAB, MG, FHL) taken from a 120 s bout of MLR-evoked fictive locomotion. Stimulation of TA and EDL (5T) was trigered by activity in the SmAB nerve. Vertical lines mark the onset and the offset of stimulus train (33 pulses at 200 Hz) delivered every third step. Oddly, TA stimulation inhibited ongoing extension and then evoked a new extensor burst without intermittent flexor activity.

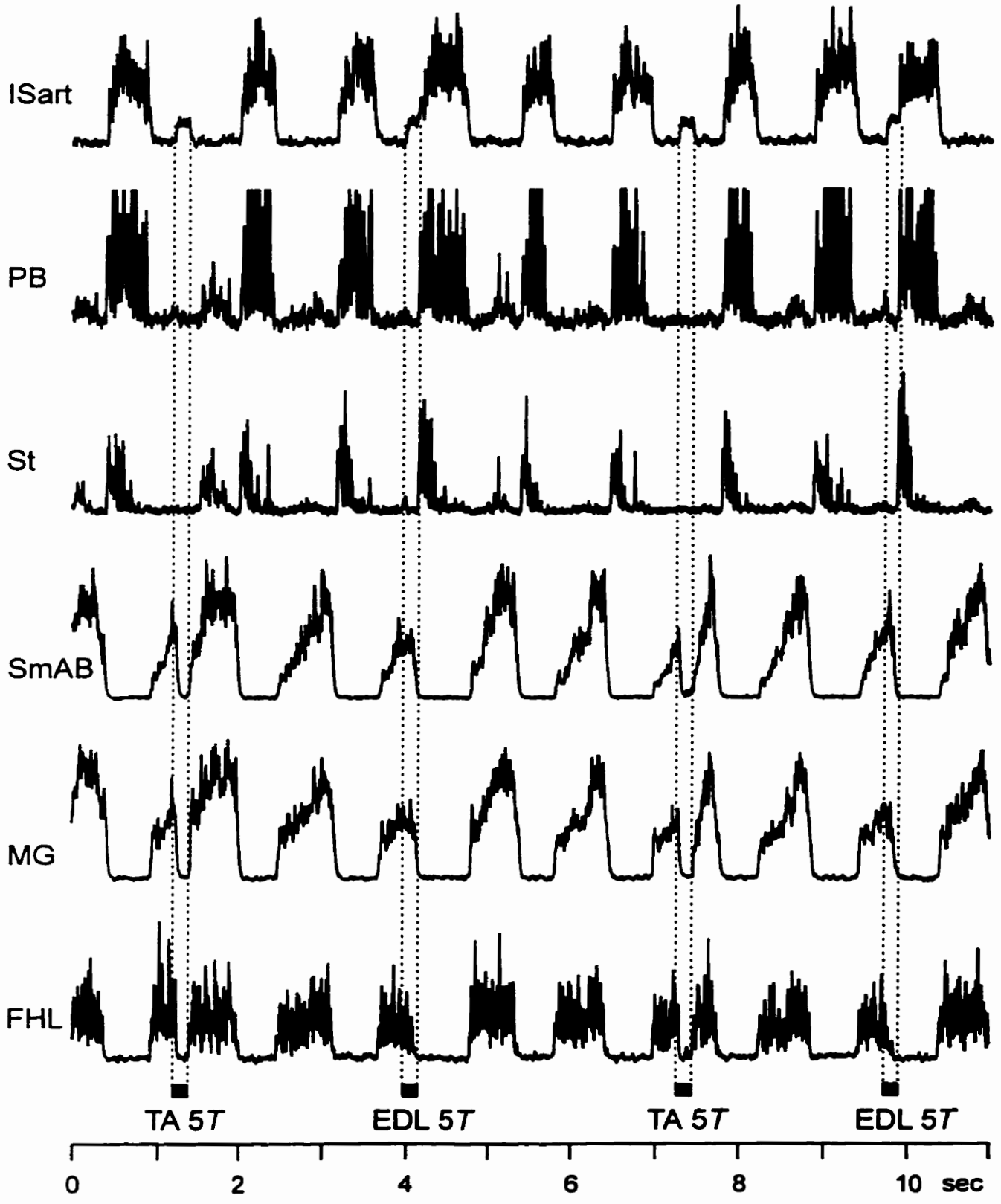


Figure 5. TA and EDL effects during extension.

Figure 6. Effects of other flexor nerves

A. The cycle period increased during steps with stimulation of Psoas and Sart nerves (30 pulses at 200 Hz) at 5T delivered during flexion as illustrated in 38 steps from a 70 s long bout of MLR-evoked fictive locomotion. LGS activity during steps with stimulation of either Psoas (■) or Sart (▲) had a significantly longer cycle duration ($p<0.001$) than during control steps (○).

