CENTRAL MEDIATION OF GROUP I MUSCLE AFFERENT EVOKED ADAPTATION OF THE LOCOMOTOR STEP CYCLE IN DECEREBRATE CATS

Pierre Guertin

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CENTRAL MEDIATION OF GROUP I MUSCLE AFFERENT EVOKED ADAPTATION OF THE LOCOMOTOR STEP CYCLE IN DECEREBRATE CATS

BY

PIERRE GUERTIN

A Thesis/Practicum submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

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ABSTRACT

A long time ago, the suggestion was made that inputs from proprioceptors of the limbs reflexively act on motoneurone output and hence muscle activity to rapidly adjust the basic step cycle pattern when walking over an uneven surface (Brown 1914). This hypothesis was supported by recent studies that have described in detail the actions of a powerful feedback system controlled by extensor group I and flexor group II afferents that can suddenly prolong and intensify the contraction of extensors throughout the whole limb during the stance phase (i.e., ground contact). This thesis will examine how the spinal cord mediates these reflexes by analyzing the effects of a brief train of electrical stimuli (*e.g.*, 20 pulses @ 200 Hz) at group I strength ($\leq 2T$) applied to an ankle extensor muscle nerve on the membrane potential of hindlimb motoneurones during fictive locomotion in decerebrate cats.

Group I afferent stimulation during the extensor phase (article 1) prolonged the excitatory locomotor drive potential, and further depolarized the membrane of hip, knee, ankle and digit extensor motoneurones, while delaying flexor activity. These effects were assisted by smaller short latency excitation (*e.g.*, a locomotor-related disynaptic pathway) and possibly in some cases, by the motoneurone's active 'self-sustained' conductances. Similar stimulation during flexion (article 2) evoked a premature phase 'switch' in extensor motoneurones from the on-going inhibitory to the excitatory locomotor drive potential and opposite effects on the membrane of flexor motoneurones ('resetting' to extension). Simultaneous recording in pairs of motoneurones revealed that the phase switch evoked by stimulating ankle extensor group I afferents appeared to be produced synchronously in

extensors and flexors throughout the whole limb.

Together these results demonstrate that the effects of briefly stimulating ankle extensor group I afferents during extension or flexion are largely mediated by the spinal generator for locomotion. There is also clear evidence for a single reciprocally organized generator ('half-centre') that mediates the group Ia and Ib afferent input. Finally, the analysis of the cases of shorter latency resetting revealed that the effects are mediated within five ms.

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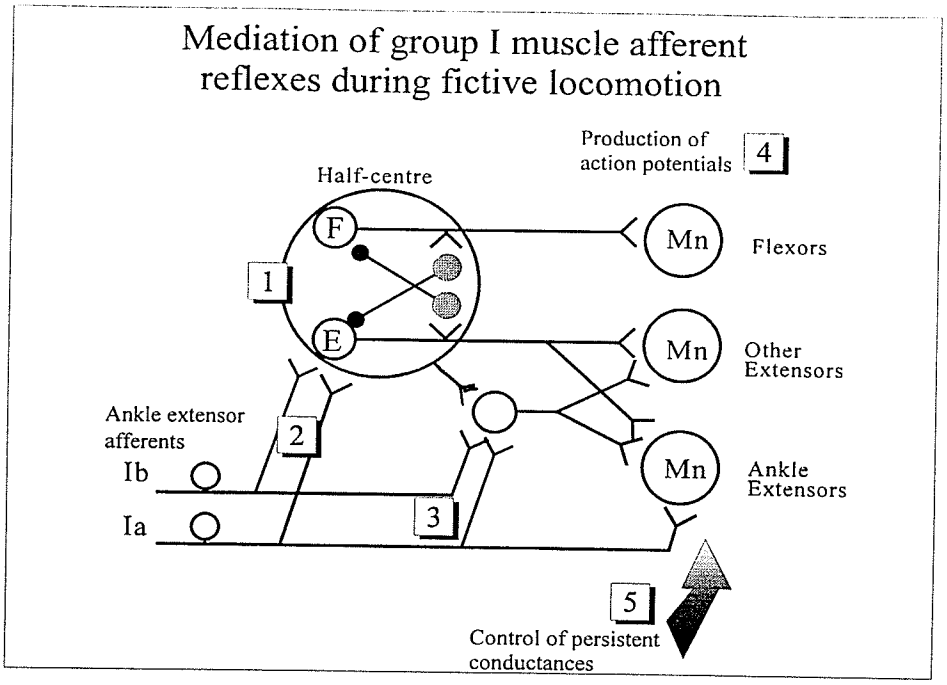
LIST OF ABBREVIATIONS

AHP	after hyperpolarizing potential
APV	2-amino-5-phosphonovalerate
Ca^{2+}	calcium ions
Co^{2+}	cobalt ions
CPG	central pattern generator
EGTA	ethylene glycol bis-Beta-amino-ethyl ether-*N*, *N'*-tetra acetic acid
EPSP	excitatory post-synaptic potential
f-I	relation between the firing frequency and the amount of injected current
FRA	flexion reflex afferent
H-reflex	Hoffman reflex
Hz	frequency, number of impulses per second
I_h	hyperpolarization-activated depolarizing current
I_k	inward rectifier current
$[K^+]$	concentration of potassium ions
L-DOPA	L-beta-3,4- dihydroxyphenylalanine
LDP	locomotor drive potential
L7	seventh lumbar segment of the cord
Mg^{2+}	magnesium ions
NMDA	*N*-methyl-*D*-aspartate
PAD	primary afferent depolarization
T	threshold for the most excitable afferent fibers
TRH	thyrotropin releasing hormone
TTX	tetrodotoxin
5-HTP	serotonin precursor, 5 hydroxy-tryptophan



This simplified circuit diagram includes the main elements believed to mediate the reflex actions evoked by ankle extensor group I muscle afferents onto hindlimb motoneurones during fictive locomotion. This diagram shows that group I afferent-evoked extension enhancement is mediated by monosynaptic connections (Eccles, Eccles & Lundberg 1957; Edgley, Jankowska & McCrea 1986; Guertin, Angel, Jimenez & McCrea in prep), locomotor-dependent disynaptic excitatory pathways (McCrea, Shefchyk, Stephens & Pearson 1995; Angel, Guertin, Jimenez & McCrea 1996), and a central pattern generator for locomotion (Guertin, Angel, Perreault & McCrea 1995; Guertin et al. in prep.). The relative contribution of each of these pathways to the powerfull group I afferent feedback reflex actions evoked during locomotion and not in resting conditions (see Pearson 1995) is addressed in this thesis. Indirect evidence suggesting that sustained active conductances in extensor motoneurones also contribute in producing group I-evoked extension enhancement. The 'General Introduction' section is organized in chapters that are numbered in association with the different parts of the diagram.

GENERAL INTRODUCTION

Even though walking appears to be a simple motor act, it is the result of a precise and complex sequence of muscle contractions. Animal models have been used to demonstrate that the pattern of motor commands sent to motoneurones, and hence to hindlimb muscles to produce basic stepping, originates from circuits of neurones located in the lumbosacral spinal cord. At the beginning of the century, Sir Charles Sherrington (1910) provided evidence suggesting that feedback input from proprioceptors of the limbs supports the genesis of the basic locomotor rhythm. A few years later, Graham Brown (1914) used a deafferented animal model to demonstrate that locomotor activity could be evoked in the absence of feedback input from the periphery. He suggested that proprioception plays a role in assisting the pattern generator to adapt and modulate each step and to carry the animal over uneven surfaces during normal unrestrained walking. This was recently confirmed by studies showing that brief activation of the group I and II muscle afferents excites the motoneurones of extensor muscles and inhibits those to flexors throughout the ipsilateral limb during fictive locomotion (Guertin, Angel, Perreault & McCrea 1995; Perreault, Angel, Guertin & McCrea 1995) and treadmill walking (see review, Pearson 1995). Several investigations are now underway to determine how spinal cord neurones mediate proprioceptor feedback for the adaptation and regulation of the locomotor step cycle. The results obtained in this study provide strong evidence that ankle extensor group I afferent reflexes evoked during the extensor or flexor phase are in both cases mainly mediated by the spinal pattern generator for locomotion.

1 Spinal generator for locomotion

Sherrington's (1910) work on spinalized cats and dogs provided evidence that the basic motor pattern for walking was the result of reflex actions from proprioceptors onto spinal centres. After a brief period of spinal shock following transection of the spinal cord, the hindlimbs are capable of executing movements called 'reflex stepping' which strikingly resemble those of the natural step. In the case of natural walking or reflex stepping, a step cycle is defined by rhythmic movements of alternating flexion (i. e., period of swing forward) and extension (i. e., period of ground contact) of each limb accompanied by an out-of-phase relationship with the corresponding contralateral limb. In order to produce stepping movements in decerebrate, acute spinal preparations, the animals were lifted from the ground with the spine vertical and the hindlimbs pendent which under their own weight sufficed to elicit stepping that could be stopped by passively flexing one limb at the hip joint. Because stepping could still be obtained after cutting all hindlimb cutaneous nerves, Sherrington believed that the locomotor rhythmicity was supported by cyclic input from proprioceptors of the hip flexor muscles and other reflexes (i. e., extensor thrust and '*umkehr*'). Sherrington already knew that reflex stepping was not solely the result of peripheral input mediated via the flexion and crossed extension reflex pathways since passive immobilization of one hindlimb during vigourous stepping did not prevent stepping in the contralateral limb. Since stepping could also be evoked by continuous stimulation of the skin (e. g., pinching the perineum or ear's pinna) or the spinal cord (i. e., faradization of the cut surface), Sherrington suggested the existence of specialized neurones in the cord that transform the tonic stimulus input into basic rhythmic stepping motor

commands.

This was later confirmed by Graham Brown (1914) who was able to demonstrate that the basic pattern for stepping was generated entirely in the spinal cord in the absence of peripheral afferent input in spinalized cats. The animals, under general anaesthesia, were lying on one side when stepping movements in the hindlimbs were spontaneously evoked ('narcosis progression') after spinalization of the cord at the lower thoracic level. Since the level of anaesthetic used was shown to abolish proprio- and extero- ceptive reflexes but not locomotor activity, Brown proposed a "half-centre" model made of two groups of spinal neurones reciprocally organized and mutually inhibiting each other, that was capable of producing the basic pattern for stepping. Activity in the first group of neurones (*e. g.*, extensor half-centre) would send motor commands to motoneurones (to excite extensors), and simultaneously inhibit the reciprocal group of neurones (flexor half-centre) preventing the excitation of antagonists (silence in flexors). After a period of 'depression' (*e. g.*, fatigue, adaptation, post-inhibitory rebound) of the extensor half-centre, the flexor half-drive would predominate for a new phase of activity.

Drs. Jankowska and Lundberg have provided the first direct evidence supporting the existence of Brown's model (Jankowska, Jukes, Lund & Lundberg 1967a, b). Interneurones located in the lumbar segments of the cord (lamina VII) were found to be active following flexion reflex afferent (FRA) stimulation. One group of neurones is activated by ipsilateral FRA, a second group by contralateral FRA, and a third group by both ipsi- and contralateral stimulation. After injection of L-DOPA and nialamide in spinal cats, FRA stimulation evokes a high frequency burst followed by a long-lasting self-sustained series of discharges. Some

neurones did not show any short latency effect during the stimulus train. These interneurones were found to be monosynaptically excited by ventro-lateral funiculus stimulation which contains descending fibers from the reticular formation. One of the most important feature that was revealed is the reciprocal organization between these interneurones since coFRA stimulation abolishes the long-latency discharges evoked by ipsilateral FRA. Finally, it was proposed that Ia interneurones could participate in producing the locomotor pattern by receiving strong excitatory input from FRA interneurones.

Sharing similarities with the half-centre model, Miller and Scott's hypothesis (1977) proposed that Renshaw cells rather than fatigue are responsible for the alternation between flexion and extension. Increasing activity in one pool of motoneurones (e.g., extensors) would be gradually inhibited by a corresponding increase of recurrent inhibition. Simultaneously, Renshaw cells would remove reciprocal inhibition of antagonists (recurrent facilitation) via their monosynaptic inhibitory input onto Ia interneurones allowing the flexor excitatory drive to take over for a new phase of activity (i.e., flexion).

Other studies have in turn suggested that Renshaw cell activity was inhibited during locomotor activity (Orlovski, Severin & Shik 1966; Bergman, Burke & Lundberg 1969. Intracellular recording from Renshaw cells and Ia interneurones showed that Ia interneurone activity is modulated by Renshaw cells rhythmicity during fictive locomotion (McCrea, Pratt & Jordan 1980) although the input of both classes of neurones is not essential to produce a basic locomotor pattern in motoneurones (Pratt and Jordan 1987). Other evidence against the Miller and Scott model was provided by Noga, Shefchyk, Jamal and Jordan (1987) who showed that the basic locomotor pattern and Ia interneurone activity

remain after i.v. injection of the nicotinic antagonist mecamylamine (MEC), which greatly abolishes Renshaw cell activation.

The highly conceptual 'ring' model (Székely, Czéh and Voros 1969; Gurfinkel and Shik 1973) was proposed to explain the existence of a more complex pattern of locomotor activity. It is made of a closed chain of at least five groups of neurones (e.g., 2 pure extensors, 2 pure flexors and 1 bifunctional) among which an excitatory drive is cyclically propagated. The propagation would travel at different speeds from one group to the other depending on the excitability (e.g., modulated by afferent input) of the path ('ring') interconnecting them. A slow-propagated drive would activate neurones of a group for a longer period of time (*e.g.*, pure extensor during the stance phase) whereas a fast-propagated one would activate a group of neurones for a brief moment in motoneurones to bifunctional muscles (Shik and Orlovsky, 1976).

Pearson and Duysens (1976) proposed a flexor burst generator model for insects and cats. This model consists of a rhythmic excitatory drive from the flexor burst generator to populations of flexor motoneurones. The burst generator would inhibit via an inhibitory interneurone the activity of extensors otherwise activated during the flexor silence by a tonic excitatory input. This model has been abandoned in favor of a symmetrical bipartite organization of the CPG in cats (review, Pearson 1995). This change is likely related to the description of a powerful feedback system from ankle extensor group I afferent activation which strongly excites pools of hindlimb extensor motoneurones (Guertin *et al.* 1995; Whelan *et al.* 1995).

The 'unit burst generator' model emerged a few years after the demonstration of the

existence of a pattern generator for locomotion located in the spinal cord that produces the basic pattern of motor commands even in absence of peripheral input. First, Székely, Czéh and Voros (1969) showed that the locomotor pattern (forelimb) in freely moving newts was similar before and after a bilateral section of the dorsal roots. This was also shown in decerebrate cats (Grillner and Zangger 1974). It was then suggested that a central pattern generator (CPG) could exist for each joint of each limb (Edgerton, Grillner, Sjöström & Zangger 1976, Grillner 1981). Activity from these 'units' would be tightly coupled during 'normal' walking but individually controlled by supraspinal inputs to produce different types of motor patterns. This model emerged after analyzing more complex patterns of locomotor activity such as backward walking, climbing, etc. For instance, it was occasionally observed that one hindlimb motor nerve can display tonic activity while the others display normal rhythmic pattern during fictive locomotion. Also, the activity of pluriarticular muscle nerves such as semitendinosus is sometimes in phase with extensors, or flexors, or both, which some authors found difficult to explain with a bipartite half-centre type of model (Edgerton *et al.* 1976).

Detailed analysis of nerve and muscle activity during spontaneous walking or evoked locomotor activity in paralyzed decorticated cats allowed Perret and Cabelguen (1980) to propose a bipartite (half-centre like) model that explains the complex activity in motoneurones innervating the bifunctional muscle semitendinosus. They proposed that both half-centres would send motor commands to 'bifunctional' motoneurones. The variety of the motoneurone pattern could be produced by modulating the half-centres output 'en route' to these motoneurones. They also suggested that a rhythm generator would be

functionally separated from a pattern generator since the rhythm and the amplitude of the locomotor drive potentials appear to be two distinct characteristics that can be independently and spontaneously changing.

Kriellaars (Ph.D. thesis) and Jordan have also proposed a functional separation of pattern and amplitude modules for the CPG in the cat. They have shown that locomotor drive potentials monitored simultaneously in pairs of motoneurones covary in amplitude among homonymous motoneurones while antagonist motoneurones inversely covary. This subject is further explored in the second article of this thesis. The complex locomotor pattern in 'bifunctional' motoneurones receiving input from both half-centres would be sculpted by controlling the amplitude of the flexor and extensor locomotor drive 'en route' to these motoneurones.

Although, there are a number of hypotheses regarding the structure and organization of the spinal pattern generator for locomotion, none have been refuted except maybe the Miller and Scott model (1977) and the flexor burst generator model (by membrane conductance measurements, MEC and strychnine injections, see Jordan 1983). As will be discussed the results presented in this thesis can all be interpreted in the context of a symmetric, bipartite reciprocal half-centre model (Brown 1914; Lundberg 1981). It is important to note that this thesis does not present evidence against the existence of other CPG models, but uses the simplicity of the half-centre model to discuss the experimental findings.

2 Proprioceptor-evoked reflex actions on the locomotor rhythm and pattern

Several studies, mostly done in cats, have shown that Golgi tendon organ and muscle spindle afferents from hindlimb muscles are part of a "length and force" feedback system promoting the activity of homonymous and synergist muscles during locomotion (review, Pearson 1995). This is a complete reversal of the effects mostly inhibitory reported in the literature (e.g., Jami 1992) and all medical textbooks. There is evidence suggesting that the effects from proprioceptors on the step cycle period could be partially mediated by the spinal circuit of neurones that produces the basic locomotor pattern and rhythm for locomotion (Review, Pearson 1995; Rossignol 1996).

It has been shown during fictive locomotion in decerebrate, paralyzed cats, that small amplitude displacements of the hip joint (passive movements) are capable of entraining the locomotor rhythm. This was shown not to originate from hip joint afferents (i.e., they were transected), but from low threshold stretch-sensitive afferents in hip extensor muscles whose action is to prolong the activity of other extensors (Kriellaars, Brownstone, Noga & Jordan 1994). Entrainment of locomotion at a faster or slower rhythm can be evoked by phasically stimulating ankle extensor muscle nerves at group I strength. This further illustrates the ability of proprioceptor group I afferents to act upon the central pattern generator for locomotion (Pearson, Ramirez & Jiang 1992; Pearson and Collins 1993).

Regulation of the step cycle pattern by proprioceptors was shown by stimulating the plantaris nerve at group I strength during flexion which resets the step cycle to a new extension phase by abruptly terminating the on-going activity in flexor nerves and prematurely initiating a new phase of activity in extensor nerves (Conway, Hultborn &

Kiehn 1987). Similar stimulation delivered during extension was shown to promote extensor activity by increasing the on-going activity of a synergist extensor nerve (Pearson and Collins 1993). Stimulation of quadriceps group I afferents can also regulate the step cycle in both *in vivo* cat (Conway et al. 1987; Gossard, Brownstone, Barajon & Hultborn 1994) and *in vitro* neonatal rat (Kiehn, Iizuka & Kudo 1992). Recently, It has been shown that reflex actions from group I afferents of ankle extensors (Guertin, Angel, Perreault & McCrea 1995, this article is not a part of the present thesis) or group II afferents of flexors (Perreault, Angel, Guertin & McCrea 1995) are extensively distributed, promoting the activity of extensors and inhibiting flexors throughout the entire limb during fictive locomotion in decerebrate cats. Similar results have been reported during spontaneous treadmill walking in decerebrate cats following stimulation of group I afferents from ankle extensor nerves which enhances stance and delays the onset of swing (Whelan, Hiebert & Pearson 1995).

Although there is evidence suggesting that reflex actions from stimulating muscle nerves at group I strengths are evoked by Golgi tendon organ Ib afferents, recently it was demonstrated with selective Ia activation (*i.e.*, small amplitude vibration of the triceps surae and plantaris muscles), that muscle spindle Ia afferents strongly contribute to these effects during fictive locomotion (Guertin *et al.* 1995). The group Ia afferents of flexor muscles can also promote synergist activity since small amplitude vibration of flexor muscles (e.g., iliopsoas, tibialis anterior) can promote flexor activity by resetting the step cycle to a new flexion in walking decerebrate cats (Hiebert, Whelan, Prochazka & Pearson, 1995).

In lampreys, a lower vertebrate, mechano-sensitive receptors are not located in muscles but along the lateral margin of the spinal cord. Their activation, by bending the caudal part of the cord in the *in vitro* spinal cord preparation, entrains the fictive swimming pattern evoked pharmacologically (Grillner, McClellan & Perret 1981). In the stick insect, stimulation of the stretch-sensitive flexor-like chordotonal organ was found to excite flexor motoneurones during locomotor activity but not at rest (Bassler 1993).

It has been also suggested that proprioception in humans might be involved in the compensation of ground irregularities at distinct phases of gait (Yang, Stein & James 1991, review, Dietz 1992). In summary, it is clear that proprioceptive feedback to the spinal cord can reflexively adapt the basic step cycle by promoting the activity of homonymous and synergist muscles and inhibiting the activity of antogonists during locomotion in mammals and other species.

3 Group Ia and Ib short latency reflex pathways

This thesis examines the contribution of the group I short latency reflex pathways such as the monosynaptic and the locomotor-related disynaptic excitatory pathways to the group I-evoked extension enhancement and resetting of the step cycle. Since these results will demonstrate that group I short latency pathways play a minor role in mediating these large effects, this section will only briefly summarize the main features of the reorganization of short-latency group I reflex pathways during locomotion (for more details, see also 'Introduction' of each article).

Group I afferent fibers of ankle extensor muscles make monosynaptic connections onto

several homonymous and close ankle synergist populations (Eccles, Eccles & Lundberg 1957) and only restricted projections exist to other extensor motoneurones at the knee and hip joints (Edgley, Jankowska & McCrea 1986). Monosynaptic excitation from ankle extensor group Ia afferents to hindlimb extensor motoneurones remain operational during fictive locomotion and only small phasic modulation, if any, was found during the step cycle in decerebrate, paralyzed cats (Shefchyk, Stein and Jordan 1984; Angel, Guertin, Jiménez & McCrea 1996).

The existence of other excitatory short latency reflex pathways revealed only during locomotion (McCrea, Shefchyk, Stephens & Pearson 1995; Angel *et al.* 1996) were shown recently to replace the classical group I non-reciprocal disynaptic inhibitory pathways (review, Jami 1992). During fictive locomotion in decerebrate cats and acute spinal preparations, selective activation of the group Ia afferents in ankle extensor muscles (*i.e.*, small amplitude vibration) or group I strength stimulation of any ankle extensor nerve evokes disynaptic excitatory post-synaptic potentials (EPSPs) onto close synergist motoneurones (McCrea *et al.* 1995) and other extensors throughout the ipsilateral hindlimb (Angel *et al.* 1996) replacing the classical non-reciprocal disynaptic inhibition (McCrea *et al.* 1995). This locomotor-related group I reflex pathway was found to be inhibited during flexion since the disynaptic EPSPs are relatively large (1 mV) during extension and usually absent during flexion with only one shock to the group I afferent fibers even though they reappear after a few successive shocks (minimum 3 shocks @ 100-200 Hz, Angel *et al.* 1996).

Another locomotor-related excitatory reflex pathway exhibiting polysynaptic latencies

(3.5-4.0 ms) was found from extensor group Ib afferents to synergist motoneurones in acute DOPA cats (Gossard, Brownstone, Barajon & Hultborn 1994). This pathway is phasically modulated during locomotion being larger during flexion than extension and is also voltage-dependence since EPSPs mediated by this pathway are further increased in amplitude at more depolarized membrane potential level (Brownstone, Gossard & Hultborn 1994).

It has been shown that extension enhancement and resetting of the step cycle can be evoked by Ia and Ib afferents (Angel *et al.* 1996; Guertin *et al.* 1995; McCrea *et al.* 1995). The failure of previous studies to demonstrate the contribution of Ia afferents can be explained by the fact that only single nerve stimulation was tested (review, Pearson 1995). Consequently the terms 'group I extension enhancement', 'group I resetting' (Guertin *et al.* 1995) and 'group I disynaptic reflex pathway' (Angel et al. 1996) have been proposed to describe the effects evoked by group I strength stimulation of ankle extensor nerves during locomotion. It remains unclear whether or not the Ia and the Ib afferents are mediated via the same reflex pathways. But as will be discussed the effects mediated by the locomotor-related short latency group I reflex pathways are not essential even though participating occasionaly in producing resetting and extension enhancement in the decerebrate cat.