B. Rectified and integrated ENGs over a 7 s period of MLR-evoked fictive locomotion showing alternation of flexor (Sart, TA, EDL, FHL) and extensor (SmAB, LGS) activity. Stimulation of PerL (25 shocks at 200 Hz) was triggered by Sart ENG activity. PerL stimulation increased amplitude but not burst duration of other flexor nerves. The activity in LGS during the stimulus delivery is most likely a stimulus artifact.

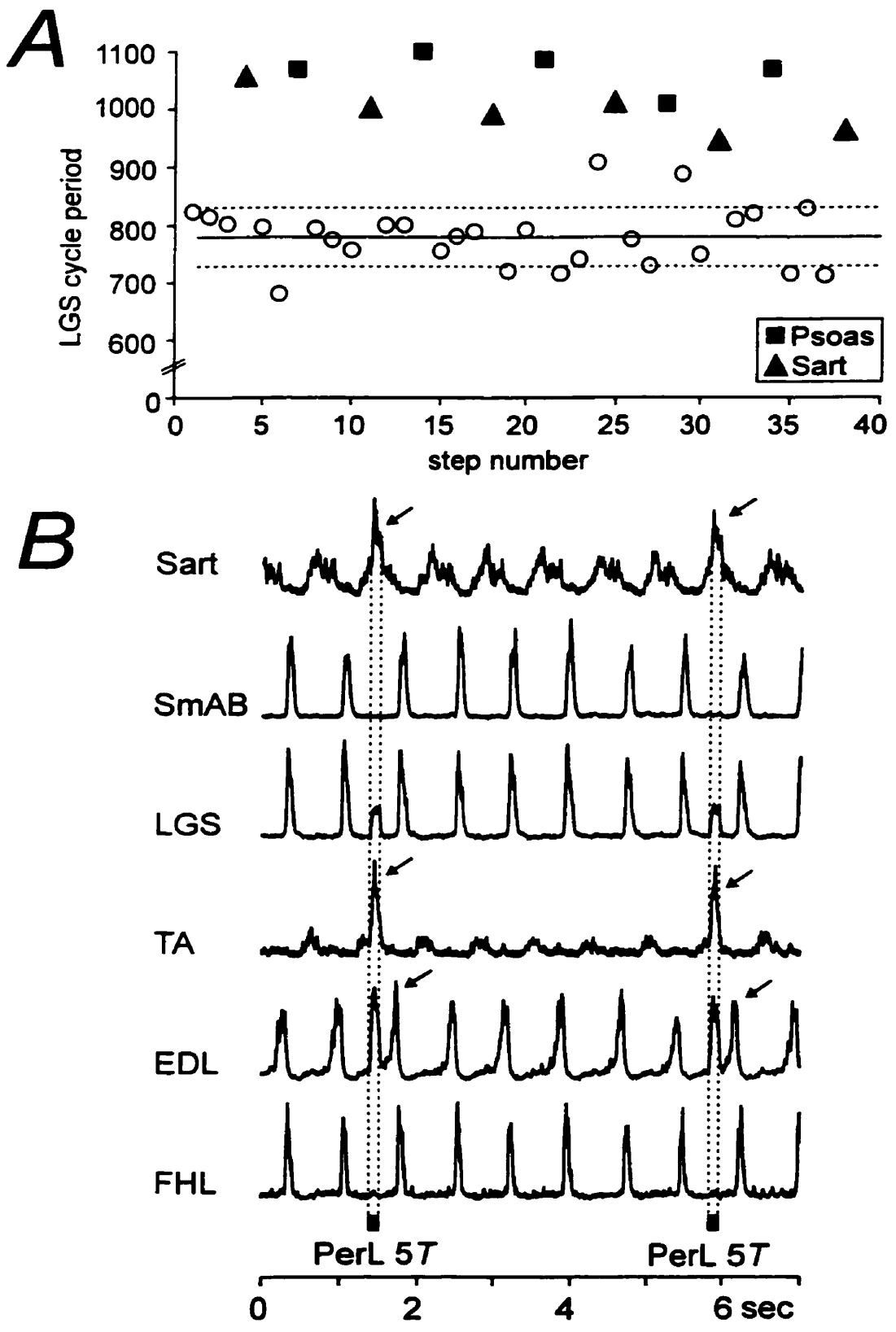


Figure 6. Effects of other flexor nerves

Figure 7. EDL stimulation <2T is not effective

Averaged ENG activity from selected flexor and extensor nerves during fictive locomotion (500 ms before and 700 ms after stimulus delivery) is shown in each panel. Vertical dashed lines mark the stimulus train (25 shocks at 200 Hz) triggered by PerL ENG activity. EDL nerve stimulation at strengths lower than 2T had only short latency excitation in PerL as seen in panels **A** and **B**. Stimulation of EDL at 2T and 5T resulted not only in amplitude changes but also in increased burst duration of other flexors (Sart and PerL) (see panels **C** and **D**).

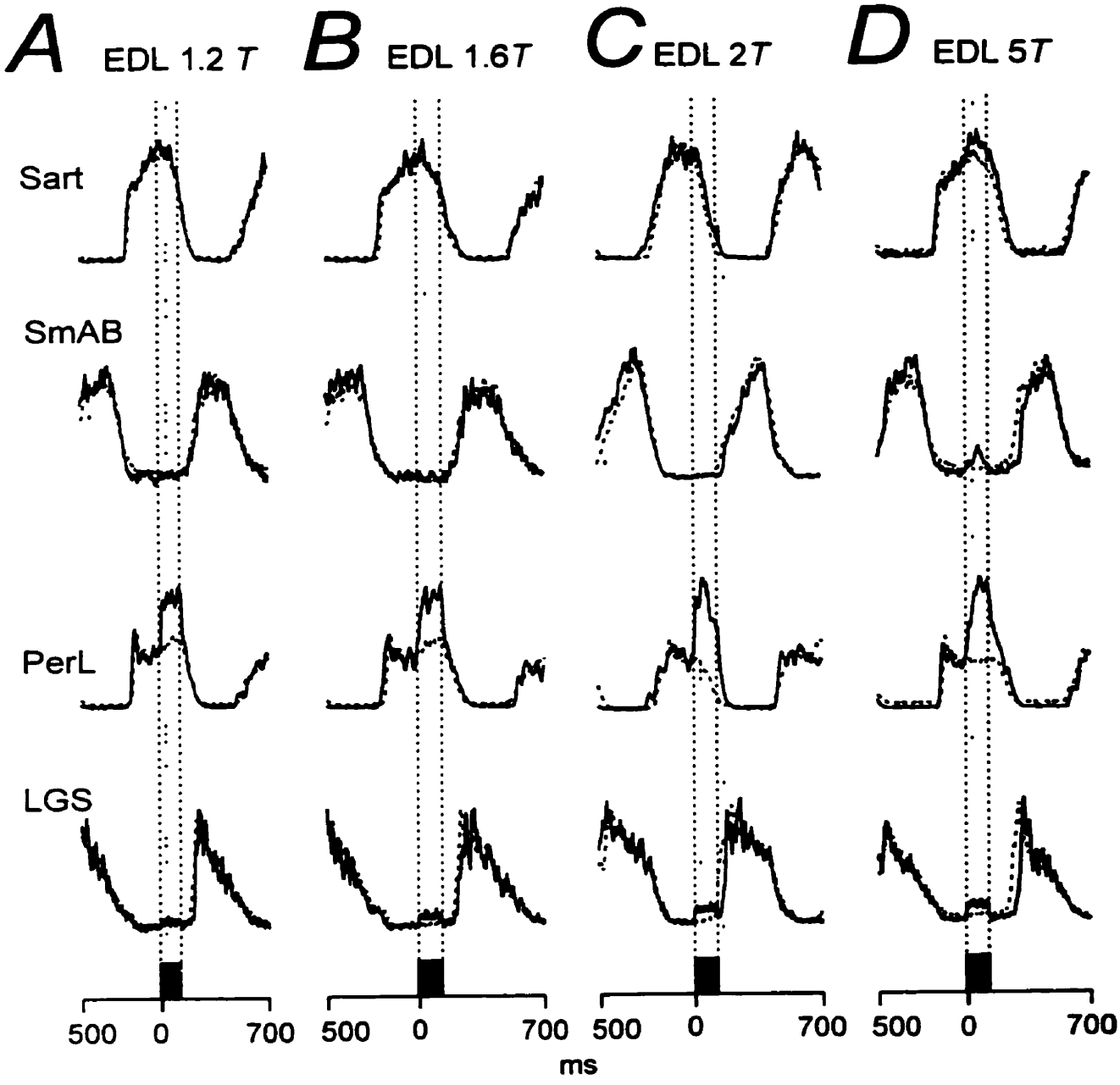


Figure 7. EDL stimulation <2T is not effective.

Figure 8. Variable effects of TA and EDL

Rectified and integrated ENGs recorded during MLR-evoked fictive locomotion showing alternating flexor (EDL, PerL, Psoas) and extensor (SmAB, GS) activity.