4 Motoneurone properties and production of action potentials

This section introduces several concepts related to the processing of sensory-evoked synaptic inputs onto motoneurones that can increase motor activity. Adrian and Bronk

(1929) reported that the discharge activity in single motoneurones increases with the intensity of afferent stimulation. They analyzed this by examining the rate of motoneurone firing discharge monitored from the motor nerve (*i.e.*, teased down to one motor unit) following stimulation of contralateral cutaneous nerves in anaesthetized spinal cats. The frequency of firing discharge increased proportionally with the stimulus strength but only when stimulation rates were lower than 10 Hz. Adrian and Bronk also suggested that motoneurone activity is dependent on constant asynchronous excitatory bombardments from premotor interneurones onto motoneurones. The more intense the bombardment, the faster the frequency of firing discharge in a single motoneurone.

Eccles and Hoff (1932) suggested, however, that motor output activity is in part controlled at the motoneurone level since antidromic spikes were shown to momentarily change the on-going frequency of firing monitored from single motoneurones. Assuming that antidromic spikes do not travel trans-synaptically, they believed that motoneurones transform and regulate constant excitatory bombardment from interneurones into repetitive firing, producing the final motor output after integration of the synaptic input.

Intracellular techniques from squid giant axons (Hodgkin and Huxley 1939), single muscle cells (Graham and Gerard 1946) and mammalian motoneurones (Brock, Coombs & Eccles 1952) have allowed the examination of membrane events responsible for action potentials and post-spike potentials. Coombs, Eccles & Fatt (1955) described two distinct post-spike hyperpolarizing potentials; a brief one of about 5 ms and a long lasting after hyperpolarizing potential (AHP) of about 100 ms. The AHP was found to be produced by an outward potassium current since intracellular injections of hyperpolarizing and

depolarizing currents were shown to decrease and increase the AHP respectively. Also an injection of sodium ions to reduce the [K+], shifts the equilibrium potential to the depolarizing direction. It was later shown that the outward potassium current underlying the AHP is dependent on inward calcium current following the action potential since intracellular iontophoretic injections of ethylene glycol bis-Beta-amino-ethyl ether-*N*, *N*'-tetra acetic acid (EGTA) largely reduce the AHP by binding to free calcium (Krnjevic, Puil & Werman 1978).

Intracellular techniques have also allowed the description of several other motoneurone properties that have aided understand and predict motoneurone output. Kolmodin and Skoglund (1958) have shown that motoneurones start firing at a similar membrane potential (threshold). Increased firing evoked by progressively increasing passive movement of the hindlimb is accompanied by a linear increase of the membrane potential, decrease of spike heights and AHP duration in decapitate, unanaesthetized cats.

Methods of intracellular current injection has helped the understanding of the linear relation between the amount of current injected and the frequency of motoneurone firing (f-I curve). Granit, Kernell & Shortess (1963) have used such techniques to show that motoneurones usually start firing with a period of relatively high frequency, followed by a reduction in firing to a steady-state level of activity after intracellularly injecting a constant continuous current (adaptation). The level of firing during both the 'early' or 'steady state' phase is directly proportional to the amount of continuously injected current in lumbar motoneurones of anaesthetized rats (Granit *et al.* 1963) and cats (Kernell 1965a). Kernell (1965b) has also shown that motoneurones can suddenly enter a state of much

higher discharge rates (secondary range) with stronger steady currents. This is reflected by a sudden increase of the linear f-I slope (2-6 times steeper). Using the same paralyzed anaesthetized cat preparation, Kernell (1965b,c) also showed that the AHP duration is linearly correlated with the stimulus strength and the rate of firing discharge; the AHP decreases when the firing frequency increases.

It was later confirmed by Kernell and his colleagues that the f-I relation found with intracellular current injections remains similar with natural synaptic excitation in anemically paralyzed and narcotized cats. In fact, Granit, Kernell & Lamarre (1966a) have shown that within the primary but not the secondary range (Granit, Kernell & Lamarre 1966b), a continuous synaptic inhibition or excitation evoked by muscle stretch or electrical nerve stimulation has the same effect on the regulation of motoneurone repetitive firing as the addition or subtraction of a constant amount of injected current. Similar results were found by stimulating the red nucleus or by muscle stretching (Schwindt and Calvin 1973), but a slight shift of the f-I curve was reported by stimulating reticulospinal cells (Shapovalov 1972). The algebraical summation of synaptic input in the primary range was interpreted as being produced by conductance changes that releases current in parallel along the cell membrane. The current injected and the current produced synaptically 'all contribute their own share to the activation of the common spike generator engaged in summing up their net value' (Granit et al. 1966a). In his book Granit (1970) said:

> "The onset of a motor act finds the motoneurone prepared to deliver a high-frequency response in the secondary range serving to accelerate the rate of rise of contraction and to potentiate motor endplate action. This is rapidly replaced by firing at an adapted steady rate. This final phase of stabilization has been interpreted as the consequence of an expansion of the firing

zone eliciting AHP that is the main stabilizing process which holds the motoneurone to firing within the primary range and endows it with the important property of operating by algebraical summation of discharge frequencies".

Evidence that the AHP conductance regulates repetitive firing was reported by Ito and Oshima (1962) who have shown that a decrease in number of successive spikes during the adaptation is accompanied by a corresponding increase of the membrane conductance. Baldissera and Gustafsson (1971a) have confirmed this by reporting that the conductance change after a single spike and after two spikes is the same in proportion to the change in intensity of injected current needed to produce a first and second intervals of the same duration.

It can not be assumed that the mechanisms underlying repetitive firing that have been described in resting preparations also apply during locomotion. The next section will in fact demonstrate that the AHP is drastically changed during locomotor activity and does not solely control motoneurone firing in non-resting preparations.

5 Active intrinsic membrane properties

There are other mechanisms by which motoneurones can increase their output activity other than by an increase of the synaptic input. This thesis will present indirect evidence suggesting that sustained intrinsic conductances, presumably voltage-dependent, contribute in increasing motoneurone output during group I-evoked extension enhancement.

One of the best known 'active' mechanism intrinsic to motoneurones is called the 'plateau potential'. The action of sustained conductances was first reported in the

decerebrate cat by Hultborn, Wigström & Wängberg (1975) while delivering a short train (30 ms up to 1 sec) of small amplitude stretches (10 μm and up, 200 Hz) to the soleus muscle (already under tension to threshold for the stretch reflex). This stimulation triggered a long latency and increase of the soleus activity that persisted for several minutes after the end of stimulation. The soleus contraction could be abruptly terminated by brief stimulation of the common peroneal nerve. This is not without remembering the long lasting 'after discharge' effect evoked by cutaneous stimulation (flexor reflex afferents) described by Sherrington (1910) in the decerebrate cat (decerebrate rigidity). Similar effects in soleus efferents could be evoked by stimulating (300 Hz) the medial gastrocnemius nerve at $1.2T$ or the quadriceps nerve at $1.1T$ suggesting that prolonged and sustained muscle contraction can be evoked with relatively large and small monosynaptic excitation. Hultborn and his colleagues (1975) first attributed these effects to Ia afferents polysynaptic pathways that could prolong muscle excitation after the train of stimuli via a reverberating loop.

Subsequently, intracellular recording of motoneurones has demonstrated that the sustained effect evoked by brief afferent stimulation was not mediated by a reverberating loop but by self-sustained active conductances that are intrinsic to motoneurones. This was suggested after observing that similar long-lasting effects could be evoked in the absence of afferent stimulation by injecting a small brief depolarizing pulse (2 nA) in a lateral gastrocnemius-soleus motoneurone (Hounsgaard, Hultborn, Jespersen & Kiehn 1984). The repetitive firing could be terminated spontaneously a few seconds later or by injecting a brief hyperpolarizing pulse. Interestingly, the effects were also abolished by i.v. injection

of a small dose of anaesthetic or just after spinalizing the animal. The active depolarizations were called 'plateau potentials'. Plateau potentials lost after spinalization could return by systemic injection of the serotonin precursor, 5 hydroxy-tryptophan (5-HTP) (Hounsgaard, Hultborn, Jespersen & Kiehn 1988) or L-beta-3,4-dihydroxyphenylalanine (L-DOPA, 20-120 mg/kg) with or without monoamine oxydase inhibitor (nialamide, 10-50 mg/kg) or clonidine (0.5-1 mg/kg) (Conway, Hultborn, Kiehn & Mintz 1988).

The role of biogenic amines in inducing plateau potentials was confirmed in slices of turtle spinal cords (Hounsgaard and Kiehn 1989). In a bath of normal Ringer, intracellular injection of a depolarizing pulse in lumbar motoneurones evokes an initial high frequency burst followed by a slower steady state (adaptation) firing activity during the pulse. With serotonin in the bath, injection of a depolarizing current produces similar effects during the pulse but is also accompanied by self-sustained steady firing activity after the stimulus offset (abolished with 5-HT blocker methysergide, but not with the 5-HT_2 or 5-HT_3 receptor blocker ritanserin and ICS205-930 respectively).

It was shown that even with tetrodotoxin (TTX) in the bath which abolishes the genesis of action potentials (*i.e.*, isolating the motoneurones from synaptic input), a long-lasting plateau-like membrane potential remains after stimulation. This demonstrates the self-generated character of plateau potentials and the role of an active, sustained conductance in producing motoneurone activity (Hounsgaard and Kiehn 1989). The conductance underlying plateau potentials (input resistance 2-4 fold lower during the plateau) was shown to be Ca^{2+} dependent since the effects are abolished in a calcium-free bath replaced

by Co^{2+} or Mn^{2+}. Specific slow inactivating Ca^{2+} L-type channels were found to be involved since the specific L-type channel blocker nifedipine (1-15 μM) abolishes plateau potentials. It was suggested that plateau potentials are regulated via the AHP Ca^{2+} dependent K^+ conductance (Hounsgaard and Kiehn 1989). There is some evidence for the contribution of other currents to plateau potentials like the I_k inward rectifier, I_h hyperpolarization-activated depolarizing current (rat: Takahashi and Berger 1990; crustacean: Kiehn and Harris-Warrick 1992a,b; review in Binder *et al.* 1993).

The control of active intrinsic properties by descending monoaminergic pathways was shown by Baldissera and Gustafsson (1971b) in the decorticated anaesthetized cat who reported that a secondary range of firing could not be evoked after thoracic spinalization. This idea is also supported by the findings that stimulation of the locus coeruleus (White, Fung and Barnes 1991) or the raphe nucleus (Brownstone and Hultborn 1992) in cats can evoke a slow depolarization of lumbar motoneurones with a reduction of the AHP and an increased steepness of the f-I slope. Systemic serotonin application increases the intensity of motor output in spinal cats walking on a treadmill (Barbeau and Rossignol 1991) and in paralyzed rabbits (5-HTP, Viala and Buser 1969). In *in vitro* preparations, bath application of 5-HT reduces the AHP, depolarizes the membrane and increases the input resistance of neurones (neonatal rat motoneurones, Lindsay and Feldman 1993, Elliott and Wallis 1992; lamprey motoneurones, Wallén, Buchanan, Grillner, Hill, Christenson & Hökfelt 1989). There is evidence suggesting that inputs from the central pattern generator onto motoneurones, independent of bulbospinal monoaminergic projections, can also reduce the AHP and decrease the input conductance (Schmidt 1994).

Neuropeptides that are co-localized with monoaminergic descending systems like substance P and TRH (with serotonergic terminals) or enkephalin and neuropeptide Y (with noradrenergic terminals) were also found to modulate motoneurone excitability (refs. in Hultborn & Kiehn 1992).

The control of motoneurone conductances during fictive locomotion (presumably by descending systems) drastically changes the f-I slope (absence of a relationship) and the response of motoneurone discharge to depolarizing current injection (Brownstone, Jordan, Kriellaars, Noga & Shefchyk 1992). Brownstone and colleagues have shown that the AHP is almost abolished during fictive locomotion and that intracellular injection of depolarizing current does not change the AHP nor the motoroneurone firing frequency.

Another active property was found recently in mammalian motoneurones and interneurones. In presence of *N*-methyl-*D*-aspartate (NMDA) and depolarizing current, the membrane potential of motoneurones oscillates cyclically in *in vitro* neonatal rats (Squalli-Housaini, Cazalet & Clarac 1993). This voltage-dependent property is insensitive to bath application of TTX demonstrating the self-generated 'pacemaker-like' character of this activity (Hochman, Jordan & Schmidt 1994). Similar results were found in interneurones near the central canal using spinal cord slices (Hochman, Jordan & MacDonald 1994).

In summary, in presence of serotonin (or noradrenaline, neuropeptides), motoneurones can be shifted from a simple state where the pattern of activity is closely related to the amount of synaptic input, to a new state where motoneurone activity becomes largely governed by active sustained voltage-dependent currents. In this thesis I will show how

group I afferent stimulation delivered during fictive locomotion can alter the LDP and firing of hindlimb motoneurones. It will be argued that these effects are mediated in parallel by the activation of locomotor-dependent short latency reflex pathways, direct effects of the afferents on the CPG, and changes in motoneurone intrinsic conductances.

Paper I

INTRACELLULAR ANALYSIS OF GROUP I-EVOKED ENHANCEMENT OF EXTENSOR MOTONEURONE ACTIVITY DURING FICTIVE LOCOMOTION IN THE CAT.

P. Guertin, M.J. Angel, I. Jiménez† and D.A. McCrea

Department of Physiology

University of Manitoba

730 William Avenue

Winnipeg, Manitoba, R3E 3J7

Canada

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†Present Address: Department of Physiology, Biophysics and Neurosciences, Centro de Investigacion y de Estudios Avanzados del IPN, México 07000, D.F. México

Summary

1. Intracellular events in extensor motoneurones were examined during the increase in hindlimb extensor motoneurone discharges produced by activation of ankle extensor group I afferents during fictive locomotion evoked in decerebrate, paralyzed cats by stimulation of the mesencephalic locomotor region. Action potentials were blocked in the impaled motoneurones by intracellular diffusion of QX-314 to allow better assessment of the locomotor drive potential (LDP).

2. Ankle extensor nerve stimulation (20 pulses, 100-200 Hz, $\leq$ 2 times threshold) delivered during the extension phase of the fictive locomotor step cycle increased the duration and or amplitude (by up to 10 mV) of the ongoing LDP. The increase in duration of the enhanced LDP was accompanied by a delay in the onset of flexion and sometimes by a resetting of the step cycle. Selective activation of ankle extensor group Ia afferents with Achilles' tendon vibration produced LDP enhancement similar to that evoked by electrical stimulation recruiting both tendon organ and primary muscle spindle afferents.

3. During both unperturbed fictive locomotion and group I-evoked extension enhancement, there was a striking similarity between the shape of a motoneurone's LDP and the shape of the integrated, rectified electroneurogram (ENG) recorded in the homonymous peripheral nerve. This similarity between a randomly sampled LDP and action potential production in the homonymous motoneurone pool suggests that the synaptic input to motoneurones during fictive locomotion is widely distributed to most members of a motoneurone pool. It also shows that integrated, rectified ENG recordings

can be a good monitor of the shape and size of LDPs occurring in homonymous motoneurones.

4. In some motoneurones group I strength stimulation produced a series of stimulus-locked EPSPs. The greatest enhancement of LDP amplitude was seen in those motoneurones in which mono and disynaptic EPSPs were evoked. It is argued that short-latency EPSPs increase motoneurone excitability by direct depolarization and by facilitating intrinsic motoneuronal conductances that contribute to the LDP.

5. Group I-evoked LDP enhancement also occurred in the absence of short-latency excitatory reflex pathways and in motoneurones in which IPSPs were evoked during the stimulus train. This suggests that the group I-evoked extension enhancement of hindlimb extensors results in part from effects mediated through the extensor portion of the locomotor central pattern generator.

Introduction

Until recently it was assumed that activity in proprioceptors influenced motoneurone activity during locomotion through activation of the same short-latency reflex pathways that had been characterized in non-locomoting preparations. Several observations have forced a reappraisal of the way in which proprioceptors, and group Ia muscle spindle and group Ib Golgi tendon organ afferents in particular, regulate motor output during locomotion. During fictive locomotion in the cat, stimulation of ankle extensor group Ia and Ib afferents during the flexion phase of the locomotor cycle can terminate ongoing flexion and initiate a premature extension phase (Conway Hultborn & Kiehn 1987; Guertin, Angel, Perreault & McCrea 1995) or entrain the locomotor cycle (Pearson, Ramirez & Yang 1992). Stimulation of the same afferents during extension increases extensor activity (Conway *et al.* 1987; Pearson & Collins 1993) in motoneurones operating at the ankle, knee and hip joints during MLR-evoked fictive locomotion (Guertin *et al.* 1995) and treadmill locomotion (Whelan, Hiebert & Pearson 1995). This widespread group I-evoked augmentation of extensor activity has been termed "group I extension enhancement" and can be produced by group I-strength electrical stimulation of ankle extensor afferents or by selective activation of Ia muscle spindle afferents by tendon vibration (Guertin *et al.* 1995). Both the resetting of the step cycle from flexion to extension and the perturbation of the step cycle period during extension enhancement are evidence that during locomotion, group I afferents can influence motoneurone activity by accessing and perturbing the operation of the CPG circuitry itself (see also Pearson 1995).

In non-locomoting conditions, the reflex actions of ankle extensor group Ia and Ib afferents on extensor motoneurones are, in addition to the group Ia-evoked monosynaptic excitation of synergists (Eccles, Eccles & Lundberg 1957a), predominantly non-reciprocal inhibition of ankle and other hindlimb extensors (Eccles, Eccles & Lundberg 1957b; Jankowska, McCrea and Mackel, 1981; further references in Jami 1992). Such group I inhibitory actions have been discussed as being organized into a negative feedback system limiting motoneurone excitation (see Jami, 1992). Non-monosynaptic group I-evoked extension enhancement is, therefore, a reorganization of group I reflexes into an excitatory system that reinforces extensor activity during locomotion (Pearson & Collins, 1993; Guertin *et al.* 1995; McCrea, Shefchyk, Stephens and Pearson 1995; Pearson 1995; Whelan, Hiebert & Pearson 1995; Angel, Guertin, Jiménez and McCrea, 1996).

The pathways responsible for group I-evoked extension enhancement have not been completely identified but during fictive locomotion there is both a reduction of non-reciprocal inhibition (Gossard, Brownstone, Barajon & Hultborn 1994; McCrea *et al.* 1995; Angel *et al.* 1996: Angel, Jankowska & McCrea, 1996b) and a recruitment of two recently characterised non-monosynaptic excitatory reflex pathways during fictive locomotion. The first is a disynaptic (1.1 - 1.8 ms latency) excitation of ankle (McCrea *et al.* 1995; Angel *et al.* 1996) and hip and knee motoneurones (Angel *et al.* 1996). This disynaptic excitation is evoked through interneurones that can be recruited during the extension phase of the fictive locomotor step cycle but not during flexion or at rest (Angel *et al.* 1996b). Disynaptic excitatory pathways are thus a potentially important mechanism underlying extension enhancement. The second route by which group I afferents could

evoke a widespread enhancement of extensor activity is through a longer latency (3.5-4.0 ms) excitation (Gossard *et al.* 1994; see also McCrea *et al.* 1995; Angel *et al.* 1996) that has been hypothesized to be evoked through interneurones within spinal central pattern generator (CPG) circuitry. Unlike extension-related disynaptic EPSPs, these longer latency EPSPs are largest during flexion (Gossard *et al.* 1994) and are voltage dependent; increasing in amplitude with motoneurone depolarization (Brownstone, Gossard & Hultborn 1994). While stimulation of ankle extensor nerve group Ia afferents will evoke monosynaptic excitation of close synergists, monosynaptic excitation of knee and hip extensors is weak (Edgley, Jankowska & McCrea 1986) and insufficient to evoke enhanced activity of hip and knee extensor motoneurones.

Studies during fictive locomotion show that lumbar motoneurones are subject to a pattern of rhythmic depolarization and hyperpolarization during the extensor and flexor phases of the step cycle. This pattern has been termed the locomotor drive potential (LDP) and is presumed to be produced by activity in the CPG (see Jordan 1983). The main objective of the present study was to investigate the intracellular events in motoneurones that accompany extension enhancement evoked by stimulation of ankle extensor group I afferents during fictive locomotion. In particular, we wished to examine the way in which extensor motoneurone LDPs are affected during extension enhancement and to determine whether short latency, locomotor-dependent group I excitatory reflexes are responsible for extensor motoneurone excitation during extension enhancement. Preliminary results have been presented in abstract form (Guertin, Angel, Jiménez & McCrea 1994).

Methods

Experiments were performed on 14 male or female cats weighting 2.0 - 3.25 kg and anaesthetized with halothane delivered in a nitrous oxide/oxygen mixture (70%-30%) for the dissection. Following a mid-collicular decerebration with removal of rostral brain tissue, anaesthetic was discontinued and fictive locomotion evoked by electrical stimulation of the mesencephalic locomotor region (MLR) in paralyzed animals. Further details of the preparation and methods for evoking locomotion are provided elsewhere (McCrea *et al.* 1995, Guertin *et al.* 1995). Combinations of at least eight ipsilateral hindlimb nerves and two contralateral nerves were dissected, cut and mounted on bipolar silver chloride hook electrodes for monitoring locomotor activity and for stimulation. These included semimembranosus (Sm) and anterior biceps (AB, usually put together as SmAB), posterior biceps and semitendinosus (PBSt), Sartorius (Sart, medial and lateral branches combined), quadriceps (Q) including the rectus femoris portion, plantaris (Pl), medial gastrocnemius (MG), lateral gastrocnemius (LG) and soleus (Sol, most often combined as LGS), the remaining part of the posterior tibial (Tib) including the muscular and cutaneous innervation of the plantar foot, and tibialis anterior (TA). The Q and Sart nerves were placed in bipolar cuff electrodes. Remaining femoral, sciatic and obturator nerve branches as well as tendons around the hip were cut bilaterally.

The dura was opened to expose the lumbar segments and glass microelectrodes filled with 50mM QX-314 in 2M sodium citrate (tip diameter 1.8-2.5 μm; resistance 2-3 MΩ) were used for intracellular recording from antidromically identified hindlimb extensor

motoneurones. Intracellular diffusion of QX-314 was used to block motoneurone action potentials (Frazier, Narahashi & Yamada 1970) and permit assessment of the locomotor drive potential (LDP, Jordan 1983). Locomotion was evoked by continuous MLR stimulation (10-15 Hz, 100 - 200 μA) for 1 - 2 minutes. Extension enhancement was produced by ankle extensor nerve stimulation (typically 100 Hz, 20 pulses) at group I strength ($\leq 2T$, where T is threshold for the most excitable afferents as recorded at the cord dorsum). Peripheral nerve stimulation was delivered independently of MLR stimulation and at a fixed delay from the onset of extensor nerve activity during fictive locomotion and usually only every third step. In three experiments, trains of small, rapid stretches of the Achilles' tendon (100 Hz, 30 stimuli, amplitude 30 μm, initial tension $<$ 5N) were used to activate group Ia muscle spindle receptors in isolation from group Ib tendon organ afferents (see also Guertin *et al.* 1995).

Electroneurograms (ENGs) were amplified and filtered (gain 5000-50000, -3 dB at 30 Hz and 3 kHz) before rectification and integration (envelope follower with a time constant of 100 ms). Intracellular and cord dorsum records and ENG activity were digitized on-line (10 kHz, 2 kHz and 500 Hz sampling rates respectively) along with markers for the MLR and peripheral nerve stimulation and extensor ENG activity (using a Concurrent 5450 computer and software developed at the Winnipeg Spinal Cord Research Centre). Subsequent analysis consisted of selecting periods of stable fictive locomotion and measuring the duration of the depolarized phase of the LDP and the duration of ENG activity. This analysis software also allowed calculation of pretriggered averages with the

and LDP ENG traces aligned to the onset of activity in a particular extensor ENG. Latencies of averaged PSPs produced by ankle extensor nerve stimulation or muscle stretch were calculated from the incoming cord dorsum volley. Results are presented as mean ± S.D. Statistics were calculated with a two tailed student's *t*-test.

Afferent systems mediating group I-evoked extension enhancement.