A. Alternating stimulation (25 shocks at 200 Hz) of TA nerve at both 2 and 5T was triggered by PerL ENG activity. The vertical dashed lines mark the stimulus trains. The first 5T stimulus evoked resetting to extension by inhibiting ongoing flexor bursts and initiating premature extensor activity (arrows in SmAB and GS). The second train of 5T stimuli evoked flexion enhancement by increasing the flexor ENG amplitude and prolonging the ongoing flexor burst duration (arrows in EDL and PerL). Note that TA 2T stimulation also evoked flexion enhancement.

B. Alternating stimulation of the EDL nerve (15 shocks 200 Hz) at 2 and 5T was triggered by TA ENG activity. The first set of stimulation at 5T evoked termination of ongoing flexor activity and a premature extensor burst (see arrow in SmAB). The second train of 5T stimuli evoked the enhancement and the prolongation of ongoing flexor activity (arrow in Psoas). Note that EDL 2T effects were also affective in evoking flexion enhancement.

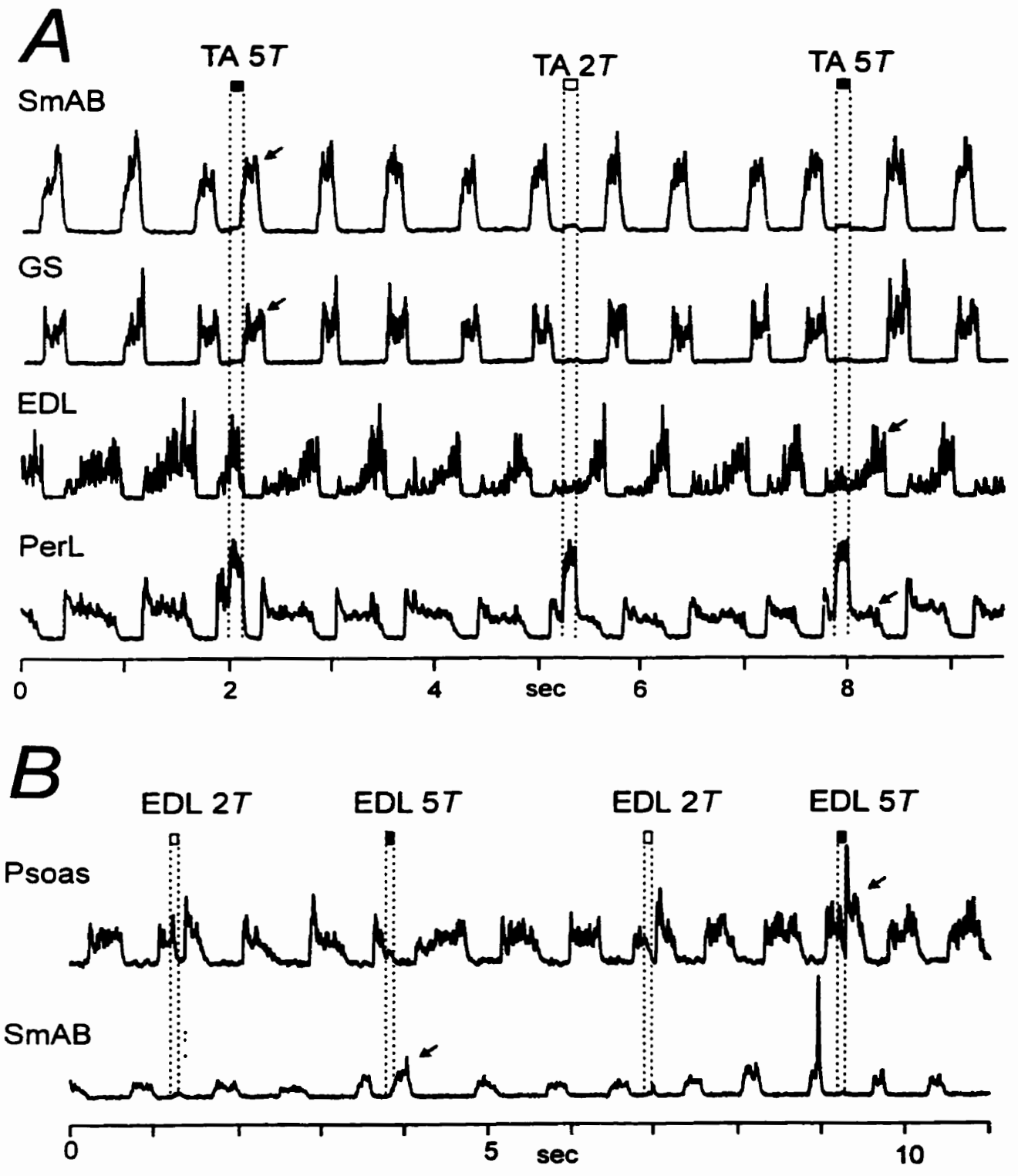


Figure 8. Variable effects of TA and EDL

Table 1. Summary of effects evoked by flexor nerve stimulation (5T) during flexion