As in the preceding studies (Guertin *et al.* 1995; McCrea *et al.* 1995; Angel *et al.* 1996) electrical stimulation of ankle extensor nerves at strengths between 1.6*T* and 2*T* during the extension phase of the fictive locomotor step cycle was used to evoke locomotor-dependent excitatory reflexes in hindlimb extensors. Such intensities will recruit a substantial proportion of group I afferents while activating few group II afferents (Jack, 1978). Previous experiments (Guertin *et al.* 1995) have shown that extension enhancement can be evoked at intensities below 1.5*T*, (i. e., below threshold for activation of any group II afferents). Using such stimulus intensities with the ventral roots intact will produce antidromic activation of some motoneurones which theoretically could result in a disturbance of the locomotor cycle (Hammond, Miller & Scott 1981). In order to determine whether the extension enhancement under investigation was the result of antidromically activated systems, the rostral portion of the L7 ventral root was sectioned and the proximal portion stimulated during the extension phase of the fictive locomotor step cycle in one experiment. This stimulation was without effect on either the timing of the fictive locomotor step cycle or activity of extensor motoneurones. Stimulation of the Pl nerve remained effective in producing extension enhancement in this experiment. Stimulation of

the L4 and L5 ventral roots is also ineffective in perturbing the fictive locomotor step cycle (Perreault, Angel, Guertin & McCrea 1995). Activation of Ia muscle spindle afferents in isolation from Ib tendon organ afferents by vibration of the Achilles' tendon was also used to produce extension enhancement while avoiding antidromic activation of motoneurone axons. Thus while the contribution of antidromically activated systems to extension enhancement cannot be completely ruled out it seems more likely that the effects reported here are due to the orthodromic activation of group Ia and Ib afferents in ankle extensor nerves (see also Guertin *et al.* 1995).

Results

Results are presented from preparations in which stimulation of ankle extensor nerves (MG, LGS and Pl) at group I strength ($\leq 2T$, 100 - 200 ms trains at 200 Hz) during the extensor phase of fictive locomotion produced an increase in hindlimb extensor motoneurone activity and delayed the onset of flexion (Guertin *et al.* 1995). The effects of such stimuli on the LDPs of 33 motoneurones (6 Pl, 4 LGS, 2 MG, 1 Sol, 6 Q, 9 SmAB, 3 FDHL, and 2 Tib) and on the activity of motoneurone populations as recorded from rectified, integrated ENGs were examined. The LDPs of all motoneurones examined were depolarizing only during activity of hindlimb extensor nerves (e. g., SmAB, MG, Pl, LGS); motoneurones with depolarizing LDPs during both flexion and extension were not analyzed. The peak to peak amplitudes of LDPs were 5 -15 mV during unperturbed fictive locomotion. Latencies for the onset of group I-evoked extension enhancement as measured in the ENG recordings are of 10-40 ms using a similar preparation (Guertin *et al.* 1995). Analysis of the difference in latencies measured intracellulary and in the ENG during the locomotor step cycle are presented in the companion manuscript in this thesis. An assessment of the minimum latencies of motoneurone depolarization during extension enhancement is complicated by the presence of monosynaptic and disynaptic excitation in some extensor motoneurones. Intracellular latency measurements, therefore, are presented only occasionally in the present study but will be analyzed and reported elsewhere (preliminary results in Guertin, Perreault & McCrea 1995).

Comparison of the duration of activity in populations of motoneurones with the LDP duration in single motoneurones during unperturbed fictive locomotion.

-- Figure 1 near here --

Fig. 1 shows intracellular records from an LGS motoneurone (top trace, truncated records) and integrated-rectified extensor (LGS, Pl, SmAB and coSmAB) and flexor (Sart) ENGs during an episode of fictive locomotion evoked by continuous MLR stimulation at 12 Hz. In this example and unlike the rest of the material to be presented, a microelectrode without QX-314 was used in order to illustrate motoneurone action potentials during fictive locomotion. In this locomotor run there was a delay of several seconds from the onset of MLR stimulation to the appearance of the rhythmic hyper- and depolarizing LDP characteristic of fictive locomotion. About 1 second of this period is illustrated on the left of Fig. 1. The appearance of the LDP and action potentials in this motoneurone before detectable activity in the LGS ENG (second trace from top in Fig. 1) indicates that this motoneurone was among the first LGS motoneurones recruited during fictive locomotion. The low gain of the LGS ENG recording prevented detection of this motoneurone's activity but was necessary to avoid amplifier clipping as fictive locomotion developed. Once this motoneurone was recruited, LDP amplitude and action potential frequency remained relatively constant despite substantial variations in the duration of the depolarizing portion of the LDP. As fictive locomotion developed there was a progressive increase in the number of action potentials recorded in the periphery as evidenced by the increasing size of the integrated-rectified ENGs.

The duration of the depolarizing portion of the LDP was defined as either the portion of the LDP above the membrane potential prior to MLR stimulation (e. g., horizontal dotted line in Fig. 1 and Fig. 3B) or, in cells in which the membrane potential prior to MLR stimulation was not recorded or suspected of drifting, the depolarizing portion of the LDP was defined as that duration above one-half peak to peak amplitude (e. g., the data illustrated in Fig 2 C-D). Comparisons of the duration of the depolarizing portion of the LDP to the duration of extensor ENG activity during fictive locomotion showed that spontaneous variations in LDP duration were associated with similar variations in the duration of action potential production in both ankle (LGS and Pl) and hip extensor motoneurone (SmAB) pools (e. g., see the LDPs labelled A and B in Fig. 1).

--Figure 2 near here--

Figure 2 presents a further analysis of the relationship between the duration of ENG activity and the duration of the depolarizing portion of the LDP in four extensor motoneurones from three experiments. Data from locomotor runs without peripheral nerve stimulation are indicated by open symbols. Figure 2A plots data obtained from the locomotor run illustrated in Fig. 1 and shows that spontaneous variations in the duration of action potential production in the LGS motoneurone pool from 175 to 350 ms were accompanied by corresponding changes in the duration of the depolarizing portion of the LDP recorded in this LGS motoneurone (see Fig. 2 legend for the equations of the linear regression lines). In panels B-D the relationships between the duration of firing in a motoneurone pool and LDP duration were obtained in motoneurones in which action

potentials were abolished by intracellular diffusion of QX-314. Panel B plots data for another LGS motoneurone and the homonymous ENG. Panel C plots data for a hip extensor (SmAB) LDP and its homonymous ENG and panel D for the LDP of a motoneurone innervating the plantar foot (Tib) and a heteronymous (hip extensor) ENG. In all examples in Fig. 2 there is a strong association between the duration of action potential activity in the population of motoneurones (i. e., ENG bursts) and the duration of the depolarizing portion of the LDP recorded in randomly sampled motoneurones. Qualitatively, there was good correspondence between the depolarization of a single motoneurone and action potential production in homonymous or close synergist motoneurone pools for all 33 LDPs examined.

LDP prolongation during group I-evoked extension enhancement.

--Figure 3 near here--

Figure 3A shows the effects of a group I intensity stimulus train (200 Hz, 30 pulses) to the MG nerve on the LDP of an LGS motoneurone (top trace) and the activity of extensor and flexor (TA) ENGs during fictive locomotion. Stimulation delivered every third step cycle during extension produced an increase in the amplitude and duration of both the LDP in this LGS motoneurone and of ENG bursts in LGS and SmAB nerves. There was a corresponding delay in the onset of the subsequent flexor (TA) discharge with the increase in the duration of extensor motoneurone activity. The LGS ENG and LDP durations during group I strength nerve stimulation are shown with the filled symbols in Fig. 2B. Mean duration of the LDP in this LGS motoneurone was increased from 576 ± 86

(n=25) to 687±43 ms (n=14; $p < .01$) with this stimulation. Corresponding mean values for the increase in LDP duration with ankle extensor nerve stimulation for the SmAB LDP in panel 2C are from 669 ± 119 to 906 ± 117 ms (n=18; $p < .01$) and for the Tib motoneurone in panel 2D from 165 ± 24 to 215 ± 21ms (n=28, $p < .01$). In all examples the duration of the depolarizing portion of the LDP is associated with a similar duration of activity in the extensor nerve during both spontaneously occurring variations in the step cycle and during group I-evoked extension enhancement. In 29 of the 33 motoneurones examined, it was possible to compare the mean duration of the depolarizing portion of the LDP during extension enhancement with the mean duration of activity in the homonymous or close synergist ENG. In all 29 cases mean LDP and close synergist ENG durations were within 5% of each other. In the 7 hip or knee motoneurones where a comparison between LDP and the heteronymous ankle ENG duration was made, LDP duration was within 15% of the ankle ENG and in 6 of 7 cases the LDP was shorter than the ankle extensor ENG duration. These observations show that the duration of action potential production recorded in the integrated, rectified ENG is a good measure of the LDP duration recorded in randomly sampled motoneurones with a closer correspondence between homonymous (e. g., 2A-C) than heteronymous (2D) nerve activity and LDP durations.

Changes in LDP amplitude and shape during extension enhancement.

Increases in the amplitude of extensor motoneurone LDPs during group I-evoked extension enhancement are evident both in the raw records in Fig. 3A and in the averaged

traces in 3B. The effects of nerve stimulation on the LDP were variable; for example in Fig. 3A there is a large and maintained increase in the amplitude of the LDP with first (left) but not the second stimulus train presentation. These LDP variations during extension enhancement are accompanied by qualitatively similarly responses in the ENG. The solid line traces in 3B are averages of 14 steps in which the MG nerve was stimulated and the dotted traces are averages of 25 unstimulated (control) steps during the period of locomotion displayed in Fig. 2B and in part in 3A. Soon after the onset of MG nerve stimulation (vertical dotted line) the amplitude of the LGS LDP increased nearly 10 mV above control levels and remained increased for a period following termination of nerve stimulation. Except for the initial rising phase, the time course and general shape of the LDP during extension enhancement is accompanied by a similarly shaped increase in homonymous (LGS) nerve activity (Fig. 3B second trace from top). The onset of the stimulus-evoked depolarization in this LGS motoneuron (Fig. 3B) occurred at a latency of 0.7 ms which corresponds to the arrival of the first of a series of monosynaptic EPSPs (inset, also described later). In averaged records there was a good correspondence between the shape of the LDP and the homonymous ENG during both unperturbed and perturbed fictive locomotion. The horizontal arrows in Fig. 3B show that this stimulation prolonged the duration of activity in both ankle and hip extensors and delayed the onset of flexion a similar amount, i. e., that it was effective in evoking extension enhancement (Guertin *et al.* 1995). Unlike the more uniform prolongation of activity in all ipsilateral hindlimb extensors, the shapes of LDPs and rectified ENGs were most similar for homonymous motoneurones during group I-evoked extension enhancement. For example in Fig. 3, the

shape of the LGS motoneurone LDP is not similar to the activity profile recorded in the SmAB nerve.

--Figure 4 near here--

Two more examples of the changes in extensor LDP and ENG activity produced by group I strength stimulation of ankle extensor nerves are shown in Fig. 4. In both cases (A, C) ankle nerve stimulation at group I strength prolonged the duration of hindlimb extensor activity. Figure 4A shows the averaged LDP in a SmAB motoneurone with (n=18) and without (n=36) stimulation of the MG nerve (1.8*T*, 30 pulses, 100 Hz). While the duration of this LDP was increased by MG nerve stimulation (filled symbols in Fig. 2C) the modest increase in LDP amplitude only occurred following termination of MG nerve stimulation. During MG nerve stimulation there was a slight reduction in LDP amplitude in this motoneurone. While no attempt was made to identify the source this slight hyperpolarization it could be due to either recurrent inhibition from MG to SmAB (Hultborn, Jankowska & Lindström 1971) or weak non-reciprocal inhibition. As seen in the SmAB ENG (Fig. 4A, inset) action potential production in the SmAB motoneurone pool was also not increased until after the stimulus train.

In these preparations Tib motoneurones behaved as extensors with their activity always in phase with ankle and hip extensors. Figure 4C shows the enhancement of a Tib motoneurone LDP produced by Pl nerve stimulation. Compared to LDPs in steps without Pl stimulation (n=56), LDPs were about 2 mV larger (n=28) following the onset of stimulation (vertical dotted line) and prolonged by about 50 ms (measurements in Fig. 2D).

The increased depolarization was evident after a latency of about 5 ms. In this example, the stimulus train evoked an extension enhancement and delayed the onset of flexion, but flexor activity returned before the end of the stimulus train (the onset of flexion is indicated by the arrow F in 4C). The intracellular records show the increase in LDP amplitude during extension followed by a fall to a less depolarized period during the normally hyperpolarized flexion phase. The averaged SmAB ENG records inset in Fig. 4C suggest that the effects of Pl nerve stimulation were qualitatively similar in hip extensors and the Tib motoneurone. In this example the latency of the increase in SMAB ENG activity was about 30 ms.

-- Figure 5 near here--

The data in Figs. 3 and 4 illustrate a range of effects seen in motoneurones during group I-evoked extension enhancement. There is a good correspondence between the prolongation of the LDP in the motoneurone and increased duration of activity in the ENG. No attempt was made to quantify the changes in shape of the LDP during the group I strength stimulus train. Qualitatively, however, there was often a strong similarity between action potential production in the homonymous motoneurone pool as indicated by the shape of the rectified-integrated ENG and the shape of the LDP in the impaled motoneurone (e.g., Figs. 3, 4A). In Fig. 5A, on the other hand, the increase in activity in the motoneurone pool during extension enhancement was not reflected by an increased level of depolarization in the homonymous motoneurone that was impaled. In this example, electrical stimulation of the Pl nerve (right portion of Fig. 5A) increased the duration of

both the LDP and homonymous ENG activity substantially but had no effect on LDP amplitude. In 7 of 11 ankle extensor motoneurones and 7 of 13 hip, knee or toe extensor motoneurones where the comparison could be made, the shape of the LDP was similar to that of the ENG recorded in the homonymous or close synergist (same joint) nerve. The correspondence between LDP shape and a heteronymous extensor ENG was less striking (e.g., Fig. 3B) with only 7 of 31 showing good similarity.

In four motoneurones (two experiments) triceps surae and Pl group Ia spindle afferents were activated in isolation from Ib tendon organ afferents by the use of Achilles' tendon vibration (see Methods). Vibration during extension produced extension enhancement similar to that evoked by combined activation of group Ia and Ib afferents with electrical stimulation; prolonging and in some cases further depolarizing the LDP. In the example shown in Fig. 5A (left), vibration produced effects in the SmAB and Q nerves and in this SmAB motoneurone that were, if anything, larger than those resulting from electrical stimulation of the Pl nerve (Fig. 5A right). The duration of hip, knee and ankle extensor activity was prolonged beyond the end of the stimulus and the onset of flexor motoneurone (Sart) activity was delayed (see also Guertin *et al.* 1995). Neither vibration nor electrical stimulation resulted in further depolarization of the LDP in this SmAB motoneurone but as judged from the ENG records, both increased the discharge of other SmAB as well as Q motoneurones. The increase in extensor ENG activity occurred with latencies of approximately 10-30 ms following the first shock.

The effects of vibration in a MG motoneurone are shown in the averaged records in

Fig. 5B. Selective activation of group Ia afferents resulted in a sustained depolarization that was maintained following the end of vibration before falling to a hyperpolarizing level with the onset of activity in the Sart nerve. The sustained activity in the MG motoneurone pool following vibration (bottom records, Fig. 5B) resembles the maintained plateau of depolarization in this motoneurone. The increase in MG nerve activity during vibration in panels A and B is likely the result of both increased efferent activity and afferent activity produced by vibration of the MG tendon. Afferent activity during vibration may account for the sharp increase in MG nerve activity in Fig. 5A during vibration but not electrical stimulation of the Pl nerve. The averaged Sart records show a sharp increase in flexor nerve activity immediately following the step in which extension enhancement was evoked (solid lines). Such increases were infrequent during both these and previous (Guertin *et al.* 1995) experiments on extension enhancement.

Short-latency postsynaptic potentials during group I-evoked extension enhancement.

As mentioned in the Introduction, activation of ankle extensor group I afferents will evoke a variety of stimulus-locked short-latency postsynaptic potentials in extensor motoneurones. In order to examine such reflexes, averaged intracellular records during the stimulus train are illustrated in Figs. 3-5 at high-gain and on an expanded time scale. The inset in Fig. 3B shows the PSPs evoked in this LGS motoneurone at the start (section 1) and near the end (section 2) of the stimulus train. Stimulation of the MG nerve evoked a monosynaptic EPSP (latency 0.7 ms, filled arrows) followed by another EPSP with a latency of approximately 1.8ms (open arrows). This later EPSP is the group I disynaptic

EPSP that is activated by extensor group I afferents during the extension phase of MLR-evoked fictive locomotion (McCrea *et al.* 1995; Angel *et al.* 1996). Towards the end of the stimulus train in Fig 3B, the disynaptic EPSP became smaller, almost disappearing at the peak of the LDP while the monosynaptic EPSP at this time became slightly larger. While not definitive, this reduction in disynaptic EPSP size is further argument against a strong voltage dependence being responsible for the gating of disynaptic excitation to only the extension portion of the fictive locomotor step cycle (see Angel *et al.* 1996). Following the stimulus train and in the absence of monosynaptic and disynaptic excitation, the LDP fell in amplitude but remained more depolarized than control records (dotted trace).

The examples in Fig. 4 B and D show short-latency group I PSPs with an inhibitory component. In the SmAB motoneurone illustrated in Fig. 4A, MG nerve stimulation evoked a small (≈0.5 mV) oligosynaptic EPSP at a latency of 2.5 ms both early (Fig. 4B) and later (not illustrated) in the stimulus train. This EPSP was followed by an IPSP with the net effect being a slight hyperpolarization during stimulation (Fig. 4A). From the reduction in firing in the SmAB nerve during the stimulus train one could infer that similar effects occurred throughout the population of SmAB motoneurones active during fictive locomotion (Fig. 4A, inset). Another mixture of PSPs occurred in the Tib motoneurone illustrated in Fig. 4C and D. While the net effect of Pl nerve stimulation was a depolarization that began soon after stimulus onset; the dominant short-latency, stimulus locked effect was inhibition that followed the small monosynaptic (0.9 ms, 0.5 mV) EPSP (Fig. 4D1). Following the monosynaptic EPSP there is IPSP with a latency of about 1.9

ms. Later in the train, and following the onset of flexion (arrow), this IPSP was almost abolished (Fig. 4D2). This motoneurone is thus an example of LDP enhancement where the motoneurone was depolarized despite the short-latency reflexes being predominately hyperpolarizing.

In the motoneurone illustrated in Fig. 5A no short-latency reflexes were detectable in either the continuous or averaged records (not shown). In Fig. 5B each vibration produced large, stimulus-locked EPSPs. The early portion of the LDP enhancement shown at higher gain in C reveals the presence of a large monosynaptic EPSP (≈5 mV, 0.7 ms latency, black arrows, Fig. 5C) followed by a probable small disynaptic EPSP (≈1-1.9 ms). The action potentials occurring in some of the traces comprising this average were a result of incomplete diffusion of QX-314. In both Fig. 5A and B, LDPs were prolonged beyond the stimulus train with the one in Fig. 5B appearing very much as a plateau-shaped event.

Figures 3-5 thus illustrate that in some cases there was little relationship between the nature and size of short-latency, stimulus locked group I reflexes and the enhancement of the LDP as seen in the impaled motoneurone or as inferred from the integrated, rectified ENG. LDPs could be increased in amplitude despite the nature or presence of PSPs but the largest increases during extension enhancement were often found in ankle extensor motoneurones. This not surprising since compared to other extensors, ankle extensor motoneurones receive the largest Ia monosynaptic excitation following ankle extensor nerve stimulation (Eccles *et al.* 1957a). The maintenance of a steady, plateau-like depolarized LDP and ENG activity beyond the end of the stimulus train during extension

enhancement is consistent with the hypothesis that premotoneuronal elements maintain or permit the expression of depolarizing active conductances in the motoneurones.

Discussion

Group I intensity stimulation of ankle extensor nerves delivered during the extension phase of the fictive locomotor step cycle increases the activity of extensor motoneurones operating at hip, knee and ankle joints (Guertin *et al.* 1995; present results). A main finding of the present study is that this extension enhancement involves an increase in the duration and/or level of the depolarizing portion of the LDP that is distributed throughout the hindlimb to most extensor motoneurones. Earlier studies argued against a contribution of Ia afferents to perturbations of the fictive locomotor step cycle (Conway *et al.* 1987; Pearson & Collins 1993; Gossard *et al.* 1994) but this view can no longer be supported. Selective activation of group Ia afferents resets the step cycle, increases extensor activity (Guertin *et al.* 1995; present results Fig. 5) and evokes disynaptic excitation of hindlimb extensors (McCrea *et al.* 1995; Angel *et al.* 1996; Fig. 5B). Achilles' tendon vibration which recruits group Ia afferents in four muscle nerves produces effects during locomotion that are similar and in some cases larger than combined activation of Ia and Ib afferents in a single nerve using electrical stimulation (Fig 5A; also Guertin *et al.* 1995). The activation of group Ia afferents in only a single ankle extensor nerve in other studies (Conway *et al.* 1987; Pearson & Collins 1993; Gossard *et al.* 1994) is the likely explanation for previous failures to show a contribution of group Ia afferents to step cycle regulation.

The relationship between the LDP and extensor motoneurone activity.

Comparison of homonymous motoneurone LDPs and ENGs show good correspondence

between the profile of depolarization recorded in randomly sampled motoneurones in which action potentials were blocked and the profile of action potential production in the homonymous motoneurone pool. The notion that all members of a motoneurone pool receive similar synaptic drive during locomotion has been previously made during studies in which the firing of single motor units were compared to whole muscle EMG activity (Hoffer Sugano Loeb Marks O'Donovan & Pratt 1987). A similar suggestion has also been made during voluntary movements in man (De Luca, Le Fever, McCue & Xenakis 1982). According to this viewpoint (reviewed in De Luca & Erim 1994) some motor programs produce a common excitation that is distributed to all motoneurones in a pool. The recruitment of individual motor units, however, varies according to their individual membrane properties. Increases in this common drive result in both increased firing of already recruited motor units and recruitment of new units. Present results provide direct support for these ideas. There was a high correlation between both homonymous and heteronymous LDP and ENG durations (Fig. 2) and good correspondence between homonymous LDP and ENG shapes following the stimulus train. During the stimulus train, differences in short-latency reflex effects between motoneurones likely contribute to a weaker correspondence between LDP and ENG shapes (e.g., Fig 5A but note the good correspondence in Fig. 3B and 5B).

The present observations on the general correspondence between increased action potential production measured in the ENG and the motoneurone LDP profile during both unperturbed and perturbed step cycles show that there can be a rather simple relationship

between motoneurone depolarization and action potential production. This conclusion is supported by earlier studies showing that during slowly rising depolarizations, the repetitive firing of motoneurones is linearly related to the level of the depolarization (Kolmodin and Skoglund, 1958). Further work has shown that repetitive firing rates of motoneurones can be predicted from the level of depolarization induced by intrasomatic current injection that in turn activates currents underlying the afterhyperpolarization (AHP) (reviewed in Gustafsson 1984). Thus in non locomoting preparations the control of repetitive firing in motoneurones is dominated by the AHP. During fictive locomotion, however, motoneurone AHPs are suppressed and there is little correlation between motoneurone firing frequency and somatic depolarizations resulting from intracellular current injection (Brownstone Jordan Kriellaars Noga & Shefchyk 1992). This results in motoneurone firing rates being faster during locomotion than those in non-locomoting preparations and suggests that firing during locomotion is not simply the result of the addition of depolarizing current by the CPG (Brownstone *et al.* 1992). The induction of an intrinsic, voltage-dependent excitation of motoneurones would cause the relationship between somatic current injection and firing to become non-linear (Brownstone *et al.* 1992). We propose that the increase in firing during extension enhancement results partly from an induction of voltage-dependent currents and this occurs throughout the motoneurone pool. Accordingly, even though there are non-linear relationships between applied currents in motoneurones and firing during locomotion (Brownstone *et al.* 1992), there could still be a linear relationship between the voltages resulting from these currents and firing. Membrane depolarization would then be a linear reflection of changes in firing

in the homonymous motoneurone pool (i. e. ENG). The present evidence of a simple relationship between motoneurone depolarization and firing should assist the development of realistic models of motoneurone activity during locomotion.

The use of QX-314 facilitated the examination of the LDP but by abolishing action potential production, prevented an assessment of motoneurone recruitment. The present experiments could not, therefore, determine the extent to which enhanced ENG activity during group I stimulation resulted from an increased firing of already recruited motoneurones or from a recruitment of additional units. The prolongation of the LDP following group I stimulation would suggest that extension enhancement partly involves prolonged activity of already recruited motoneurones.

Neuronal pathways responsible for group I-evoked extension enhancement

The Introduction reviewed the changes in group I reflex pathways that can occur during fictive locomotion and the emergence of group I-evoked disynaptic excitation of extensors. Our previous studies on disynaptic excitation (McCrea *et al.* 1995; Angel *et al.* 1996) used single nerve shocks which do not produce extension enhancement that is obvious in the ENG recordings. The present study used trains of stimuli which could evoke disynaptic excitation and summate to contribute substantially to extension enhancement. An important observation, therefore, is that extension enhancement can be produced without evoking obvious stimulus-locked EPSPs in motoneurones (e.g.,Fig 5A). Thus extension enhancement can be evoked by activation of either premotoneuronal or excitatory intrinsic motoneuronal mechanisms that are not expressed as discrete, stimulus-locked events.

Premotoneuronal mechanisms would include excitatory actions of group I afferents on the CPG circuitry which is discussed here as a half-centre model of the spinal CPG. The essential elements of this organization are two excitatory systems, one for flexor and one for extensor motoneurones with mutual inhibition between these excitatory systems (e.g., Lundberg 1981). In agreement with others (Gossard *et al.* 1994; Pearson 1995; McCrea *et al.* 1995; Angel *et al.* 1996) the present data argues strongly for excitatory actions of group I afferents on extensor-related elements in the CPG. Continued and prolonged operation of the extensor portion of the CPG that was triggered by peripheral nerve stimulation is consistent with the observation of an increased LDP duration when stimulus-locked effects are over in motoneurones (i. e., beyond the end of stimulation). Furthermore, the strong similarity between the larger or longer duration LDPs in randomly sampled motoneurones and action potential production in the homonymous motoneurone pool is suggestive of effects being evoked through a CPG organized to distribute a common excitation to hindlimb extensors. Although the present data were limited to an examination of extensor motoneurones, unpublished observations show that accompanying the excitation of extensor motoneurones during extension enhancement there is inhibition of all flexor motoneurones (Guertin *et al.* 1995; Guertin, Perreault & McCrea 1995). Again this is consistent with a half-centre organization of the CPG.

In those cases where group I strength stimulation produced stimulus locked, short-latency PSPs, these would summate with depolarizing effects evoked through the CPG circuitry. For example, monosynaptic group Ia excitation would combine with the more

widely distributed disynaptic, group I-evoked excitation (Angel *et al.* 1996) to contribute to extension enhancement. Accordingly during extension enhancement produced by ankle nerve stimulation, the larger monosynaptic excitation of ankle extensors (Eccles *et al.* 1957) could result in a greater depolarization than seen in heteronymous synergists (compare the LGS and SmaB ENGs in Fig. 3). Proprioceptive feedback from group Ia or Ib afferents in several extensor nerves during real locomotion could result in substantially more extensor motoneurone depolarization than demonstrated here with single nerve stimulation. Thus in addition to determining the timing of the step cycle through actions on CPG circuitry, proprioceptive extensor group I afferent activity may regulate the intensity of extensor bursts throughout the hindlimb. The ability of extensor group I afferents to increase extensor activity has also been shown in decerebrate cats during treadmill locomotion (Whelan *et al.* 1995) and in awake animals with perturbations evoked during quiet standing (Pratt, 1994).

As mentioned in the Introduction, locomotion appears to involve the induction and regulation of voltage dependent motoneurone conductances that may result in persistent motoneurone depolarization in the absence of continuous excitatory synaptic bombardment. Accordingly, the CPG may enable voltage-dependent conductances during the depolarizing portion of the LDP (Brownstone *et al.* 1994). Because no attempt was made to examine the nature or voltage dependency of the LDP, we cannot comment on whether the shape of the enhanced LDP is partly determined by a Ca^{2+} dependent conductance characteristic of the plateau potential (Hounsgaard *et al.* 1988). Observations such as those in Figs. 3B

and 5B, however, show that "plateau-shaped" depolarizations may be maintained at different levels during and after the group I stimulus train. Thus even small depolarizations evoked by short-latency segmental reflexes such as mono- and disynaptic excitation may summate to bring the motoneurone to a depolarizing potential that can sometimes be maintained by intrinsic conductances (e. g., Fig. 5B). Differences in the expression or saturation of such conductances between motoneurones could result in a discrepancy between the shapes of an LDP and the synergist ENG (e.g., Fig. 5A).

In summary, motoneurone excitability during locomotion is influenced by a number of factors including; 1) synaptic input arising from activity in the CPG circuitry responsible for the basic locomotor pattern, 2) the control of intrinsic motoneurone conductances that promote or regulate motoneurone depolarization and action potential production, 3) input from supraspinal systems, 4) and sensory feedback produced by interactions between the limbs and the environment. The present and companion studies (Guertin *et al.* 1995; McCrea *et al.* 1995; Angel *et al.* 1996) provide evidence that group I afferents can increase extensor motoneurone activity by actions that are evoked through the CPG and by recruiting short-latency, locomotor dependent reflex pathways organized in parallel to the CPG. An understanding of the role that intrinsic motoneurone currents play in responding to the reflex actions of group I afferents must await experiments designed to study such conductances. The net result of these mechanisms is a reflex reversal from a system of inhibitory group I actions at rest to a system that promotes the extensor phase and excites extensor motoneurones during locomotion. In more natural conditions,

imposing a brief and rapid stretch to ankle extensor muscles during walking increases the intensity of extensor contraction during stance in humans (Yang, Stein & James 1991).

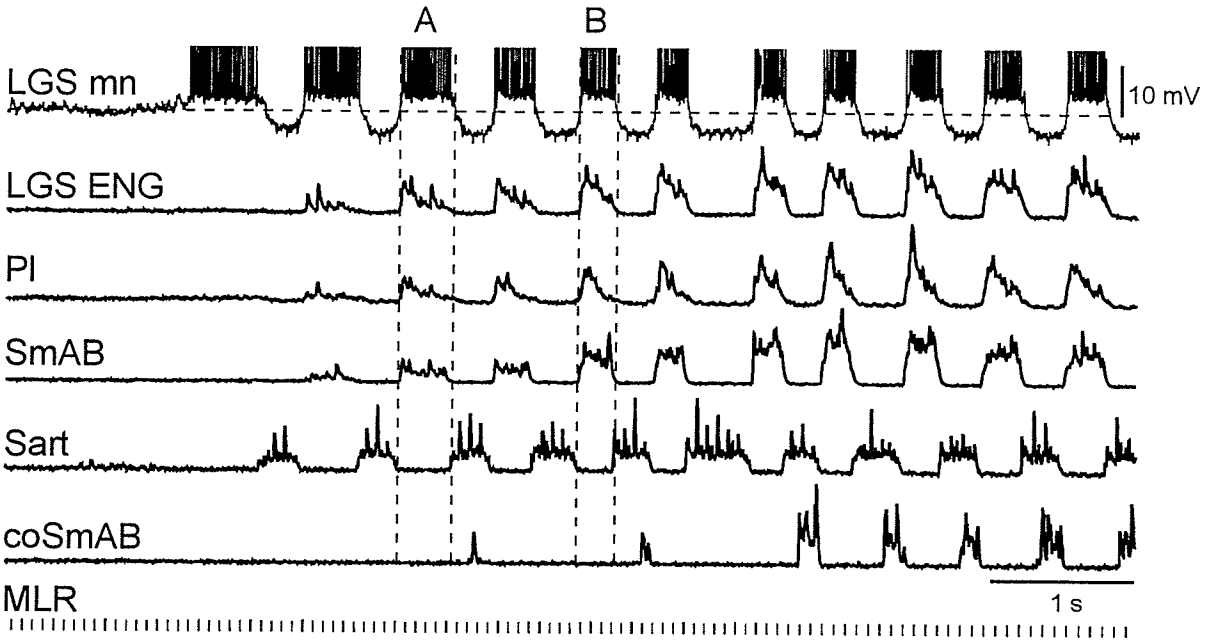

Figure 1. *Activity recorded in a LGS motoneurone and from hindlimb muscle nerves during fictive locomotion evoked by MLR stimulation*
The top trace is an DC-coupled intracellular record from a LGS motoneurone with a membrane potential before locomotion of -60 mV (horizontal dashed line). Action potentials were > 70 mV and are truncated for illustration purposes. Except for the bottom trace, the other traces are integrated and rectified (sampling rate 500 Hz) ENGs from ipsilateral extensor (LGS, Pl, Q, SmAB), flexor (Sart) and contralateral extensor nerves (coSmAB). The amplitude of the ENG traces are uncalibrated. The bottom trace shows markers indicating the stimuli to the MLR (100 μA, 1 ms). The start and end of depolarized phase of two LDPs labelled A and B are represented by the vertical dotted lines plotted where the LDP trace crossed the pre-locomotor membrane potential. Spontaneous variations in LDP duration are accompanied by similar changes in the duration of extensor motoneurone activity recorded in the ENGs.

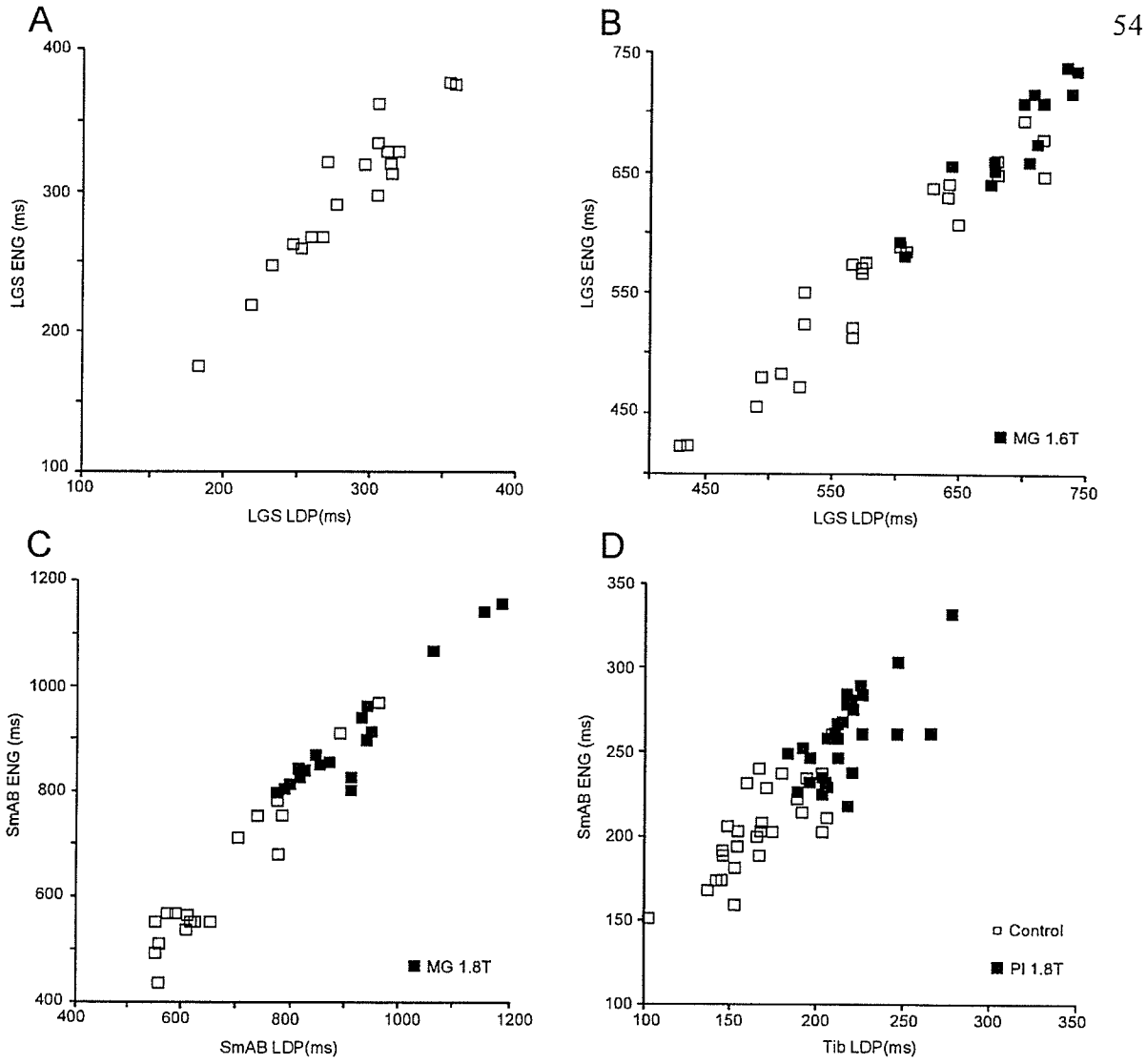

Figure 2. *Variations in the duration of the depolarizing portion of a LDP are associated with similar variations in action potential production in motoneurone populations*
The duration of extensor ENG activity during fictive locomotion is plotted against the duration of the depolarized phase of a LDP during a series of successive steps. Measurements obtained with and without group I intensity stimulation of an ankle extensor nerve are represented by solid and open symbols respectively. The motoneurones from which LDP measurements were made in panels A-D are illustrated in Figs. 1, 3, 4A and 4C respectively. In all examples there is good correspondence between extensor ENG and LDP durations. The linear regression equations are:

A: □ LGS_{ENG} ms = -21 + 1.12 • LGS_{LDP}; r^2 = .90
B: □ LGS_{ENG} ms = 31 + .915 • LGS_{LDP}; r^2 = .94
■ LGS_{ENG} ms = -33 + 1.026 • LGS_{LDP}; r^2 = .93
C: □ $SmAB_{ENG}$ ms = -146 + 1.162 • $SmAB_{LDP}$; r^2 = .93
■ $SmAB_{ENG}$ ms = 83 + .897• $SmAB_{LDP}$; r^2 = .91
D: □ $SmAB_{ENG}$ ms = 68 + .816 • Tib_{LDP}; r^2 = .58
■ $SmAB_{ENG}$ ms = 82 + .815 • Tib_{LDP}; r^2 = .67

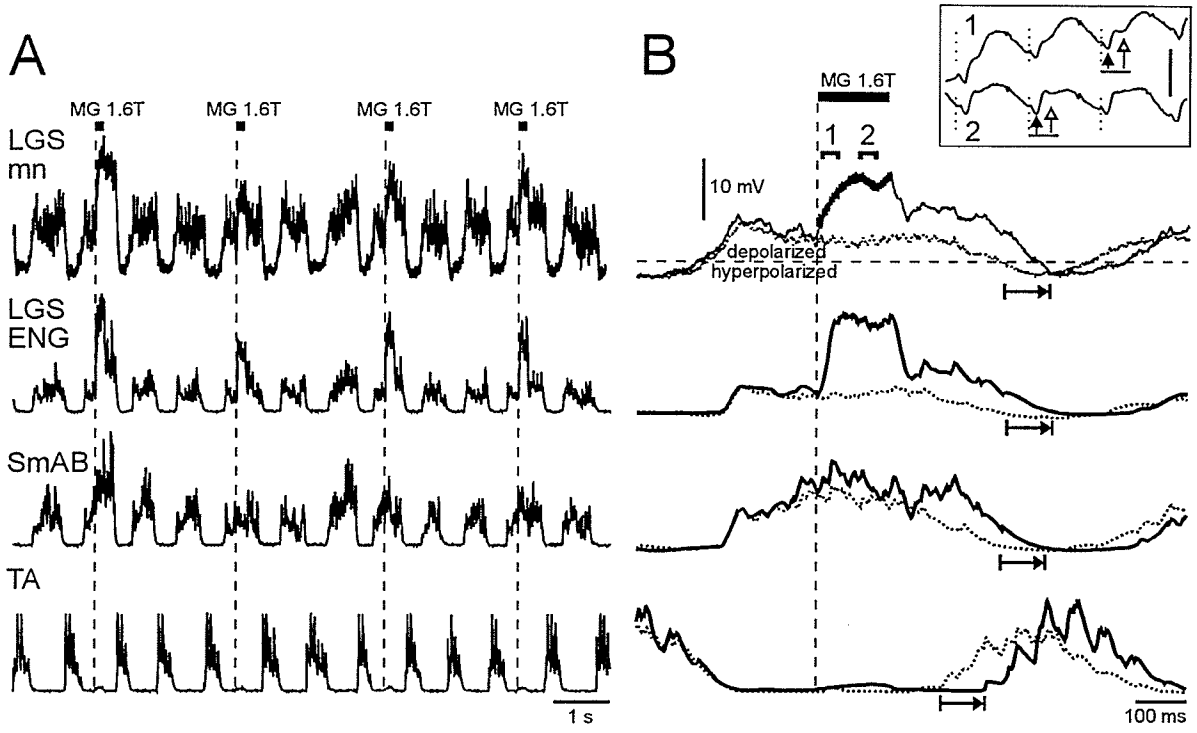

Figure 3. *Group I stimulation of ankle extensor nerves during extension prolongs and increases the LDP of synergist extensor motoneurones*

A train of pulses was delivered every third step to the MG nerve (1.6T, 200 Hz, 30 shocks, pulse duration 0.1 ms) about 300 ms after the onset of activity in the LGS ENG. The vertical dotted lines show the onset of the stimulus train. In this and the following figures, action potentials were blocked in the impaled motoneurone with intracellular diffusion of QX-314. A, continuous intracellular records from a LGS motoneurone (top trace) and ENG activity from extensor (LGS, SmAB) and flexor (TA) nerves. B, averaged records during control (dotted) and with MG stimulation (solid) are superimposed. The horizontal dashed line shows the pre-locomotor membrane potential (65 mV). The regions of the LDP labelled 1 and 2 are shown on an expanded scale in the inset. Calibration bars are 2 mV and 2 ms. Vertical dotted lines in the inset show the time of arrival of the afferent volley at the cord dorsum. Filled arrows indicate the monosynaptic Ia EPSP and the open arrows the disynaptic group I EPSP.

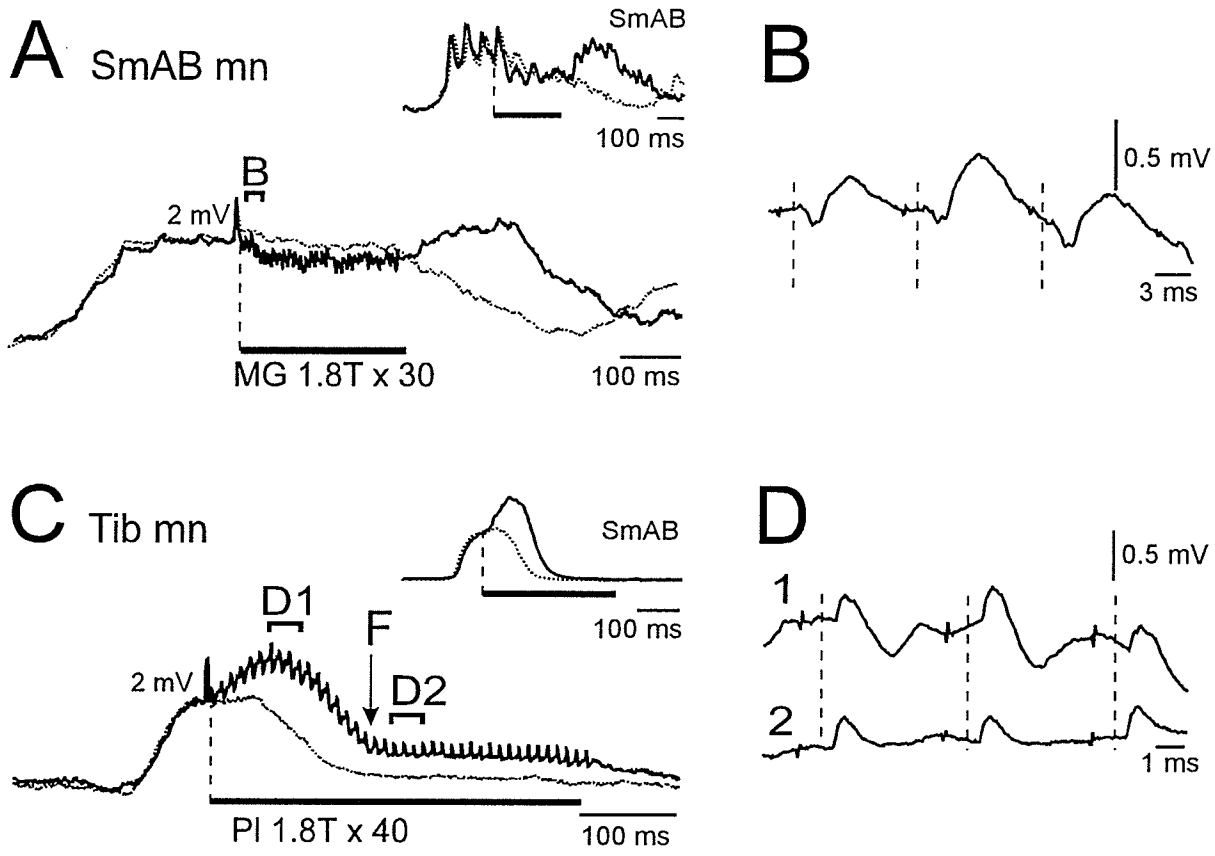

Figure 4. *Ankle extensor nerve stimulation enhances the LDPs of heteronymous extensor motoneurones*

A, C. averaged control intracellular records (dotted traces) are superimposed on those obtained following group I stimulation (duration indicated by the thick horizontal lines) of ankle extensor nerves (solid traces). Averaged ENG records obtained simultaneously are inset. B, D, expanded portions of the intracellular averages during nerve stimulation for panels A and C respectively. Other details as in Figure 3. A. 36 control and 18 stimulation trials were used to calculate these averages which show an increase in the amplitude and duration of the LDP following the stimulus train. B, during stimulation a combination of excitatory and inhibitory PSPs resulted in a small hyperpolarization C, stimulation of the PI nerve every third step resulted in depolarization despite the predominately hyperpolarizing nature of the stimulus locked PSPs shown in panel D.

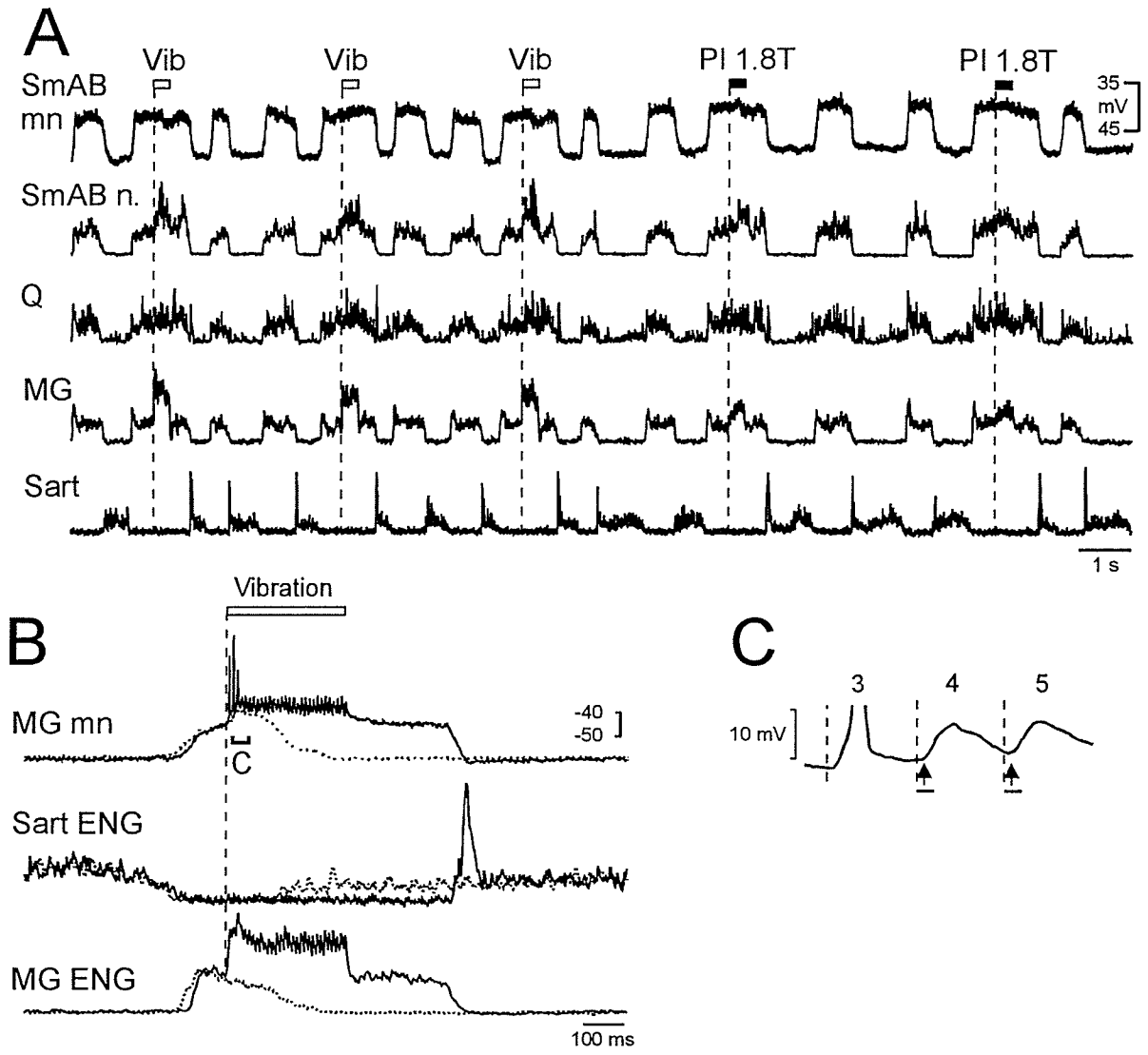

Figure 5. *Activation of muscle spindle primaries also evokes enhancement of extensor motoneurone LDPs*

A. shows continuous records from a SmAB motoneurone (top) and extensor (SmAB, MG) and flexor (Sart) ipsilateral nerves during an episode of fictive locomotion. Vibration of the Achilles' tendon (initial tension 300 grams, 35 μm) is indicated by the open box and electrical stimulation of PI by the solid box. While there were no obvious stimulus locked PSPs in this motoneurone, enhanced activity of extensor motoneurones was produced both by activation of muscle spindle primaries with vibration and by activation of both Ia and Ib afferents with electrical stimulation. B, averaged records (n=7 control; n=3 with vibration) show that vibration resulted in a substantial prolongation of the MG motoneurone LDP and MG ENG activity while delaying the onset of flexor (Sart) activity. The arrival of the cord dorsum volleys is shown as vertical dotted lines and monosynaptic EPSPs by vertical arrows. C, expanded records of the LDP during the 3-5th stretch of the Achilles tendon. Horizontal bars indicate 2 ms.