The nerves stimulated during flexion are listed in the rows. The columns show the number of experiments in which the stimulation of the respective nerve prolonged and enhanced flexor activity; reset to extension or had no effects at all from the total number of experiments it was tested in. The number of experiments, in which the stimulation of the respective nerve produced enhancement of flexor ENG amplitude (EFA) without significantly modulating the cycle duration, are in parenthesis.

Table 1

Nerve stimulated	Prolong & enhance flexion	Resetting to extension	No effects
EDL	**9/13**	2/13	4/13 (2/4 EFA)
TA	3/12	**11/12**	1/12
Psoas	3/3		
Sart	3/4		1/4
PerL			4/4 (3/4 EFA)

DISCUSSION

Flexor afferents affect the central pattern generator circuitry

The main finding of this study is that during MLR evoked fictive locomotion, stimulation of flexor muscle nerves can enhance the ongoing activity of flexor motoneurons. Enhancement of flexor activity was seen following stimulation of the EDL, PerL, Psoas and Sart nerves. The relatively brief stimulus trains to single nerves used in this study had powerful effects on flexor activity. We suggest that during real locomotion with proprioceptive feedback from several muscles, reflexes evoked from flexor muscle afferents may contribute substantially to the activity of flexor motoneurons even during unperturbed over ground locomotion. This suggestion is in keeping with the observation that during treadmill locomotion, electrical stimulation of EDL (1.8- 5T) increases the duration of the ongoing flexor activity (Hiebert et al. 1996).

Stimulation of either the EDL, Poas or Sart nerves elicits long lasting excitation of limb flexors that persists after the termination of the stimulus train. Therefore these effects are not simply stimulus-locked reflexes but actions exerted on the central organization responsible for the timing of locomotor bursts as well as responsible for regulating the amount of flexor motoneuron activity. Furthermore, the observation that stimulation of a single flexor nerve increases the activity of not only flexor motoneurons operating at all limb joints but also delays the onset of limb extensor activity is a strong argument that this stimulation affects the central locomotor circuitry (CPG). The present results suggest the existence of a set of locomotor-dependent reflexes to flexor motoneurons that are analogous to the system described for the maintenance and

augmentation of extensor activity during both fictive and real locomotion (see Introduction). Similar to the actions of extensor afferents which promote extensor motoneurone activity, the amplitude and timing of flexor activity appears to be regulated by sensory input arising from flexor muscle afferents. There is, however, a clear difference in the intensity of stimulation required to evoke flexion and extension enhancement. Extension enhancement is evoked by group I afferents and although the role of Ib tendon organ afferents was originally stressed (Conway et al. 1987, Gossard et al. 1994) it is now clear that muscle spindle primaries (Ia) can also promote extensor activity (Guertin et al. 1995). Flexion enhancement during fictive locomotion on the other hand, is only evoked with higher intensity electrical stimulation (e.g. Fig 5) and as will be discussed, it appears that in some nerves flexion enhancement requires the activation of muscle spindle secondaries.

The ability of hip flexor (Anderson and Grillner 1983; Kriellaars et al. 1994; Hiebert et al. 1996) as well as ankle flexor (Hiebert et al. 1996) muscle afferents to entrain or prevent the transition from swing to stance during fictive locomotion is well documented. The idea that reflexes evoked by hip flexor muscles afferents may also be an integral part of the generation of flexor motoneuron activity during the swing has not, to our knowledge, been discussed before. The conclusion that flexor muscle afferents may have a powerful regulatory role to assist motoneuron activity during the swing phase may appear at odds with previous findings using the same preparation employed here (Perreault et al. 1995). In that study, stimulation of the TA, and Sart (at 5T) nerves inhibited the ongoing flexion phase and initiated a premature extensor phase (i. e. reset to

extension). The effects of EDL, PerL and Psoas stimulation were not examined previously. However, the extension promoting effects of TA stimulation in the present and earlier study (Perreault et al. 1995) were the same. Thus one of our conclusions is that there are two distinct sets of reflexes evoked from flexor muscle afferents during fictive locomotion; a resetting to extension (e. g. from the TA nerve) and a resetting and enhancement of flexion evoked by PerL, EDL and Psoas stimulation.