References

Angel, M.J., Guertin, P., Jiménez, I. & McCrea, D.A. (1996a). Group I extensor afferents evoke disynaptic EPSPs in cat hindlimb motoneurones during fictive locomotion. *Journal of Physiology* **494**, 851-861.

Angel, M.J., Jankowska, E. & McCrea, D.A. (1996b). Fictive locomotor activity of candidate interneurones mediating disynaptic excitation of extensors in the cat. *Journal of Physiology* submitted.

Brownstone, R. M., Gossard, J.- P. & Hultborn, H. (1994). Voltage-dependent excitation of motoneurones from spinal locomotor centres in the cat. *Experimental Brain Research* **102**, 34-44.

Brownstone, R.M., Jordan L. M., Kriellaars D. J., Noga B. R., & Shefchyk S. J. (1992) On the Repetitive Firing in Lumbar Motoneurones During Fictive Locomotion in the Cat. *Experimental. Brain. Res.* **90**: 441-55.

Conway, B. A., Hultborn, H. & Kiehn, O. (1987). Proprioceptive input resets central locomotor rhythm in the spinal cat. *Experimental Brain Research.* **68**, 643-656.

De Luca, C.J. & Erim, Z. (1994). Common drive of motor units in regulation of muscle force. *Trends in Neuroscience* **17** (7), 299-305.

De Luca, C.J., Le Fever, R.S., McCue, M.P. & Xenakis, A.P. (1982). Control scheme governing concurrently active human motor units during voluntary contractions.

Journal of Physiology **329**, 129-142.

Eccles, J.C., Eccles, R.M. & Lundberg, A. (1957a). The convergence of monosynaptic excitatory afferents onto many different species of alpha motoneurones. *Journal of Physiology* **137**, 22-50.

Eccles, J. C., Eccles, R. M. & Lundberg, A. (1957b). Synaptic actions on motoneurones caused by impulses in Golgi tendon organ afferents. *Journal of Physiology* **138**, 227-252.

Edgley, S., Jankowska, E. & McCrea, D.A. (1986). The heteronymous monosynaptic actions of triceps surae group Ia afferents on hip and knee extensor motoneurones in the cat. *Experimental Brain Research* **61**, 443-446.

Frazier, D.T., Narahashi, T. & Yamada, M. (1970). The site of action and active form of local anesthetics. II. Experiments with quaternary compounds. *The Journal of Pharmacology and Experimental Therapeutics* **171** (1), 45-51.

Gossard, J- P., Brownstone, R. M., Barajon, I. & Hultborn, H. (1994). Transmission in a locomotor-related group Ib pathway from hindlimb extensor muscles in the cat. *Experimental Brain Research* **98**, 213-228.

Guertin, P., Angel, M. J., Perreault, M- C. & McCrea, D. A. (1995). Ankle extensor group I afferents excite extensors throughout the hindlimb during MLR-evoked fictive locomotion in the cat. *Journal of Physiology* **487**, 197-209.

Guertin, P., Angel, M. J., Jiménez, I. & McCrea, D. A. (1994). Both disynaptic and longer latency interneuronal pathways mediate extension enhancement evoked by group I muscle afferents during fictive locomotion. *Society for Neuroscience Abstracts* **20**, 648.8.

Guertin, P., Perreault, M-C. & McCrea, D.A. (1995) Organization of the CPG revealed by perturbation of the step cycle evoked by group I afferents. *Society for Neuroscience Abstracts* **21**, 277.8.

Gustafsson, B. (1984). Afterpotentials and transduction properties in different types of central neurones. *Archives Italiennes de Biologie* **122**, 17-30.

Hammond, G.R., Miller, S. & Scott, P.D. (1981). The effect of antidromic stimulation of hindlimb nerves during fictive locomotion in low spinal cats to test a model of a spinal locomotor generator. In *Motion and Organization Principles*, vol 1, *Advances in Physiological Science. Regulatory Functions of the CNS.* ed. Szentagothai, J., Palkovits, M. & Hamori, J., pp. 213-217. Pergamon Press, Budapest.

Hoffer, J.A., Sugano, N., Loeb, G.E., Marks, W.B., O'Donovan, M.J. & Pratt, C.A. (1987). Cat hindlimb motoneurons during fictive locomotion. II. Normal activity patterns. *Journal of Neurophysiology* **57** (2), 530-553.

Hounsgaard, J., Hultborn, H., Jespersen, B. & Kiehn, O. (1988). Bistability of alpha-motoneurones in the decerebrate cat in the acute spinal cat after intravenous 5-hydroxytryptophan. *Journal of Physiology* **405**, 345-367.

Hultborn, H., Jankowska, E. & Lindstrom, S. (1971) Relative Contribution From Different Nerves to Recurrent Depression of Ia IPSPs in Motoneurones. *Journal of Physiology* **215**, 637-64.

Kolmodin, G.M. and Skoglund, C.R. (1958). Slow membrane potential changes accompanying excitation and inhibition in spinal moto- and interneurons in the cat during natural activation. *Acta Physiologica Scandinavia* **44**, 11-54.

Jack, J. J. B. (1978). Some methods for selective activation of muscle afferent fibres. In *Studies in Neurophysiology,* ed. Porter, R., pp. 155-176. Cambridge University Press, UK.

Jami, L. (1992). Golgi tendon organs in mammalian skeletal muscle: functional properties and central actions. *Physiological Reviews* **72**, 623-666.

Jankowska, E., McCrea, D. A. & Mackel, R. (1981). Pattern of 'non-reciprocal' inhibition of motoneurones by impulses in group Ia muscle spindle afferents in the cat. *Journal of Physiology* **316**, 393-409.

Jordan, L.M. (1983). Factors determining motoneuron rhythmicity during fictive locomotion. In. *Neural origin of rhythmic movements*, edited by Roberts and Roberts, Cambridge: Cambridge University Press, pp. 423-444.

Lundberg, A. (1981). Half-centres revisited. In *Regulatory functions of the CNS. Motion and Organization Principles,* vol.1, *Advances in Physiological Sciences* **21**, ed. Szentgothi, J., Palkovitis, M. & Hamori, J. pp. 155-167. Pergamon Adademiai Kiado,

Budapest, Hungary.

McCrea, D. A., Shefchyk, S. J., Stephens, M. J. & Pearson, K. G. (1995). Disynaptic group I excitation of synergist ankle extensor motoneurons during fictive locomotion. *Journal of Physiology* **487,** 527-539.

Pearson, K.G. (1995). Proprioceptive regulation of locomotion. *Current Opinion in Neurobiology* **5**, 786-791.

Pearson, K.G. & Collins, D. F. (1993). Reversal of the influence of group Ib afferents from plantaris on activity in medial gastrocnemius muscle during locomotor activity. *Journal of Neurophysiology* **70**, 109-117.

Pearson, Ramirez & Jiang (1992). Entrainment of the locomotor rhythm by group Ib afferents from ankle extensor muscles in spinal cats. *Experimental Brain Research* **90**, 557-566.

Perreault, M-C., Angel, M.J., Guertin, P. & McCrea, D.A. (1995). Effects of stimulation of hindlimb flexor group II afferents during fictive locomotion in the cat. *Journal of Physiology* **487**, 211-220

Pratt, C. A. (1995) Evidence of Positive Force Feedback Among Hindlimb Extensors in the Intact Standing Cat. *Journal of Neurophysiology* **73**, 2578-2583.

Whelan, P. J., Hiebert, G. W. & Pearson, K. G. (1995). Stimulation of the group I extensor afferents prolongs the stance phase in walking cats. *Experimental Brain Research* **103**, 20-30.

Paper II

CENTRAL LATENCIES OF GROUP I MUSCLE AFFERENT-EVOKED RESETTING RECORDED IN PAIRS OF MOTONEURONES DURING FICTIVE LOCOMOTION IN THE CAT.

P. Guertin, M-C. Perreault* and D.A. McCrea

Department of Physiology

University of Manitoba

730 William avenue

Winnipeg, Manitoba, R3E 3J7

Canada

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* Present address:

Department of Physiology, Panum Institute, Blegdamsvej 3, 2200-DK, Copenhagen, Denmark.

Summary

1. The present study used the decerebrate preparation in which fictive locomotion was produced by stimulation of the midbrain locomotor region to examine the central latencies of group I-evoked resetting of the step cycle. Intracellular recording was used to examine the effects of resetting on the rhythmic hyper and depolarizing locomotor drive potential (LDP) in flexor and extensor motoneurones. Trains of stimuli at group I strength (≤2 times threshold, 20-30 shocks, 200 Hz) delivered to ankle extensor nerves during flexion abruptly terminated on-going locomotor bursts in flexor nerves and initiated extensor activity. Resetting was evoked throughout the hindlimb; all effective stimulus presentations resulted in a modulation of the step cycle pattern recorded in hip, knee, ankle and digit electroneurograms (ENGs).

2. Resetting always resulted in a rapid-onset hyperpolarizing LDP in flexor motoneurones and a depolarizing LDP in extensors. The onset of the switch in the LDP to a new phase occurred simultaneously in flexor and extensor motoneurones during group I-evoked resetting of the fictive locomotor pattern.

3. Central latencies for the initiation of the reset depolarizing LDP in extensors ranged from 1 - 65 ms (mean 18.5 $\pm$ 16.7 ms, n = 19). In the fastest 8 examples, the mean latency was 4·8 $\pm$ 0.5 ms. Resetting latencies were identical in extensor motoneurone pairs recorded simultaneously (n=5) acting at proximal or distal joints. The onset of activity in extensor nerves during resetting occurred later (mean latency 40 $\pm$ 24 ms, n = 19) than the depolarization of homonymous or close synergist motoneurones.

4. These results are consistent with a 'half-centre' organization of the spinal generator for locomotion with reciprocally organized extensor and flexor portions. The synchronicity of effects in flexor and extensor motoneurones suggests parallel actions of ankle extensor group I afferents on both portions of the half-centre. Resetting effects are distributed simultaneously to motoneurones operating at proximal or distal joints. The short latencies show that resetting can be evoked through pathways consisting of relatively few interneurones interposed between group I afferents and hindlimb motoneurones.

Introduction

It is well known that sensory feedback from hindlimb extensor group I muscle afferents can modulate the step cycle during both fictive (Conway Hultborn & Kiehn 1987; Guertin Angel Perreault & McCrea 1995) and treadmill locomotion (Whelan Hiebert & Pearson 1995; see also Pearson, 1995). In discussing these effects, the term "group I" is used to emphasize that ankle extensor group Ia muscle spindle and group Ib Golgi tendon organ afferents have similar effects on the locomotor cycle (see Guertin *et al.* 1995; McCrea Shefchyk Stephens & Pearson 1995; Angel Guertin Jimenez & McCrea 1996; Paper I of this thesis). Stimulation of ankle extensor group I afferents produces different effects when delivered during the extension or flexion phase of the locomotor cycle. Delivered during extension, brief trains of electrical stimuli to an ankle extensor nerve at group I strength can entrain the step cycle (Pearson, Ramirez and Jiang, 1992) or enhance the activity of extensor motoneurones operating at hip, knee and ankle joints (Guertin *et al.* 1995). In parallel to these actions, group I afferents also evoke an extension dependent, disynaptic excitation of hindlimb extensors (McCrea *et al.* 1995; Angel *et al.* 1996). It is likely that such actions revealed during fictive locomotion in reduced preparations are also relevant to real overground locomotion. Ankle extensor group I afferents are mainly active during the extensor phase of the step cycle (Prochazka Trend Hulliger & Vincent 1989) and several studies have argued for a role of this proprioceptive group I input in augmenting the amount and duration of extensor activity during locomotion (Duysens & Pearson 1980; Pearson *et al.* 1992; Pearson & Collins 1993; Gossard, Brownstone, Barajon & Hultborn

1994; Guertin *et al.* 1995; McCrea *et al.* 1995; Pearson 1995; Whelan *et al.* 1995; Angel *et al.* 1996; Yang, Stein & James 1991).

When stimulation of ankle extensor group I afferents is delivered during the flexor phase of fictive locomotion, the step cycle is reset; on-going activity of flexors is terminated and a premature locomotor burst in extensors initiated (Conway *et al.* 1987; Guertin *et al.* 1995). These resetting actions may be more of an experimental curiosity than a reflection of a physiologically relevant regulatory process since it is unlikely that ankle extensor group I afferents would be activated during flexion. Nevertheless group I-evoked resetting may be a useful paradigm in which to examine the organization of the central pattern generating circuitry for locomotion.

Some of the reflex actions of group I afferents during fictive locomotion such as disynaptic excitation, appear to be organized in parallel to the central pattern generator (CPG) circuitry (see Guertin Angel Jimenez & McCrea, Paper I of this thesis). However, it is reasonable to conclude that peripheral nerve-evoked reflexes capable of perturbing the step cycle are at least partially mediated by the CPG for locomotion. The most useful model of the mammalian locomotor CPG to discuss such perturbations is the 'half-centre' model. In this model which was originally conceived by Graham Brown (1914) and given a tentative neuronal basis by Lundberg, Jankowska and colleagues (see Lundberg 1979, 1981), reciprocal activity in mutually inhibitory spinal circuits is responsible for the alternation of flexor and extensor activity during locomotion.

The development of the half-centre model has relied heavily on the use of the

unanesthetized (decerebrate), acute spinal, cat preparation. In this preparation following administration of L-DOPA, stimulation of the flexor reflex afferents (FRA) produces alternating discharges in flexor and extensor nerves and excites spinal interneurones whose activities are reciprocally organized (Jankowska, Jukes, Lund & Lundberg, 1967a,b). It has been these suggested that these interneurones are part of the locomotor CPG (see Lundberg 1979, 1981). In the L-DOPA preparation short-latency FRA-evoked reflexes are suppressed and replaced by longer latency reflexes presumably as part of the reorganization of reflex pathways during locomotion. In support of these ideas, Pearson & Collins (1993) found during clonidine-induced fictive locomotion that stimulation of ankle extensor group I afferents activated extensor motoneurones with latencies of 30 - 50 ms; i. e. in the range of the 'locomotor-dependent' FRA reflexes. Similar latencies have been reported for the increase in extensor nerve (ENG) activity during group I-evoked resetting in both MLR-evoked an L-DOPA-induced fictive locomotion (Conway *et al.* 1987; Guertin *et al.* 1995) and for flexor nerve, group II afferent-evoked resetting (Perreault, Angel, Guertin & McCrea, 1995).

Intracellular recordings during stimulation of group I afferents have provided better estimates of the latency of extensor motoneurone depolarization during resetting. In the L-DOPA preparation, brief stimulus trains to ankle extensor nerves evoke EPSPs in extensors motoneurones. According to the hypothesis developed, this extensor excitation (latency 3.5 - 4 ms) is evoked through interneurones that are part of the locomotor, half-centre organization (Gossard *et al.* 1994). This is an attractive hypothesis since it suggests

that group I afferent stimulation could be a useful tool for exploring the neuronal basis of the CPG. Missing from the study by Gossard *et al.* (1994) was an intracellular analysis of group I-evoked stimulation that actually reset the step cycle. Thus it is was not possible in that study to distinguish between group I-evoked activation of locomotor-dependent reflex pathways operating in parallel to the CPG circuitry or group I actions on the CPG itself (cf. Paper 1 of this thesis).

The present study was undertaken, therefore, to intracellularly characterize the group I-evoked resetting of the fictive locomotor step cycle produced by ankle extensor group I afferent stimulation during flexion. The specific goals were to examine the effects of resetting on the membrane potentials of both flexor and extensor motoneurones; to measure the minimum latencies of resetting in flexors and extensors; and to examine the similarity of effects in motoneurones innervating proximal or distal joints. In order to facilitate the comparison of data between motoneurones, intracellular recordings were obtained simultaneously from pairs of motoneurones. The observations reported here are complementary to those reported previously (Paper I of this thesis) on the intracellular effects of group I afferents during extension enhancement. Some of the present results have appeared in abstract form (Guertin, Perreault & McCrea, 1995).

Methods

Preparation

Results from experiments on eleven male or female cats weighing 2·0-3·2 kg are reported. Details of the methodology can be found in Guertin *et al.* (1995). Animals were anaesthetized with halothane (1-1.5%) in a mixture of oxygen (30%) and nitrous oxide (70%). A tracheal catheter was inserted and cannulae were placed into the right jugular and femoral veins for administration of fluids and drugs. A buffer solution was continuously infused (5 ml/hr), atropine (0·05 mg/kg) and dexamethasone (2 mg/kg) were given either s.c. and i.v. The following muscle nerves from the left hindlimb were dissected and cut: posterior biceps and semitendinosus (PBSt), semimembranosus and anterior biceps (SmAB), medial and lateral sartorius (Sart), quadriceps and rectus femoris (Q), plantaris (Pl), medial gastrocnemius (MG), lateral gastrocnemius (LG) often together with soleus (LGS), the remainder of the posterior tibial nerve including the muscular and cutaneous innervation of the foot (Tib), tibialis anterior (TA), flexor digitorum hallucis and longus (FDHL), extensor digitorum longus (EDL) and brevis (EDB) sometimes taken together. The Q and Sart nerves were placed in cuff electrodes and the remaining nerves were mounted on conventional bipolar, silver chloride, hook electrodes. The contralateral SmAB and PBSt nerves were also dissected and mounted for monitoring contralateral motor activity. Other branches of the sciatic, femoral and obturator nerves and tendons inserting around the iliac crest were cut bilaterally. After laminectomy exposing the L3-S1 segments, a craniotomy and a precollicullar-postmammillary decerebration (rostral tissue

removed) were performed. After anaesthesia was then discontinued and the animals paralysed (i.v. gallamine triethiodide 2 - 3 mg/kg/hr) and ventilated. Temperature and end tidal CO_2 were maintained within normal limits. Low blood pressures were countered by infusion of electrolyte solutions or dextran; no pressor agents were used.

Stimulation of peripheral nerves and the MLR

Fictive locomotion was evoked by uni- or bilateral stimulation (100 - 150 μA, 1ms rectangular pulses, 10-15 Hz) of the mesencephalic locomotor region (MLR)(see Guertin *et al.* 1995). Locomotor activity was confirmed by the presence of a bilateral alternating and rhythmic pattern in the muscle nerves and motoneurones. Perturbation of the fictive step cycle was accomplished by stimulation (typically 20-30 stimuli, 0.1 ms pulse @ 200 Hz) of ankle extensor nerves (Pl, MG, LGS or LG) at group I strength ($\leq$2 times the threshold for the most excitable fibres, *T*). Stimulus delivery during the flexor phase of the step cycle was controlled by a computer that detected a threshold level of integrated flexor nerve (TA or Sart) ENG activity and following a selected delay (50-100 ms), triggered the stimulus train. Stimulus trains were delivered once every 4-5 step cycles. Relays allowed switching between stimulation and recording of the peripheral nerves.

Criteria for resetting

Resetting of the fictive step cycle was defined as a perturbation of the on-going and next cycle period, with the complete termination of flexor ENG bursts and the initiation of locomotor bursts in extensors throughout the hindlimb. Only trials that met these criteria

were kept for analysis. To facilitate the illustration of resetting, the mean cycle period for steps just prior to delivery of the group I strength stimulation was calculated and plotted as open circles before and a filled circle following stimulus delivery.

Data collection and analysis

The dura was opened and small areas of pia removed in the L5-L7 spinal segments. Glass microelectrodes filled with 2 M sodium citrate (tip diameter 1.8 - 2.2 μm; resistance 1-2 MΩ) were used to impale antidromically identified extensor and flexor hindlimb motoneurones. Independent arcs and microdrives were used to position two such electrodes. MLR-stimulation was begun to obtain fictive locomotion immediately following impalement of a pair of motoneurones. Only motoneurones with membrane potentials greater than -50 mV before MLR stimulation were analysed. ENG recordings were amplified (x 5000-50000), filtered (30 Hz - 3 kHz, -3 dB) rectified and integrated (100 ms time constant). ENG records from up to 8 nerves were digitized (at 500 Hz) on-line using a Concurrent 5450 computer along with two high-gain and two low gain intracellular records (at 10 kHz), the cord dorsum volley (2 kHz) and stimulus markers (2 kHz).

Averages of ENG and intracellular records were made for step cycles that were perturbed by group I strength stimulation (shown in solid lines) and for 'control, non-stimulated' step cycles (dotted lines). Averages were aligned to the computer generated pulse indicating the onset of flexor nerve activity. Latency measurements were made within the computer from averaged traces. Central latencies in extensor motoneurones were measured as the time from the arrival of the first shock in the train at the cord dorsum and

the point at which the motoneurone membrane potential exceeded and was maintained above pre-stimulus, baseline, levels by least 0·5 mV (horizontal dotted line). The latency of resetting in flexor motoneurones was measured as the time to a sustained hyperpolarization of 0·5 mV below baseline. These latencies are indicated by vertical arrows in the figures. Results are reported as mean $\pm$ S.D.

Results

An example of the resetting behaviour investigated is shown in Fig. 1A. Ankle extensor nerve stimulation triggered by activity in the ankle flexor (TA) nerve prematurely terminated flexion and evoked discharges in hip (SmAB), knee (Q) and ankle (Pl) nerves. The connected circles below the intracellular FDHL record show the preceding (open circles) and predicted (solid circle) occurrence of the (mid) extension phase of the step cycle. In this example LGS nerve stimulation (2*T*, 30 pulses, duration indicated by the thick horizontal lines) delivered every fifth step, phase-advanced extensor activity. LGS, Pl and MG nerve stimulation was equally effective in evoking resetting from the flexion to the extension phase during fictive locomotion. Complete resetting of the step cycle was seen in nine of eleven experiments and all data reported are from these preparations. In one other preparation ankle extensor nerve stimulation evoked only a very small amount of activity in extensor nerves and in another was without effect.