The only study in which the effects of flexor nerve stimulation has been examined during real locomotion is that by Hiebert et al. (1996). In that study the effects of muscle stretch (TA, Psoas and EDL) and electrical stimulation (EDL and TA) were examined during spontaneous treadmill locomotion in decerebrate cats. From results obtained with stimulation of flexor afferents during the stance (extension) phase they concluded that activity in group Ia afferents in the EDL and Psoas nerves can shorten the duration of extensor bursts and promote the transition to the flexion phase. That study also examined the effects of nerve stimulation during the flexion phase. While stimulation of TA group I afferents and stretch of Psoas were ineffective during flexion, EDL muscle stretch prolonged the duration of flexor activity. In one (of four) preparations TA stimulation at 5T promoted the ongoing flexor activity (i. e. produced effects similar to those of EDL nerve stimulation). Unpublished work re-examining this issue (Pearson, personal communications) indicates that stretch of the Psoas muscle during flexion can enhance ongoing flexor activity during treadmill locomotion. Thus in decerebrate cats during both fictive and treadmill locomotion, activation of flexor muscle afferents can have powerful effects on the step cycle including an enhancement of ongoing flexor motoneurone

activity.

Which afferents evoke flexion enhancement?

Neither in the previous study by Perreault et al. (1995) nor in the present one was stimulation of TA nerve at less than 2T strength effective in evoking a resetting to extension. Resetting was readily evoked with 5T intensity stimulation. Resetting evoked at 2T could reflect the involvement of the least excitable group I afferents or as argued by Perreault et al. (1995), it can result from the actions of the relatively high proportion of low threshold group II afferents in the TA nerve (Jack 1978). Since the majority of group II afferents are activated when increasing the stimulation strength from 2 to 5T (Eccles and Lundberg 1959) even a small number of TA group II afferents may evoke significant actions on the locomotor pattern.

Similarly electrical stimulation of EDL below 2T did not result in powerful actions on the step cycle. The fact that stimulation at 5T was more effective than stimulation at 2T (see Fig. 7) again suggests that recruitment of group II fibres is contributing to the effects on the CPG. We suggest that the effects evoked by flexor higher threshold afferents (> 2T) are most likely due to activation of secondary muscle spindle endings. Secondary muscle spindle receptors are the most likely source of group II afferent input because they give rise to the majority of axons in group II afferents (Boyd and Davey 1968). We cannot, however, rule out a contribution from other, non spindle afferents.

The present results on Sart stimulation are somewhat at odds with those reported by Perreault et al (1995). They found that Sart stimulation at twice threshold produced

flexion enhancement similar to that reported here (see their Figure 2C) while stimulation at 5T terminated ongoing flexion and initiated extension (i. e. reset the cycle). This they argued, suggested that group I (Ia and Ib) afferents evoked the enhancement of ongoing flexion while group II (5T stimulation) afferents were responsible for the resetting to extension. In the present study 2T strength stimulation of the Sart nerve prolonged flexion but increasing the intensity to 5T did not produce the resetting to extension as reported by Perreault et al. (1995). We have no satisfactory explanation for the difference in 5T Sart stimulation in the two studies. One possible difference between the 1995 and present study is the way in which Sart afferents were dissected and mounted for stimulation. In the present study, the Sart nerve was sometimes divided into 2 branches and in all cases there was a more extensive dissection of the ventrally located nerves. This included separation of the rectus femoris nerve running alongside the Sart nerve and the use of a larger (triple) nerve cuff as well as an additional cuff for mounting the distal Psoas nerve in some experiments. It is thus possible that the present effects of 5T stimulation are from nerves subject to more trauma than in the previous study. If this were the case, then 5T stimulation intensity would actually have recruited a smaller number of group II afferents. Unfortunately, no attempts were made to minimize the nerve dissection or use higher stimulation intensities during the course of these experiments. In future experiments the use of mechanical (instead of the currently applied electrical) stimulation of a selected muscle nerve can aid in the proper assessment of the separate actions of group I and group II afferents. Despite this shortcoming on the relative actions of group I and II afferents in the Sart nerve, the contrasting effects of TA and

EDL nerve stimulation stand out as clear examples of how both a resetting to extension and a resetting to flexion can be evoked in the same preparation from stimulation of flexor muscle nerves. In both, the case of TA and EDL nerves, group II muscle spindle afferents are the most likely source of their effects on the step cycle. In the case of Sart and Psoas nerve stimulation, it appears that activity in group I afferents can evoke flexion enhancement.

Is flexion enhancement evoked through FRA pathways?

Flexion enhancement and the flexion reflex both involve a widespread activation of flexor motoneurons evoked by recruitment of group II afferents. The question arises as to whether the effects reported here during fictive locomotion reflect the emergence of locomotor- dependent reflexes that can regulate the CPG or are flexion reflexes that can also be evoked in the absence of locomotion. As mentioned in the Introduction, the FRA system is characterized by the common reflex actions of a wide variety of afferents that arise from different peripheral receptors as well as from different locations in the hindlimb. The common actions of the FRA are maintained during DOPA-induced fictive locomotion (Schomburg et al. 1998) where stimulation of cutaneous and muscle nerves can evoke a resetting to flexion.

During MLR-evoked fictive locomotion, however, several observations argue against the possibility that the effects of flexor nerve stimulation are mediated by flexion reflex pathways. (1) The FRA concept includes common reflex effects of group II afferents from both flexor and extensor nerves. During MLR evoked fictive locomotion and in contrast to the actions of flexor nerve afferents, extensor group II afferents appear

to have little effect on the step cycle (e. g. Guertin 1995, Gossard 1994). Furthermore, in the same preparation flexion reflexes evoked by single shock stimulation of both group II and cutaneous afferents are depressed (Perreault et al. 1999). (2) Stimulation of some flexor afferents resets to extension (Perreault et al. 1995). The contrast between the resetting to extension evoked by group II intensity stimulation of some nerves (TA, PbSt) and flexion enhancement evoked from other nerves (EDL, Psoas, PerL) is clearly against the commonality of reflex effects expected of the FRA system (see McCrea 1992). Thus there is a differentiation of group II flexor nerve evoked reflexes during locomotion. (3) The effects of stimulation of cutaneous nerves become highly differentiated during MLR-evoked fictive locomotion. Thus the cutaneous nerve SP evokes the stumbling corrective response during fictive locomotion in decerebrate cats (Quevedo et al. in preparation) while stimulation of the cutaneous nerve innervating the plantar surface of the foot, the tibial (Tib) nerve, enhances the activity of extensor motoneurons throughout the limb (Guertin et al. 1995). In this preparation the knee joint and sural nerves (Perreault et al. 1995) do not evoke the same effects as TA when stimulated during the flexor phase of the fictive locomotor activity. Knee joint nerve stimulation prolongs the ongoing flexor activity as does EDL stimulation. The effects of sural nerve stimulation are complex resulting in excitation followed by an inhibition in the Sart nerve. This differentiation of cutaneous reflexes further strengthens the argument against the maintenance of the FRA system during locomotion.

Traditionally the use of the term "FRA" denotes not only the afferents that may evoke the flexion reflex but also addresses the entire organization of spinal interneurons

producing such effects (McCrea 1992). As argued by Lundberg et al (1987b), the flexion reflex is just one of the possible movements evoked by the interneurons that receive input from FRA. The variety of reflexes evoked by higher threshold proprioceptive afferents suggests that during locomotion there is a large segregation of interneuronal pathways to evoke different actions based on the specific sensory information relayed through a pathway. Thus it is not surprising that theoretical framework provided by the FRA concept is not applicable to the variety of reflexes evoked by segmental afferents during locomotion. It was recognized early on that flexion reflexes must be suppressed during locomotion to avoid disrupting the step cycle (Eccles & Lundberg 1959). This is because even undisturbed walking would result in activation of many of the flexion reflex afferents, and in particular group II muscle afferents. If these afferents continued to evoke flexion reflexes this would interfere with purposeful movement (discussed in Perreault et al. 1999). The finding that group II afferents do not evoke flexion reflexes during locomotion thus makes good functional sense.

Multiple pathways available for flexor afferent actions

It appears that in decerebrate cats during both fictive and treadmill (Hiebert et al 1996) locomotion, activation of flexor muscle afferents can have powerful effects on the step cycle including an enhancement of ongoing flexor motoneurone activity. It is also apparent that both the afferent type and source (nerve origin) of these effects differs during fictive and treadmill locomotion. We suggest that some of these differences are due to the existence of multiple reflex pathways available to flexor muscle afferents whose excitability is differentially controlled in different preparations.

The contrasting effects of TA and EDL nerve stimulation during fictive locomotion are particularly intriguing in this regard. In both cases the effects on the locomotor step cycle have a threshold of about 2T with more powerful actions exerted as stimulus strength is increased to 5T. As presented in the Introduction, both muscles are powerful ankle flexors and under many conditions are active at the same time during locomotion. Despite the few cases with the variable effects (see Fig. 8), the contrasting actions of TA and EDL stimulation were surprisingly consistent (see Table 1). The different effects of these two nerves during the same run of fictive locomotion can only be explained by access to different sets of reflex pathways by these afferents.