-- Figure 1 near here--

Extensor motoneurone pairs

Intracellular recordings from a FDHL motoneurone recruited during fictive locomotion and from an unrecruited SmAB motoneurone are shown in Fig. 1A (upper portion). During resetting there is a rapid depolarization of both extensor motoneurones as well as activity in SmAB, Q and Pl nerves. Figure B shows averaged records (100 ms before and ~ 500 ms after stimulation) of both intracellular and ENG records from the run of fictive

locomotion partly illustrated in panel A. Averaged traces in the absence of LGS nerve stimulation (control) are shown as dotted lines and were calculated from a 70 second period of fictive locomotion including the control steps illustrated in Fig. 1A. Averaging distorted the shape of action potentials in the FDHL intracellular records and especially the control record in which more sweeps were averaged. The delay between the onset of activity in the solid and dotted line traces illustrates that stimulation of the LGS nerve during flexion reset the locomotor rhythm by phase-advancing extension.

The onset of membrane depolarization during resetting in the FDHL and SmAB motoneurones begins about 6 ms following the start of LGS nerve stimulation (arrows under FDHL and SmAB traces). The latency of the new (reset) activity is about 24 ms as recorded in the extensor nerves. Given a typical conduction distance from the adult cat lumbar spinal cord to the ankle extensor nerves of 160 mm and an average motoneurone conduction velocity of ~80 $m \cdot s^{-1}$, the conduction time for action potentials travelling to the recording site would be about 2 ms. Thus the difference between action potential production and the onset of depolarization of the extensor motoneurones is about 16 ms (24 - 6 -2) and reflects the average duration of the subthreshold depolarizing portion of the LDP during these conditions. Since the illustrated ENGs represent activity in motoneurones operating at the three major joints, the similar latencies of ENG activity during resetting suggest that the effects of group I stimulation are distributed simultaneously to motoneurones innervating proximal and distal muscles (see also Guertin *et al.* 1995).

-- Figure 2 near here--

Expanded, high gain, averaged intracellular records of the FDHL and SmAB motoneurones illustrated in Fig. 1 are shown in Fig. 2A. Only the response to the first six shocks in the 30 shock train is shown (the vertical bars plotted at 5ms intervals indicate the arrival of the volley produced by the 200 Hz train). The horizontal lines plotted at and 0.5 mV above the prestimulus membrane potential were used to determine the onset of depolarization (See Methods). In this example the onset of depolarization during resetting in both the hip and toe extensor motoneurones was about 6 ms following the first shock. The depolarization then increased steadily thereafter (see Fig. B). In panels B and C three of the eleven sets of individual records that comprise the averaged data in A are shown. The fluctuations in onset of depolarization (vertical arrows) may be due to other synaptic events during fictive locomotion that obscure the actual onset of resetting. Central latencies reported in this paper were, therefore, all measured from averaged records. In some cases, this procedure may have overestimated the minium latency of effects during resetting.

--Figure 3 near here--

Figure 3 shows another example of resetting in a pair of PBSt and LGS motoneurones. In this experiment the PBSt nerve was active only during the extensor phase of the locomotor cycle and this PBSt motoneurone was depolarized only during extension (see Perret and Cabelguen [1980] for discussion of the behaviour of bifunctional motoneurones during fictive locomotion). A train of stimuli delivered to the LGS nerve during flexion initiates premature activity in both the PBSt and LGS motoneurones, while terminating the

on-going activity in the Sart nerve (Fig. 3A). Figure 3B shows the averaged membrane potentials (n=9) from the first sixteen (of thirty) shocks delivered to the LGS nerve. The large stimulus-locked (vertical bars) depolarization in the LGS motoneurone is a combination of an antidromic M spike (latency of 0·3 ms) and a monosynaptic EPSP. Note that these short latency depolarizations neither summate nor lead to a persistent depolarization during the first 22 ms. At a latency of 22 ms (arrow), however, the membrane potential undergoes a net depolarization. There is a similar sustained depolarization with a 22 ms onset in the PBSt motoneurone without preceding EPSPs. These results give support for the notion that the resetting depolarization of extensors is likely a result of a synchronous output from the extensor portion of the CPG to hindlimb motoneurones that in this case had a latency of 22 ms and 6 ms in the example in Fig. 1.

The records in Fig. 3C and D were obtained while recording from the same PBSt motoneurone illustrated in panels A and D about 2 min prior to the impalement of the LGS motoneurone. During this run of fictive locomotion the LGS nerve was stimulated at 1.5T and then 2T. Averages of the resetting produced by both stimulation intensities are superimposed in panel D and are similar in shape and latency, producing a depolarization with a latency of about 30 ms. Because 1.5T stimulation strength is below that required to recruit even the most excitable group II afferents in ankle extensor nerves (Jack 1978), this data confirms the group I nature of the resetting reported here. The comparison of panels B and D also shows that the latency of resetting depolarization can change within an experiment. No attempt was made to determine the factors responsible for these latency

fluctuations.

The latency of group I-evoked resetting depolarization was the same in three other extensor motoneurone pairs (not illustrated). In a pair of SmAB motoneurones, stimulation of the Pl nerve at 2*T* (30 shocks) evoked sustained depolarizations in both at a latency of 5 ms. In another extensor pair (MG and an unidentified extensor motoneurone) Pl nerve stimulation depolarized both cells at a latency of 4·5 ms. Finally, in a pair of FDHL and Pl motoneurone resetting evoked by LG nerve stimulation at 2*T* strength appeared simultaneously at a latency of 24 ms.

In summary, whenever group I-evoked resetting from flexion to extension occurred, it evoked motoneurone activity at the same time in all extensor nerves and in all extensor motoneurones impaled. The central latency of resetting was variable between experiments and during experiments (e.g., Figs 3A vs. 3C), but for the five extensor motoneurone pairs examined, the onset of the resetting depolarization was synchronous even in motoneurones acting at different joints. In 19 extensor motoneurones including the 10 paired recordings, the mean central latency of resetting was of 18·5 $\pm$ 16·7 ms. The mean latency of resetting recorded at the same time in an homonymous or close synergist extensor ENG was 40 $\pm$ 24 ms (n = 19).

-- Figure 4 near here--

Intracellular latencies are presented in Fig. 4 which shows that resetting was evoked at latencies ranging from 1 to 65 ms. Measurements obtained simultaneously from

motoneurone pairs are plotted immediately adjacent. As mentioned, the variability in the latencies of resetting could be seen within a single experiment (e. g. experiment #1). However, in 8 extensor motoneurones, the latencies were of 6 ms or less (mean 4.8 ms). There was no relationship between the latency of resetting and the motoneuron pool investigated.

Flexor - extensor motoneurone pairs

--Figure 5 near here--

Figure 5A shows the effects of stimulating the LG nerve in an extensor (SmAB) and a flexor motoneurone pair. The flexor motoneurone was not identified antidromically but the occurrence of monosynaptic excitation upon TA and EDL stimulation suggests it was a pretibial flexor. Stimulation during flexion, depolarized the SmAB motoneurone and hyperpolarized the flexor motoneurone. Note the mirroring of effects in the two motoneurones and the 6 ms latencies of both the depolarization and hyperpolarization (arrows in Fig. 5B). In an other pair of motoneurones (Pl and TA), Pl nerve stimulation at 2T during flexion produced resetting of the rhythm (Fig. 5C), a sustained depolarization in the Pl motoneurone with a latency of approximately 1 ms and a hyperpolarization of the TA motoneurone at a latency of 1·5 ms (Fig. 5D). It is difficult to estimate the latency of the resetting in the Pl motoneurone because of the presence of the antidromic M spike (0·3 ms) followed by homonymous monosynaptic excitation and possibly disynaptic excitation. Similarly the disynaptic, reciprocal inhibition of the TA motoneurone (1.5 ms) makes an estimation of the locomotor-related resetting hyperpolarization difficult. An interpretation

of the growth of reciprocal inhibition during the train is not possible because of the increasing amplitude of the group I volley on the cord dorsum record. It appears, however, that sustained changes in membrane potential occur concomitantly in the TA and Pl motoneurone.

Group I afferent-evoked resetting was observed in three other flexor-extensor motoneurone pairs (5 in total). In a pair of TA and MG motoneurones, stimulation of the Pl nerve during flexion hyperpolarized the TA motoneurone and depolarized the MG motoneurone both at a central latency of 15 ms. In a pair of Pl and EDL-EDB motoneurones, resetting was also evoked synchronously 55 ms following the stimulus onset to the LG nerve. Finally, resetting at a latency of about 65 ms was seen in a SmAB and TA motoneurones. All observations are presented in Fig. 4 which shows that both short and long resetting latencies were seen in flexors and extensors but in all cases, the hyperpolarization and depolarization was evoked simultaneously.

Discussion

The present study is the first to report intracellular estimates of the central latencies of motoneurone membrane potential changes during group I-evoked resetting. Although the latencies were variable, the onset of the persistent depolarization of extensors and hyperpolarization of flexors often occurred with latencies of around 5 ms (Fig. 4). While not systematically analysed, the LPDs occurring immediately after group I-evoked resetting from flexion to extension were similar in shape, amplitude and duration to control LDPs (e. g. see Fig 1A).

It is unlikely that group Ia-evoked monosynaptic or group I-evoked disynaptic excitation (McCrea *et al.* 1995; Angel *et al.* 1996) contribute significantly to group I-evoked resetting (see also Conway *et al.* 1987). The distribution of monosynaptic excitation from the ankle extensors nerves is limited (Eccles *et al.* 1957; Edgley Jankowska & McCrea 1986) and is insufficient to account for the initiation of activity in extensors throughout the limb. Similarly disynaptic excitation is unlikely to account for resetting of extensor activity. Disynaptic excitation is not routinely evoked in extensor motoneurones during the flexion phase of the step cycle (McCrea *et al.* 1995; Angel *et al.* 1996) and has not been found in acute spinal preparations (McCrea *et al.* 1986) in which group I-evoked resetting can also be produced (Conway *et al.* 1987). Nevertheless, it is possible that the very rapid resetting illustrated in Fig. 5C was initiated by monosynaptic (and disynaptic) excitation of ankle extensor motoneurones and concomitant reciprocal inhibition of antagonist (TA) motoneurones. The full pattern of resetting of the activity of motoneurones

acting at other joints would have then been evoked in parallel at a longer latency by group I input onto the CPG. This suggestion is supported in Fig. 5C by the appearance of slight activity in the LG nerve soon after stimulus onset followed 50-60 ms later by a large burst of activity in the LG and SmAB nerves. However, a sequentially evoked initiation of ankle and then other extensor motoneurones was the exception and not the rule. Overall the evidence argues against a significant contribution from short-latency reflexes to resetting.

Intrinsic motoneurone conductances underlying the LDP (Brownstone Gossard & Hultborn 1994) are also unlikely to account for the earliest portion of the depolarization of the LDP in extensors during resetting because they would be inactivated at hyperpolarized membrane potentials during flexion. The induction of intrinsic motoneurone conductances could, however, contribute to the recruitment of extensor activity seen in the ENG which occurred at a mean latency of 40 ms (see also Guertin *et al.* 1995; Pearson & Collins 1993). The hyperpolarization of flexors during resetting may be due to a termination of voltage-dependent depolarizing conductances during flexion. This is consistent with the suggestion that the group I-evoked hyperpolarization of flexors in a preparation capable of locomotion is primarily a disfacilitation (Conway *et al.* 1987; for conductances underlying unperturbed LDPs see Jordan 1983, Pratt & Jordan 1987). The identical latencies of flexor motoneurone hyperpolarization and extensor depolarization in the present study show that the control of both these processes is tightly regulated during fictive locomotion. The variable latency of the coupled resetting of flexor and extensor activities (Fig. 4) most likely results from changes in the excitability of interneurones

involved in resetting or the effectiveness of group I afferents in activating these interneurones. In summary, it is most likely that the short-latency effects evoked during resetting result primarily from activity in CPG pathways that resets the locomotor rhythm and thereby perturbs the membrane potentials of flexor and extensor motoneurone throughout the limb (see Pearson 1995). This is unlike the enhancement of the LDP amplitude and duration that occurs when group I stimulation is delivered during extension. During extension enhancement short-latency excitatory reflex pathways and intrinsic motoneurone conductances both contribute to the CPG-evoked enhancement of extensor activity (Paper I).

If group I-evoked resetting is mediated by CPG pathways, then the present results may offer insight into the organization of the locomotor CPG. The similarity of resetting latencies seen in randomly sampled motoneurone pairs was striking (Fig. 4) and suggests that the effects of resetting are distributed synchronously to all ipsilateral (hindlimb) motoneurones. Thus present results provide no support for the existence of a locomotor CPG organization that sequentially excites extensors in the limb (see discussion of 'ring model' of locomotion in Edgerton Grillner Sjöström & Zangger 1976). Results obtained in flexor and extensor motoneurone pairs also show the strict reciprocal organization of the CPG in which excitation of extensors is simultaneously coupled with inhibition of flexors. This is similar to unperturbed fictive locomotion where there is a high (inverse) correlation between the simultaneously recorded depolarization of extensors and hyperpolarization of flexor motoneurones (Kriellaars 1992). Thus during both unperturbed

locomotion (Kriellaars 1992) and resetting, the motor commands from the half-centre are sent synchronously to flexor and extensor motoneurones. CPG organization is usually represented by a symmetrical arrangement of centres affecting flexor and extensor motoneurones (Jankowska *et al.* 1967b; Edgerton *et al.* 1976; Lundberg 1981; Gelfand Orlovsky & Shik 1988). Schematic diagrams that attempt to incorporate the actions of group I afferents during locomotion imply that group I-evoked perturbation of the step cycle affects the extensor portion of the CPG before affecting the flexor portion (e. g. Gossard *et al.* 1994; Pearson 1995; McCrea *et al.* 1995). This is an asymmetrical organization of group I input to the CPG emphasizing that extensor group I afferents have a dominant action on the extensor portion of the CPG (Gossard *et al.* 1994; Guertin *et al.* 1995; McCrea *et al.* 1995; Pearson 1995). The present results, however, suggest that group I actions are not mediated sequentially from extensors to flexors because the latencies of flexor hyperpolarization and extensor depolarization are identical. Thus there should be equal path lengths from extensor group I afferents to flexor and extensor motoneurones. This suggestion does not argue against a symmetrically organized half-centre model of the locomotor CPG. It does suggest that group I afferents can access flexor and extensor portions of the CPG circuitry in a manner more complex than previously thought.

Present results support the suggestion of Gossard *et al.* (1994) that the 3.5 to 4.0 ms latency, group I-evoked excitation of extensors in L-DOPA preparations may be evoked through CPG circuitry. That pathway, only operational in locomotor or locomotor-like

states, is phasically modulated (i.e., larger during the flexion phase) and is accompanied by a similar latency hyperpolarization of flexors (Gossard *et al.* 1994). The present results show that the depolarization of extensors during actual resetting can occur at similar latencies and is accompanied by a corresponding hyperpolarization of flexors. It is therefore, likely that the pathway activated during resetting corresponds to that reported by Gossard *et al.* (1994). Short resetting latencies suggest that there is a short neuronal path-length through which activity in group I ankle extensor afferents can perturb the step cycle. If results obtained during resetting reflect the operation of CPG circuitry, then the CPG itself may consist of a relatively few layers interneuronal organization. The examples of short latency resetting (5 ms) could be explained by effects through a pathway with about four (or fewer) interposed interneurones between the group I afferent fibres and the motoneurones. For reasons already discussed, some or all neurones interposed in this pathway are likely part of the locomotor half-centre.

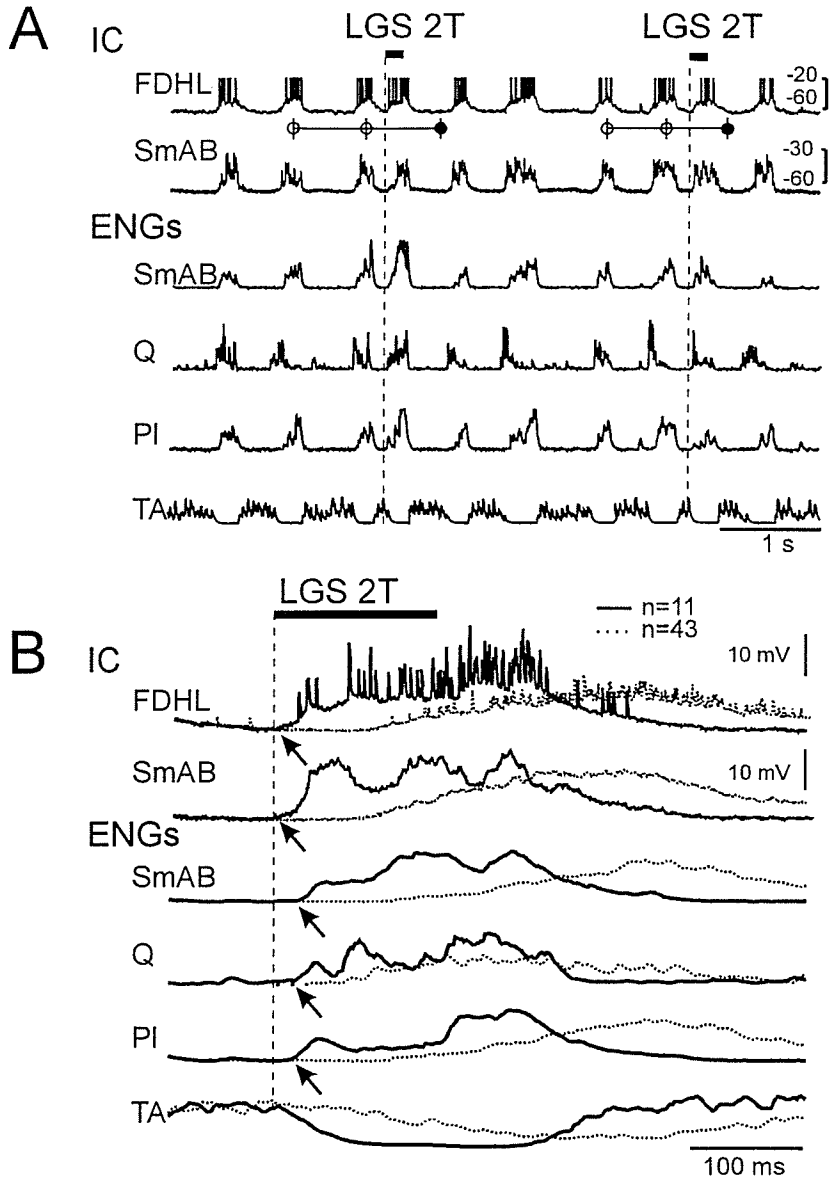

Figure 1. *Resetting of locomotor activity evoked by group I strength stimulation of the LGS nerve in a pair of extensor motoneurones and in motor nerves.*

The two top traces are DC-coupled intracellular records from a FDHL and a SmAB motoneurone. Action potentials in the FDHL motoneurone were about 60 mV and are trucanted for clarity. The remaining traces are integrated and rectified (sampling rate 500 Hz per trace) electroneurograms (ENGs) from ipsilateral hindlimb extensor (SmAB, Q, Pl) and flexor (TA) muscle nerves. The amplitude of the ENGs is uncalibrated. The vertical dashed lines indicate the onset of the LGS nerve stimulation (rectangles). **A**. Raw intracellular and ENG records. The circles beneath the top trace mark the extensor rhythm to illustrate where the post-stimulus extensor activity (black filled circles) should have happened in absence of stimulation. **B**. Corresponding averaged records made of eleven stimulated cycles (solid line traces) and forty-three control nonstimulated cycles (dotted line traces). The arrows indicate the earliest effects evoked in extensors during stimulated cycles compared to nonstimulated cycles.

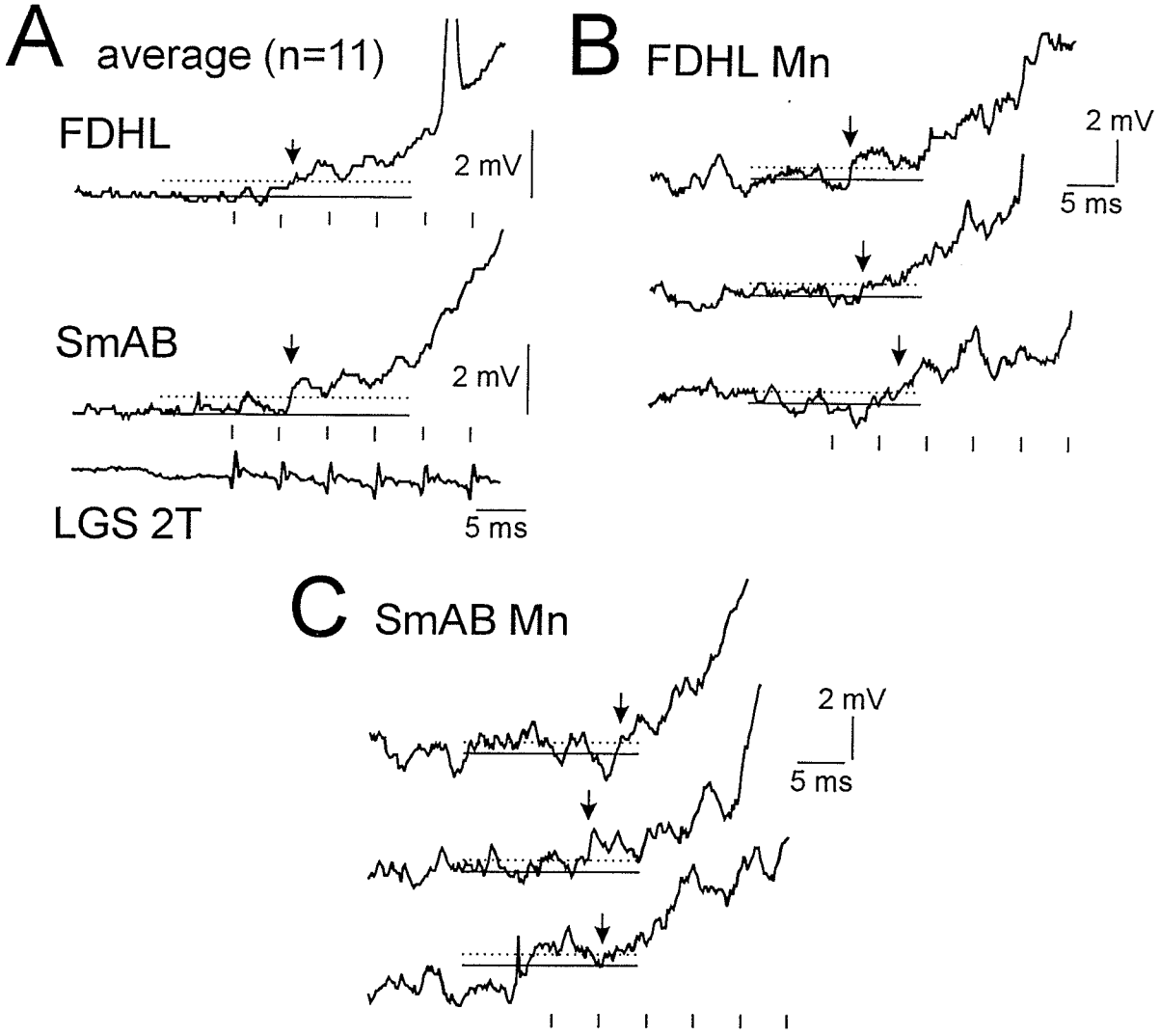

Figure 2. *High gain expanded traces of the locomotor drive potential phase switch evoked during resetting in a pair of extensor motoneurones.*

This figure shows the same pair of motoneurones used for the previous figure. The arrows show where the membrane potential was depolarized by a net 0·5 mV (horizontal dotted line) over the hyperpolarizing phase of LDP (horizontal solid line). The baseline horizontal solid line was placed by hand to best fit the membrane potential level prior the stimuli. Vertical bars indicate the stimulation (200 Hz). Central latencies were measured as the delay between the first group I afferent volley arriving at the cord dorsum (CD trace) and the arrow. **A.** Average records made from 11 stimulated cycles showing the effects of the first six in a train of 30 stimuli. The early spike evoked in the FDHL motoneurone was truncated for illustration purposes. **B.** Individual records for the FDHL motoneurone from three different stimulus trains. **C.** Corresponding raw records for the SmAB motoneurone.

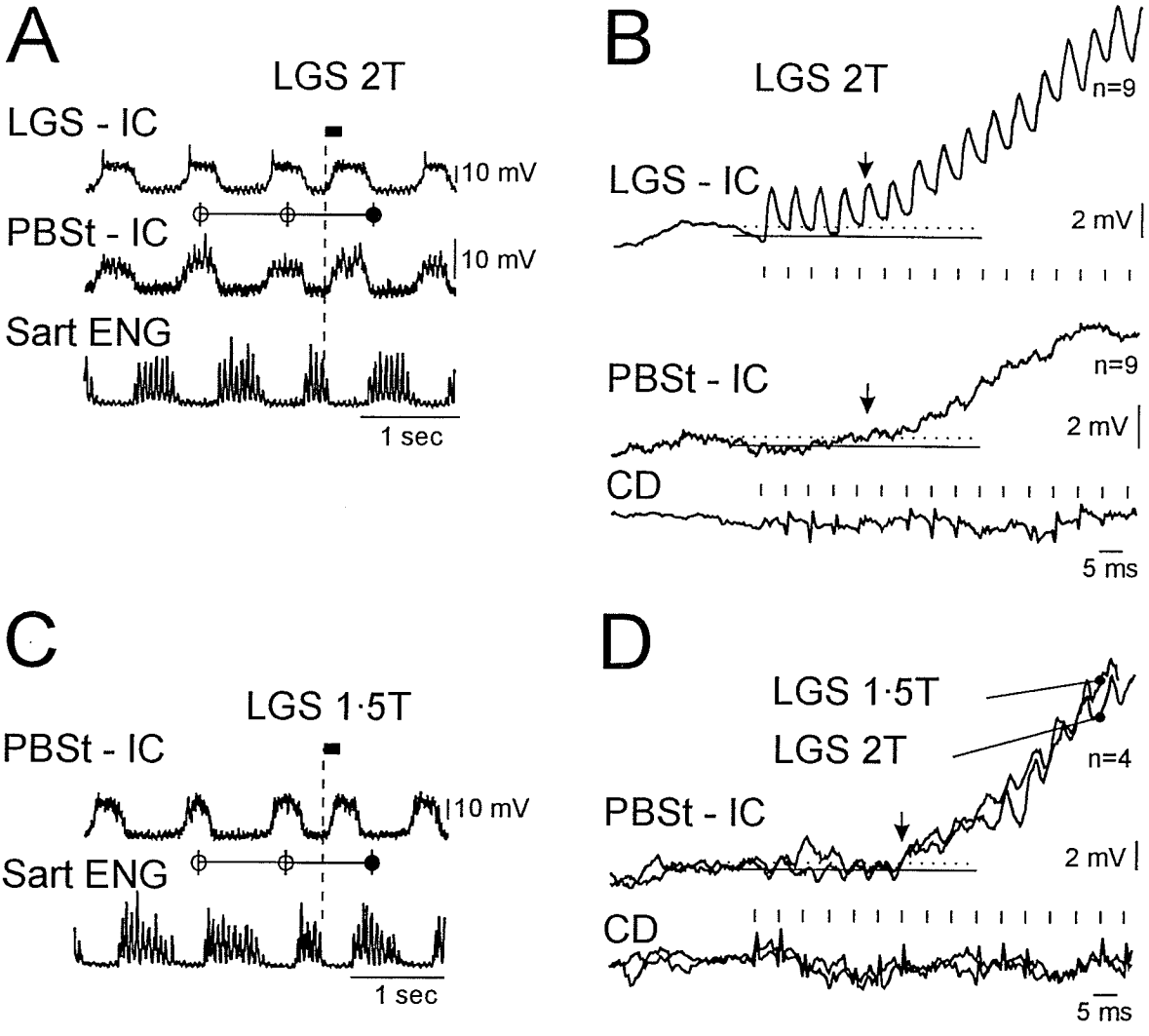

Figure 3. *Effects of LGS nerve stimulation at 1·5T and 2T in a pair of extensor motoneurones.*

Vertical dashed lines indicate the onset of a stimulus train delivered during flexion. The horizontal dotted and solid lines were placed as described in the previous figure. The arrows indicate the central latency of resetting. Vertical bars represent the cord dorsum volley of each stimulus (5ms intervals). **A-B**. Recordings from a pair made of a LGS and a PBSt (active as an extensor) motoneurone. Stimulation of the LGS nerve at 2*T* during flexion. **C-D**. In another bout of locomotion with only the PBSt motoneurone, the effects of stimulating the LGS nerve at 1·5*T* and 2*T* strengths are compared.

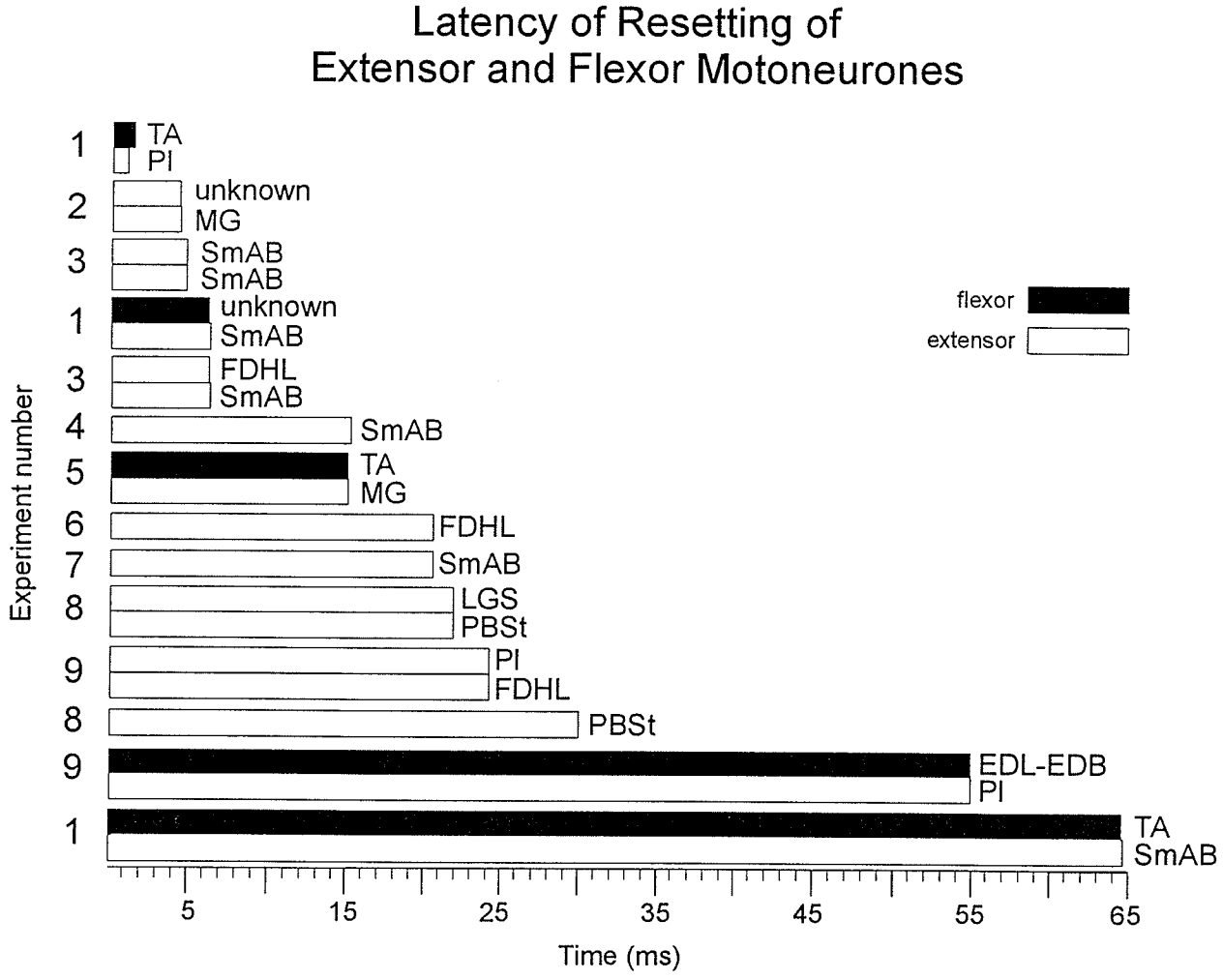

Figure 4. *Bar graph summarizing the latency of resetting in extensor and flexor motoneurones.*

Pairs of motoneurones are plotted as adjacent bars. Data from 9 different experiments were used during which resetting could be successfully evoked by group I strength stimulation to ankle extensor nerves during flexion.

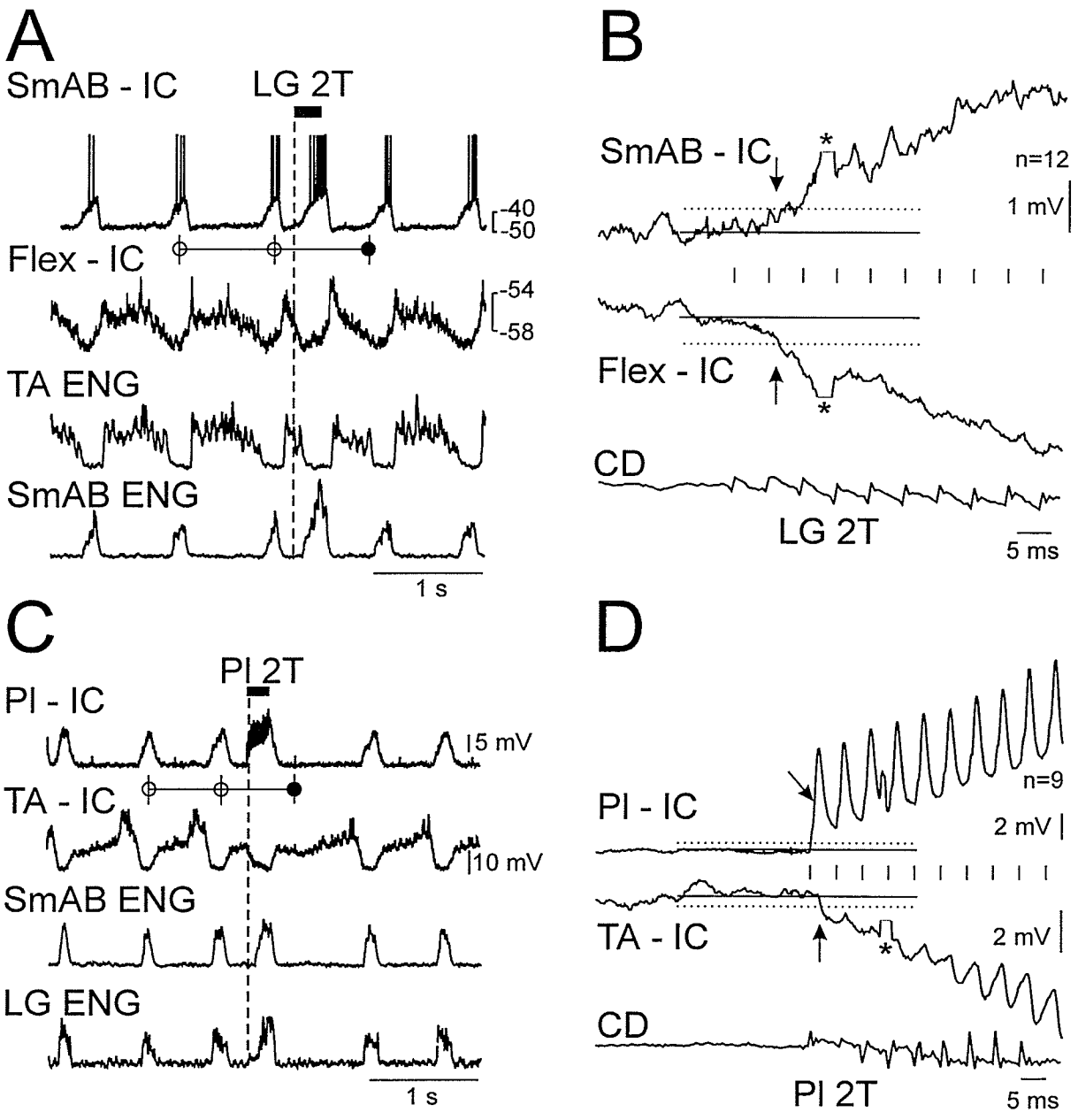

Figure 5. *Group I resetting in pairs of extensor and flexor motoneurones.*

A,C. Raw records showing the effect of one stimulus train on the locomotor activity of flexor (TA, Flex) and extensor (SmAB, Pl, LG). The filled circles illustrate the phase advanced resetting to extension evoked by stimulation of the Pl or LG afferents at 2*T* strength. **B,D**. Average traces made of 12 and 9 stimulated cycles respectively. The arrows indicate the central latencies measured from the first group I volley to a net change of the membrane potential of +0·5 mV for extensors and -0·5 mV for flexors (horizontal dotted lines). * indicates calibration pulses that have been truncated and removed from the illustration.

References

Angel, M. J. Guertin, P. Jiménez, I. & McCrea. D. A. (1996) Group I extensor afferents evoke disynaptic EPSPs in cat hindlimb motoneurones during fictive locomotion. *Journal of Physiology* **494**, 851-861.

Brown, G. (1914). On the nature of the fundamental activity of the nervous centres; together with an analysis of the conditioning of rhythmic activity in progression, and a theory of the evolution of function in the nervous system. *Journal of Physiology* **48,** 18-46.

Brownstone, R. M., Gossard, J.-P. & Hultborn, H. (1994). Voltage-dependent excitation of motoneurones from spinal locomotor centres in the cat. *Experimental Brain Research* **102**, 34-44.

Conway, B., Hultborn, H. & Kiehn, O (1987). Proprioceptive input resets central locomotor rhythm in the spinal cat. *Experimental Brain Research* **68**: 643-656.

Duysens, J. and Pearson K. G. (1980). Inhibition of flexor burst generator by loading ankle extensor muscles in walking cats. *Brain Research* **187**, 321-332.

Eccles, J. C., Eccles, R. M. & Lundberg, A. (1957). The convergence of monosynaptic excitatory afferents onto many different species of alpha motoneurones. *Journal of Physiology* **137**: 22-50.

Edgerton, V. R., Grillner, S., Sjöström, A. & Zangger, P. (1976). Central generation of locomotion in vertebrates. *Neural Control of Locomotion* **18**, 439-464.New York: Plenum Press.

Edgley, S. Jankowska, E. & McCrea, D. A. (1986). The heteronymous monosynaptic actions of triceps surae group Ia afferents on hip and knee extensor motoneurones in the cat. *Experimental Brain Research* **61**: 443-446.

Gelfand, I. M., Orlovsky, G. N. & Shik, M. L. (1988). Locomotion and scratching in tetrapods. *Neural control of rhythmic movements in vertebrates.* p 167-199. edited by A H Cohen, S. Rossignol and S. Grillner. New York: John Wiley and Sons.

Gossard, J.-P. Brownstone, R. M., Barajon, I. & Hultborn, H. (1994). Transmission in a locomotor-related group Ib pathway from hindlimb extensor muscles in cat. *Experimental Brain Research* **98**, 213-228.

Guertin, P. Perreault, M.-C. & McCrea, D.A. (1995). Organization of the CPG revealed by perturbation of the step cycle evoked by group I afferents. *Society for Neuroscience Abstract* **21,** 277.8, p.688.

Guertin, P., Angel, M. J., Perreault, M.-C. & McCrea, D. A. (1995). Ankle extensor group I afferents excite extensor throughout the hindlimb during fictive locomotion in the cat. *Journal of Physiol*ogy **487**, 197-209.

Jack, J.J.B. (1978). Some methods for selective activation of muscle afferent fibres. In: Studies in neurophysiology, ed. Porter R., Cambridge University Press. Cambridge, pp.155-176.

Jankowska, E., Jukes, M. Lund, S. & Lundberg, A. (1967 a). The effect of DOPA on the spinal cord. V. Reciprocal organization of pathways transmitting excitatory action to

alpha motoneurones of flexors and extensors. *Acta Physiologica Scandinavia* **70**, 369-388.

Jankowska, E., Jukes, M. Lund, S. & Lundberg, A. (1967b). The effect of DOPA on the spinal cord. VI. Half-centre organization of interneurons transmitting effects from flexor reflex afferents. *Acta Physiologica Scandinavia* **70**, 389-402.

Jordan, L.M. (1983). *Factors determining motoneuron rhythmicity during fictive locomotion*. In Neural origin of rhythmic movements, edited by Roberts and Roberts, Cambridge: Cambridge University Press, pp. 423-444.

Kriellaars, D. (1992). Generation and peripheral control of locomotor rhythm. University of Manitoba, Ph.D. thesis.

Lundberg, A. (1979). Multisensory control of spinal reflex pathways. *Progress in Brain Research* **50**, 11-28.

Lundberg, A.(1981). Half-centres revisited. *Regulatory Functions of the CNS. Motion and Organization Principles.* **1**, 155-167.J. Szentagothi, M. Palkovits and J. Hamori eds. Adv. Physiol. Sci., Budapest, Hungary: Pergamon Akademiai Kiado .

McCrea, D. A. Shefchyk, S. J. Stephens, M. J. & Pearson, K. G. (1995). Disynaptic group I excitation of synergist ankle extensor motoneurons during fictive locomotion. *Journal of Physiology* **487**, 527-539.

Pearson, K. G. & Collins, D. F. (1993). Reversal of the influence of group Ib afferents from plantaris n activity in medial-gastrocnemius muscle during locomotor activity. *Journal*

of Neurophysiology **70,** 1009-1017.

Pearson, K. G., Ramirez, J. M. & Jiang, W. (1992). Entrainment of the locomotor rhythm by group Ib afferents from ankle extensor muscles in spinal cats. *Experimental Brain Research* **90**, 557-566.

Pearson, K. G. (1995). Proprioceptive regulation of locomotion. Current Opinion in Neurobiology **5**, 786-791.

Perreault, M.-C., Angel, M. J., Guertin, P. & McCrea, D.A. (1995). Stimulation of hindlimb flexor group II afferents during fictive locomotion. *Journal of Physiology* **487**, 211-220.

Perret, C. & Cabelguen, J-M. (1980). Main characteristics of the hindlimb locomotor cycle in the decorticate cat with special reference to bifunctional muscles. *Brain Research* **187**, 333-352.

Pratt, C.A. and Jordan, L.M. (1987). Ia inhibitory interneurons and Renshaw cells as contributors to the spinal mechanisms of fictive locomotion. *Journal of Neurophysiology*. **57**(1): 56-71.

Prochazka, A., Trend, P., Hulliger, M. & Vincent, S. (1989). Ensemble proprioceptive activity in the cat step cycle: towards a representative look-up chart. *Progress in Brain Research* **80**, 61-74.

Whelan, P.J., Hiebert, G.W. & Pearson, K.G. (1995). Stimulation of the group I extensor afferents prolongs the stance in walking cats. *Exp. Brain Res.* **103**: 20-30.

Yang, Stein & James (1991). Contribution of peripheral afferents to the activation of the soleus muscle during walking in humans. *Exp. Brain Res.* 87: 679-687.

GENERAL DISCUSSION

This thesis provides several insights related to the combination and relative contribution of the reflex pathways that mediate the group I afferent-evoked effects on the locomotor pattern in the decerebrate and paralyzed cat. Some aspects of how the CPG is organized are also suggested. An intracellular analysis of the membrane potential-evoked changes in hindlimb motoneurones demonstrates that the spinal generator for locomotion is mainly responsible for mediating the 'extension enhancement' evoked by ankle extensor group I afferent stimulation during extension and the 'resetting to extension' evoked by similar stimulation during flexion. The results suggest that the mediation of extension enhancement effects can be assisted occasionally by short latency excitatory pathways (*e.g.*, locomotor-related disynaptic pathway) from ankle extensor group I afferents to hindlimb extensor motoneurones. Combined monosynaptic and locomotor-related disynaptic excitation further enhanced membrane depolarization and motor nerve activity in close synergists. Indirect evidence suggests that 'plateau potential' intrinsic conductances induced by combined short latency excitation can support the extension enhancement in some cases. Simultaneous recording in paired motoneurones during group I-evoked resetting showed that the locomotor drive potential phase switch in flexors and extensors is synchronously produced throughout the limb with a central latency often less than 6 ms. These results support the hypothesis of a 'half-centre' organization of the spinal generator for locomotion and suggest that the effects evoked by group I afferents during locomotion are mediated by only few classes of interneurones.

This section is devoted to some aspects that have not been discussed in the two articles. These topics include the relative contribution of motoneurone active conductances and the limitations of the decerebrate and paralyzed preparation. The first article provided indirect evidence that sustained motoneurone intrinsic conductances are activated in some cases during group I-evoked extension enhancement. To prove that this is the case one could inject a short constant current hyperpolarizing pulse during the enhanced membrane depolarization. The contribution of voltage-dependent conductances (*e.g.*, bistable property-like) to extension enhancement could be revealed by short hyperpolarizing pulses that terminate sustained conductances underlying the motoneurone plateau potential (Hounsgaard *et al.* 1984). A problem in assessing the role of active conductances in the group I extension enhancement is related to the fact that LDPs are themselves voltage-dependent (Brownstone *et al.* 1994). One possible way to distinguish the conductances underlying the LDPs from those related to the enhancement, is to superimpose a brief constant current hyperpolarizing pulse on top of a continuous ramp-like depolarizing current. At threshold for the activation of LDP voltage-dependent conductance, one could compare (e.g., subtract) the amplitude of the LDP prior and after stimulating group I afferents (i.e., delivered with a delay after LDP onset). If the group I afferent stimulation increases the LDP amplitude at threshold for LDP voltage-dependent conductances than it is likely that conductances other than those underlying the LDPs are involved in producing the group I extension enhancement in extensor motoneurons. The ligand-dependency of active conductances, like the plateau potential, could also be examined by applying close to the recording site, monoaminergic receptor blockers like the 5-HT

antagonist methysergide (turtle spinal cord slices; Hounsgaard and Kiehn 1989).

One can also examine the contribution of more specific channels to the group I-evoked extension enhancement. For instance, nifedipine, an L-type channel blocker that participates in motoneurone's plateau potentials (Hounsgaard and Kiehn 1989) could be used to assess the role of specific Ca^{2+} conductance. Also, apamin, a specific blocker of calcium-dependent K^+ channels, applied locally (i.e., close to the motoneurone pool) could also be usefull since it has been found to increase repetitive firing, decrease the locomotor rhythm and thus regulate the LDP duration in lampreys during NMDA-evoked fictive locomotion (Hill, Matsushima, Schotland & Grillner 1992; El Manira, Tegnér & Grillner 1994). Application of 2-amino-5-phosphonovalerate (APV, NMDA receptor blocker) might also reveal the contribution of NMDA receptor-channels in the termination of the enhanced LDP since it has been found to augment the repolarization of a depolarizing-evoked response in lamprey spinal neurones (Hill, Brodin & Grillner 1989). In adult *in vitro* rats, NMDA was also found to enhance trigeminal afferent-evoked EPSPs in abducens motoneurones (but NMDA receptor blockers don't reduce the EPSPs) and to evoke regenerative oscillations likely by allowing these EPSPs to trigger motoneuronal voltage-dependent bistability (Durand 1993).

The present results in combination with previous work provided strong evidence of a profound reorganization of the pathways mediating the group I afferent reflexes during locomotion compared with non-locomoting conditions. It is thus clear that a 'rewiring' of the group I pathways occurs when the spinal cord switches into the locomoting mode.

These insights were obtained in a decerebrate, paralyzed preparation which largely limits afferent and descending inputs to the lumbar cord where these reflexes are mediated. This allowed us to examine, in relative isolation, group I reflex actions on motoneurone activity. Consequently, this preparation does not reveal the result of multiple converging inputs on lumbar group I pathways during locomotion. The possibility remains that the rewired reflex pathways examined in this thesis differ during normal unrestrained walking. Some recent evidence however shows that group I reflex pathways are also reorganized during treadmill walking. Ankle extensor group I afferent stimulation can affect the step cycle pattern by prolonging extensor and delaying flexor muscle activity in decerebrate cats spontaneously walking on a treadmill (Whelan *et al.* 1995). These effects in walking cats are similar qualitatively to those found in decerebrate, paralyzed animals (Guertin *et al.* 1995, also described in this thesis) suggesting that the reorganization of group I reflex pathways described in the present study is operational in both preparations. The group I evoked effects were less pronounced in non-paralyzed (Whelan *et al.* 1995) than in paralyzed cats (Guertin *et al.* 1995) which might suggest that multiple converging sensory inputs present during real walking reduce the relative importance of group I reflex actions on motoneurone activity. The reduced effects found during treadmill walking could also possibly be attributed to saturation of an already active group I afferent system. An additional activation of group I afferent fibres evoked by an unexpected perturbation would not be capable of producing large increases of extensor excitability such as seen during fictive locomotion.