The relatively small number of preparations in which TA and EDL nerve stimulation during extension had significant effects, and the variability of the evoked effects in extension, do not provide clear conclusions about the effects of these nerves on the step cycle when stimulated during extension (see also Hiebert et al 1996). However, the existence of variable effects from these nerves is further evidence for the existence of multiple pathways that may be readily available for group II actions under other conditions. Figure 8 shows that even in the same run, the effects of nerve stimulation can be variable. Together, there seems to be a wide variety of effects on the CPG that can be evoked from flexor muscle nerves. A future challenge is, therefore, to determine under which circumstances these reflexes play a role in the regulation of motoneurone activity and the step cycle during real locomotion.

Studies in anaesthetized preparations have revealed four main groupings of interneurons with strong monosynaptic group II input. They are:1) mid lumbar group II

(Edgley & Jankowska 1987b), 2) caudal lumbar group II (Lundberg et al. 1987b, Riddell & Hadian 2000) , 3) sacral group II (Jankowska & Riddell 1994), and 4) contralaterally projecting lamina VIII cells (Jankowska & Noga 1990). Unfortunately, there is little information available about where the interneurons involved in locomotor-dependent group II reflexes are located. The only study to have addressed the locomotor activity of interneurons with group II input focussed on last order cells excited from quadriceps afferents with caudal projections to motoneurons in L7 or S1 (Shefchyk et al. 1990). In the case of the differential effects of TA and EDL stimulation, neurons with monosynaptic input from group II afferents in these nerves may be located in L5-7 (Edgley & Jankowska 1987a; Riddell & Hadian 2000). To our knowledge all attempts at finding interneurons with input from TA and EDL have stimulated these nerves together and not separately as would be required to address the issues raised here.

Functional implications of reflexes evoked by flexor muscle afferents

Proprioceptive feedback from extensor muscle afferents may account for a substantial amount of the activity of extensor motoneurons during real locomotion (see Introduction). The recognition that this feedback produces excitation instead of the inhibition evoked during non-locomoting conditions is arguably one of the most important recent insights into spinal motor control systems. The excitatory actions of extensor group I afferents during locomotion are likely mediated through direct effects of these afferents on extensor portions of the CPG (summarized in McCrea 1998). The ability of flexor muscle afferents to increase ongoing flexor motoneurone activity (flexion enhancement) thus appears as an analogous control over the locomotor system. By

positive feedback this system can automatically augment flexor activity during swing should increased loading or unexpected increases in muscle length occur. An assessment of the extent to which flexion enhancement might contribute to ongoing flexor activity during unperturbed locomotion will require experiments in which flexor muscles are unexpectedly unloaded or prevented from lengthening.

The flexion enhancement produced from hip flexors (Psoas and Sart) and ankle flexors (EDL and PerL) during fictive locomotion would appear to make functional sense during real locomotion. But why would the high threshold muscle afferents from two functionally similar muscles, TA and EDL evoke contrasting effects during fictive locomotion?

To this question we have no satisfactory answer. As presented in the Introduction, there are major differences in the anatomical features and locomotor activity of these two muscles. It can be speculated that if the toes were to hit an obstacle during the later part of swing, the activation of EDL group II afferents by toe stretch and ankle flexion would reflexly enhance ongoing flexor activity and help in clearing the obstacle. To some extent this reflex would be similar to the stumbling correction that occurs following cutaneous stimulation of the dorsal aspect of the foot (see Introduction). Similarly, an unexpected rearward slip of the foot at the end of stance would stretch EDL tendons by actions at both the toes and ankle. According to the results reported here (and by Hiebert et al 1996) this in turn would promote the onset of the swing phase and presumably speed up the initiation of the subsequent stance phase to increase postural stability. We are unable to guess why TA afferents do not have the same actions during fictive locomotion. It is

important to mention again, however, that during treadmill locomotion, TA afferents do not reset to extension (Hiebert et al. 1996). Thus it is possible that the actions of TA afferents reported here are a reflection of an incomplete control of the multiple reflex pathways available to group II afferents and do not necessarily represent the operation of a reflex that normally functions during overground, forward locomotion.

To summarize, the actions of flexor group II afferents during locomotion are profoundly different from those evoked in preparations not locomoting. This promotes the idea of "state-dependence" which implies that the mammalian spinal networks and their functional connectivity should be examined only when the nervous system is in the appropriate state. Going from rest to locomotion involves a transition from a state in which flexor group II afferents evoke flexion reflexes to one in which certain group II afferents promote flexion while others promote extension. We suggest that the flexion enhancing actions of group I and II fibres may be an important component of real locomotion. Identification of the neurons responsible for these actions and the systems controlling the multiple reflex pathways available to flexor muscle afferents must await future study.

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