Several mechanisms could possibly reduce or modulate reflex actions evoked by group I afferents during locomotion. Their actions could be changed by regulating the access of group I afferent inputs to the spinal cord via presynaptic inhibitory mechanisms. In decorticate and in spinal cats, Dubuc, Cabelguen & Rossignol (1987) have reported biphasic rhythmic fluctuation of hindlimb dorsal root potentials during fictive locomotion, with largest effects during flexion (*e.g.*, antidromic discharge). Intra-axonal recordings of identified primary afferent fibers in MLR-decerebrate and spinal DOPA cats have shown that the majority (89%) of the group I afferents from flexors and extensors exhibit a larger membrane depolarization during flexion. It was also shown that dorsal root reflexes are evoked only in flexor muscle afferents and only during flexion (Gossard, Cabelguen & Rossignol 1991). Group I muscle afferent-evoked dorsal root potentials (from flexors and extensors) during fictive locomotion showed a complex pattern of depolarization but generally the larger effects occurred during extension (Gossard and Rossignol 1990). Together these results suggest that various patterns of presynaptic inhibition of individual afferents subject to both centrally and peripherally-evoked primary afferent depolarization (PAD) migh be mediated by different PAD pathways. However, evidence from this laboratory shows that modulation (evoked only during extension) of the locomotor-dependent group I-evoked disynaptic excitation is evoked by CPG input rather than by PAD pathways (Angel *et al.* 1996). A similar suggestion was made for the group I-evoked monosynaptic excitation found to be variably modulated in either phase of the step cycle (Shefchyk, Stein & Jordan 1984; Angel *et al.* 1996).

Evidence for PAD was also reported in non-paralyzed walking cats (Dubuc, Cabelguen & Rossignol 1988; Beloozerova and Rossignol 1994, 1995) and humans (Stein and Capaday 1988). In humans, the amplitude of the H-reflex was found to be progressively reduced from standing, to walking, and to running with further reduction during early stance and flexion in ankle extensor muscles (Stein and Capaday 1988). More natural stimulation of extensor group I afferents (and possibly group II) was achieved by briefly stretching ankle extensor muscles with a pneumatic device attached to the foot. This stimulus increases soleus activity in a phase-dependent manner as well as H-reflexes (Yang, Stein & James 1991). Together these results show that brief group I muscle afferent-evoked reflexes are movement-, velocity-, and phase-dependent. It remains unclear how the combined actions of supraspinal, peripheral and CPG input onto PAD pathways might ultimately limit and modulate the access of group I afferent inputs to the spinal cord in intact animals (Gossard and Rossignol 1990). The amplitude of group I reflexes during locomotion likely depends on several factors and varies according to the task and the goal to be achieved.

Strong evidence exists suggesting that group I afferent reflexes are involved in regulating other aspects of the step cycle pattern. For instance, Duysens and Pearson (1980) suggested that the phase switch from extension to flexion is controlled by force-sensitive Ib afferents by showing that flexor activity can only occur when the limb is sufficiently unloaded and Ib extensor activity falls below a given threshold level. Further support of this view is found in observations that the swing phase is rapidly reinitiated in

absence of ground contact (an unexpected hole in the treadmill) and hence extensor Ib activity, thus preventing the animal from falling (Gorassini, Prochazka, Hiebert & Gauthier 1994). Similar paradigms in chronic spinal cats showed less effects than in intact animals revealing the importance of converging descending inputs to support this reflex action (Hiebert, Gorassini, Jiang, Prochazka & Pearson 1994).

Group I reflexes were also found to participate in promoting extensor activity just after foot contact. These actions help compensate for the increasing load of the limb during the stance phase (Gorassini *et al.* 1994). Even though this peak in extensor activity occurs about 30 ms after foot contact, evidence suggests that it is largely evoked centrally by the spinal generator for locomotion (Gorassini *et al.* 1994). Thus, extensor group I afferent stimulation appears to enable the spinal generator to produce this enhanced extensor activity that occurs just after foot contact (Hiebert, Whelan, Prochazka & Pearson 1995).

The modulation of group I afferent reflexes (and possibly group II) definitively regulates muscle stiffness allowing a smooth and proper walking pattern. This is revealed particularly in patients with spinal cord injuries who have an increased H-reflex that shows little if any phase-dependent modulation throughout the step cycle. This lack of group Ia reflex modulation, even at the beginning of the stance phase, could contribute to clonus seen during walking (Yang, Fung, Edamura, Blunt, Stein & Barbeau 1991). Modulation of the Ia reflex can be obtained and walking pattern partially restored in spastic paretic patients by brief conditioning cutaneomuscular stimulation of the medial plantar nerve (Fung and Barbeau 1994). Administration of antispastic agents like clonidine (Stewart,

Barbeau & Gauthier 1991) and recently shown, L-DOPA (Eriksson, Olausson & Jankowska 1995) was also found to reduce exaggerated stretch reflex (from group I and II muscle afferents) and clonus during walking.

Others have suggested that presynaptic and phase-dependent reflex modulation are part of normal locomotion. My thesis has shown how group I afferents can by accessing CPG circuitry (and via locomotor-dependent reflexes) modulate motoneurone activity and particularly extensor activity. This indicates that group I afferents can access CPG circuitry to affect both the amplitude and timing of motoneurone activity and hence muscle contraction. The proprioceptive group I afferent system is thus another mechanism that can be used to continually shape and assist the timing of muscle activity during locomotion.

REFERENCES

Adrian and Bronk (1929). The discharge of impulses in motor nerve fibres. Part II. The frequency of discharge in reflex and voluntary contractions. *J. Physiol.* 67: 119-151.

Angel, Guertin, Jiménez & McCrea (1996) Group I extensor afferents evoke disynaptic EPSPs in cat hindlimb motoneurones during fictive locomotion. *J. Physiol.* In press.

Baldissera and Gustafsson (1971a). Regulation of repetitive firing in motoneurones by the afterhyperpolarization conductance. *Brain Res.* 30: 431-434.

Baldissera and Gustafsson (1971b). Supraspinal control of the discharge evoked by constant current in the alpha-motoneurones. *Brain Res.* 25: 642-644.

Barbeau and Rossignol (1991). Initiation and modulation of the locomotor pattern in the adult chronic spinal cat by noradrenergic, serotonergic and dopaminergic drugs. *Brain Res.* 546: 250-260.

Bassler (1993). The walking- (and searching-) pattern generator of stick insects, a modular system composed of reflex chains and endogenous oscillators. *Biol. Cybern.* 69: 305-317.

Beloozerova and Rossignol (1994). Antidromic activity of dorsal root filements during treadmill locomotion in thalamic cats. *Soc. Neurosci. Abst.* 20: pp.1755.

Beloozerova and Rossignol (1995). Antidromic activity in uncut dorsal roots of the cat. *Soc. Neurosci. Abst.* 21: pp.413.

Bergmans, Burke & Lundberg (1969). Inhibition of transmission in the recurrent inhibitory pathway to motoneurons. *Brain Res.* 13: 600-602.

Binder, Heckman & Powers (1993). How different afferent inputs control motoneuron discharge and the output of the motoneuron pool. *Cur. Opin. Neurobiol.* 3: 1028-1034.

Brock, Coombs & Eccles (1952). The recording of potentials from motoneurones with an intracellular electrode. *J. Physiol.* 117: 431-460.

Brown (1914). On the nature of the fundamental activity of the nervous centres; together with an analysis of the conditioning of rhythmic activity in progression, and a theory of the evolution of function in the nervous system. *J. Physiol.* 48: 18-46.

Brownstone and Hultborn (1992). *Regulated and intrinsic properties of the motoneurone: effect on input-output relations.* In Muscle afferents and spinal control of movements. Edited by Jami, Pierrot-Deseilligny, Zytnicki. Cambridge: Pergamon Press, pp175-181.

Brownstone, Gossard & Hultborn (1994). Voltage-dependent excitation of motoneurones from spinal locomotor centres in the cat. *Exp. Brain Res.* 102: 34-44.

Brownstone, Jordan, Kriellaars, Noga & Shefchyk (1992). On the regulation of repetitive firing in limbar motoneurones during fictive locomotion in the cat. *Exp. Brain Res.* 90: 441-455.

Conway, Hultborn & Kiehn (1987). Proprioceptive input resets central locomotor rhythm

in the spinal cat. *Exp. Brain Res.* 68: 643-656.

Conway, Hultborn, Kiehn & Mintz (1988). Plateau potentials in alpha-motoneurones induced by intravenous injection of L-DOPA and clonidine in the spinal cat. *J. Physiol.* 405: 369-384.

Coombs, Eccles & Fatt (1955). The electrical properties of the motoneurone membrane. *J. Physiol.* 130: 291-325.

Dietz (1992). Human neuronal control of automatic functional movements: interaction between central programs and afferent input. *Physiol. Rev.* 72(1): 33-68.

Dubuc, Cabelguen & Rossignol (1987). *Antidromic discharges of primary afferents during locomotion.* In Motor control, edited by Gantchev, Dimitrov and Gatev. Plenum Press, pp.165-169.

Dubuc, Cabelguen & Rossignol (1988). Rhythmic fluctuations of dorsal root potentials and antidromic discharges of single primary afferents during fictive locomotion in the cats. *J. Neurophys.* 60: 2014-2063.

Durand (1993). Synaptic excitation triggers oscillations during NMDA receptor activation in rat abducens motoneurons. *Eur. J. Neurosci.* 5: 1389-1397.

Duysens and Pearson (1980). Inhibition of flexor burst generation by loading ankle extensor muscles in walking cats. *Brain Res.* 187: 321-333.

Eccles and Hoff (1932). The rhythmic discharge of motoneurones. *Proc. Roy. Soc.* (Lond)

B 110: 483-514.

Eccles, Eccles & Lundberg (1957). The convergence of monosynaptic excitatory afferents onto many different species of alpha motoneurones. *J. Physiol.* 137: 22-50.

Edgerton, Grillner, Sjöström & Zangger (1976). *Central generation of locomotion in vertebrates.* In Neural control of locomotion, edited by Herman, Grillner, Sjostrom and Zangger. New York: Plenum Press, pp439-464.

Edgley, Jankowska & McCrea (1986). The heteronymous monosynaptic actions of triceps surae group Ia afferents on hip and knee extensor motoneurones in the cat. *Exp. Brain Res.* 61: 443-446.

Elliott and Wallis (1992). Serotonin and L-Norepinephrine as mediators of altered excitability in neonatal rat motoneurons studies in vitro. *Neuroscience* 47: 533-544.

El Manira, Tegnér & Grillner (1994). Calcium-dependent potassium channels play a critical role for burst termination in the locomotor network in lamprey. *J. Neurophys.* 72 (4): 1852-1861.

Eriksson, Olausson & Jankowska (1995). Antispastic effects of L-DOPA.

Fung and Barbeau (1994). Effects of conditioning cutaneomuscular stimulation on the soleus H-reflex in normal and spastic paretic subjects during walking and standing. *J. Neurophys.* 72(5): 2090-2103.

Garcia-Rill and Skinner (1987). The mesencephalic locomotor region i. Activation of a

medullary projection site. Brain Res. 411: 1-12.

Gossard and Rossignol (1990). Phase-dependent modulation of dorsal root potentials evoked by peripheral nerve stimulation during fictive locomotion in the cat. *Brain Res.* 537: 1-13.

Gossard, Cabelguen & Rossignol (1991). An intracellular study of muscle primary afferents during fictive locomotion in the cat. *J. Neurophys.* 65(4): 914-926.

Gossard, Brownstone, Barajon and Hultborn (1994). Transmission in a locomotor-related group Ib pathway from hindlimb extensor muscles in cat. *Exp. Brain Res.* 98: 213-228.

Graham and Gerard (1946). Membrane potentials and excitations of impaled single muscle fibers. *J. Cell Comp. Physiol.* 28: 99-117.

Granit (1970). *The basis of Motor Control.* Academic Press: London and New York. pp.122-166.

Granit, Kernell & Shortess (1963). Quantitative aspects of repetitive firing of mammalian motoneurones, caused by injected currents. *J. Physiol.* 168: 911-931.

Granit, Kernell & Lamarre (1966a). Algebraical summation in synaptic activation of motoneurones firing within the 'primary range' to injected currents. *J. Physiol.* 187: 379-399.

Granit, Kernell & Lamarre (1966b). Synaptic stimulation superimposed on motoneurons firing in the 'secondary range' to injected current. *J. Physiol* (Lond) 187: 401-415.

Grillner (1981). *Control of locomotion in bipeds, tetrapods, and fish.* In: Handbook of Physiology. The nervous system II, edited by Brookhart and Mountcastle. Bethesda: Amer. Physiol. Soc., pp1179-1236.

Grillner and Zangger (1974). Locomotor movements generated by the deafferented spinal cord. *Acta Physiol. Scand.* 91: 38-39A.

Grillner, McClellan & Perret (1981). Entrainment of the spinal pattern generators for swimming by mechano-sensitive elements in the lamprey spinal cord in vitro. *Brain Res.* 217: 380-386.

Guertin, Angel, Perreault & McCrea (1995). Ankle extensor group I afferents excite extensor throughout the hindlimb during fictive locomotion in the cat. *J. Physiol.* (Lond) 487: 197-209.

Gurfinkel and Shik (1973). *The control of posture and locomotion.* In: Motor control, edited by Gydikov, Tankov, Kosarov. New York: Plenum Press, pp217-234.

Gorassini, Prochazka, Hiebert & Gauthier (1994). Corrective responses to loss of ground support during walking. I. Intact cats. *J. Neurophys.* 71(2): 603-610.

Hiebert, Gorassini, Jiang, Prochazka & Pearson (1994). Corrective responses to loss of ground support during walking. II. Comparison of intact and chronic spinal cats. *J. Neurophys.* 71(2): 611-622.

Hiebert, Whelan, Prochazka & Pearson (1995). Suppression of the corrective response to

loss of ground support by stimulation of extensor group I afferents. *J. Neurophys.* 73(1): 416-420.

Hiebert, Whelan, Prochazka & Pearson (1995) Regulation of stance phase duration by flexor muscle afferents. *Soc. Neurosci. Abst.*, 21: 1430.

Hill, Brodin & Grillner (1989). Activation of *N*-methyl-*D*-aspartate (NMDA) receptors augments repolarizing responses in lamprey spinal neurons. Brain Res. 499: 388-392.

Hill, Matsushima, Schotland & Grillner (1992). Apamin blocks the slow AHP in lamprey and delays termination of locomotor bursts. *NeuroReport* 3: 943-945.

Hochman, Jordan & MacDonald (1994). N-methyl-D-aspartate receptor-mediated voltage oscillations in neurons surrounding the central canal in slices of rat spinal cord. *J. Neurophys.* 72: 565-577.

Hochman, Jordan & Schmidt (1994). TTX-resistant NMDA receptor-mediated voltage oscillations in mammalian lumbar motoneurons. *J. Neurophys.* 72: 1-4.

Hodgkin and Huxley (1939). Action potentials recorded from inside a nerve fibre. *Nature* 144: 710-711.

Hounsgaard and Kiehn (1989). Serotonin-induced bistability of turtle motoneurones caused by a nifedipine-sensitive calcium plateau potential. *J. Physiol.* 414: 265-282.

Hounsgaard, Hultborn, Jespersen & Kiehn (1984). Intrinsic membrane properties causing a bistable behavior of alpha-motoneurones. *Exp. Brain Res.* 55: 391-394.

Hounsgaard, Hultborn, Jespersen & Kiehn (1988). Bistability of alpha-motoneurones in the decerebrate cat in the acute spinal cat after intravenous 5-hydroxytryptophan. *J. Physiol.* 405: 345-367.

Hultborn and Kiehn (1992). Neuromodulation of vertebrate motor neuron membrane properties. *Cur. Opin. Neurobiol.* 2: 770-775.

Hultborn, Wigström & Wängberg (1975). Prolonged activation of soleus motoneurones following a conditioning train in soleus Ia afferents - a case for reverberating loop? *Neurosci. Let.* 1: 147-152.

Ito and Oshima (1962). Temporal summation of after-hyperpolarization following a motoneuron spike. *Nature* (Lond) 195: 910-911.

Jami (1992). Golgi tendon organs in mammalian skeletal muscle: functional properties and cetral actions. *Physiol. Rev.* 72: 623-666.

Jankowska, Jukes, Lund & Lundberg (1967a). The effect of DOPA on the spinal cord. V. Reciprocal organization of pathways transmitting excitatory action to alpha motoneurones of flexors and extensors. *Acta Physiol. Scand.* 70: 369-388.

Jankowska, Jukes, Lund & Lundberg (1967b). The effect of DOPA on the spinal cord. VI. Half-centre organization of interneurons transmitting effects from flexor reflex afferents. *Acta Physiol. Scand.* 70: 389-402.

Jordan (1991). Brainstem and spinal cord mechanisms for the initiation of locomotion. In

Neurobiological Basis of Human Locomotion, ed. M. Shimamura, S. Grillner, V.R. Edgerton. Japan Scientific Societies Press, Tokyo. pp. 3-19.

Jordan (1983). *Factors determining motoneuron rhythmicity during fictive locomotion.* In Neural origin of rhythmic movements, edited by Roberts and Roberts, Cambridge: Cambridge University Press, pp. 423-444.

Kernell (1965a). The adaptation and the relation between discharge frequency and current strength of cat lumbosacral motoneurones stimulated by long-lasting injected currents. *Acta Physiol. Scand.* 65: 65-73.

Kernell (1965b). High-frequency repetitive firing of cat lumbosacral motoneurones stimulated by long-lasting injected currents. *Acta Physiol. Scand.* 65: 74-86.

Kernell (1965c). The limits of firing frequency in cat lumbosacral motoneurones possessing different time course of afterpolarization. Acta Physiol. Scand. 65: 87-100.

Kiehn and Harris-Warrick (1992a). 5-HT modulation of hyperpolarization- activated inward current and calcium- dependent outward current in a crustacean motor neuron. J. Neurophys. 68: 496-508.

Kiehn and Harris-Warrick (1992b). Serotonergic stretch receptors induce plateau properties in a crustacean motor neuron by a dual-conductance mechanism. J. Neurophys. 68: 485-495.

Kiehn, Iizuka & Kudo (1992). Resetting from low threshold afferents of N-methyl-D-

aspartate-induced locomotor rhythm in the isolated spinal cord-hindlimb preparation from newborn rats. Neurosci. Let. 148: 43-46.

Kolmodin and Skoglund (1958). Slow membrane potential changes accompanying excitation and inhibition in spinal moto- and interneurons in the cat during natural activation. *Acta Physiol. Scand.* 44: 11-54.

Kriellaars (1992). *Generation and peripheral control of locomotor rhythm.* Manitoba, University of Manitoba, Ph.D. thesis.

Kriellaars, Brownstone, Noga & Jordan (1994). Mechanical entrainment of fictive locomotion in the decerebrate cat. *J. Neurophys.* 71: 1-13.

Krnjevic, Puil & Werman (1978). EGTA and motoneuronal after-potentials. *J. Physiol.* 275: 199-223.

Lindsay and Feldman (1993). Modulation of respiratory activity of neonatal rat phrenic motoneurones by serotonin. *J. Physiol* (Lond) 461: 213-233.

Lundberg (1981). Half-centre revisited. In *Adv. Physiol. Sci., vol 1., Motion and Organization Principles*, ed. J. Szentagothai, M. Palkovits, J. Hamori, pp. 155-167.

McCrea, Pratt & Jordan (1980). Renshaw cell activity and recurrent effects on motoneurons during fictive locomotion. *J. Neurophys.* 44: 475-488.

McCrea, Shefchyk, Stephens & Pearson (1995). Disynaptic group I excitation of synergist ankle extensor motoneurons during fictive locomotion. *J. Physiol* 487: 527-539.

Miller and Scott (1977). The spinal locomotor generator. *Exp. Brain Res.* 30: 387-403.

Noga, Kriellaars & Jordan (1991). The effects of selective brainstem or spinal cord lesions on treadmill locomotion evoked by stimulation of the mesencephalic or pontomedullary locomotor regions. J. Neurosci. 11(6): 1691-1700.

Orlovski, Severin & Shik (1966). Effect of speed and load on coordination of movements during running of the dog. *Biophysics* 11: 414-417.

Pearson (1995). Proprioceptive regulation of locomotion. *Cur. Opin. Neurobiol.* 5: 786-791.

Pearson and Collins (1993). Reversal of the influence of group Ib afferents from plantaris on activity in medial-gastrocnemius muscle during locomotor activity. *J. Neurophys.* 70: 1009-1017.

Pearson and Duysens (1976). Function of segmental reflexes in the control of stepping in cockroaches and cats. *In Neural control of locomotion*, edited by Herman, Grillner, Stein & Stuart, New York: Plenum Press, pp. 519-538.

Pearson, Ramirez & Jiang (1992). Entrainment of the locomotor rhythm by group Ib afferents from ankle extensor muscles in spinal cats. *Exp. Brain Res.* 90: 557-566.

Perreault, Angel, Guertin & McCrea (1995). Stimulation of hindlimb flexor group II afferents during fictive locomotion. *J. Physiol.* 487: 211-220.

Rossignol (1996). Neural control of stereotypic limb movements. *In Integration of motor,*

circulatory, respiratory and metabolic control during exercise, Handbook of Physiology, edited by Rowell and Shepard. Bethesda: Amer. Physiol. Soc., pp173-216.

Pratt and Jordan (1987). Ia inhibitory interneurons and Renshaw cells as contributors to the spinal mechanisms of fictive locomotion. *J. Neurophy.* 57(1): 56-71.

Schmidt (1994). Afterhyperpolarization modulation in lumbar motoneurons during locomotor-like rhythmic activity in the neonatal rat spinal cord in vitro. *Exp. Brain Res.* 99: 214-222.

Schwindt and Calvin (1973). Equivalence of synaptic and injected current in determining the membrane potential trajectory during motoneuron rhythmic firing. *Brain Res.* 59: 389-394.

Shapovalov (1972). Extrapyramidal monosynaptic and sisynaptic control of mammalian alpha-motoneurons. *Brain Res.* 40: 105-115.

Shefchyk, Stein and Jordan (1984). Synaptic transmission from muscle afferents during fictive locomotion in the mesencephalic cat. *J. Neurophys.* 51(5): 986-997.

Sherrington (1910). Flexion reflex of the limb, crossed extension reflex, and reflex stepping and standing. *J. Physiol.* 40: 28-121.

Shik and Orlovsky (1976). Neurophysiology of locomotion. In *Physiological Reviews*, vol. 56. ed. The American Physiological Society, Maryland, pp. 464-501.

Squalli-Housaini, Cazalet & Clarac (1993). Oscillatory properties of the central pattern

generator for locomotion in neonatal rats. *J. Neurophys.* 70: 803-813.

Stein and Capaday (1988). The modulation of human reflexes during functional motor tasks. *TINS* 11: 328-332.

Stewart, Barbeau & Gauthier (1991). The effects of clonidine in the modulation of the locomotor pattern and spasticity in spinal cord injured patients: a preliminary study. *Can. J. Neurol. Sci.* 18: 321-331.

Székely, Czéh & Voros (1969). The activity pattern of limb muscles in freely moving normal and deafferented newts. *Exp. Brain Res.* 9: 53-62.

Takahashi and Berger (1990). Direct excitation of rat spinal motoneurones by serotonin. *J. Physiol.* 423: 63-76.

Viala and Buser (1969). The effects of DOPA and 5-HTP on rhythmic efferent discharges in hindlimb nerves in the rabbit. *Brain Res.* 12: 437-443.

Wallén, Buchanan, Grillner, Hill, Christenson & Hökfelt (1989). Effects of 5-Hydroxytryptamine on the afterhyperpolarization, spike frequency regulation, and oscillatory membrane properties in lamprey spinal cord neurons. *J. Neurophys.* 61: 759-768.

Whelan, Hiebert & Pearson (1995). Stimulation of the group I extensor afferents prolongs the stance in walking cats. *Exp. Brain Res.* 103: 20-30.

White, Fung & Barnes (1991). Norepinephrine effects on spinal motoneurons. *Prog. Brain*

Res., Amsterdam Elsevier Science Publishers B.V. 88: 343-350.

Yang, Stein & James (1991). Contribution of peripheral afferents to the activation of the soleus muscle during walking in humans. *Exp. Brain Res.* 87: 679-687.

Yang, Fung, Edamura, Blunt, Stein & Barbeau (1991). H-reflex modulation during walking in spastic paretic subjects. *Can. J. Neurol. Sci.* 18: 443-452.