CHARACTERIZATION OF A DISYNAPTIC EXCITATORY REFLEX PATHWAY FROM GROUP I AFFERENTS DURING FICTIVE LOCOMOTION IN THE CAT

By

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A Thesis

Presented to the

University of Manitoba

In Partial Fulfilment of the Requirements

for the Degree

Doctor of Philosophy

in

Physiology

University of Manitoba

Department of Physiology

730 William Avenue

Winnipeg, Manitoba

Canada

July, 1996

ISBN 0-612-16080-7

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CHARACTERIZATION OF A DISYNAPTIC EXCITATORY REFLEX PATHWAY FROM GROUP 1 AFFERENTS DURING FICTIVE LOCOMOTION IN THE CAT

BY

MICHAEL JOSHUA ANGEL

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

DOCTOR OF PHILOSOPHY

"The scientific journey has no end. It has only halting places--
points at which the traveller can look round and survey."

-Sir Charles S. Sherrington (1857-1952)

Abstract

The textbook description of interneuronally mediated reflexes evoked from Golgi tendon organ (Ib) and muscle spindle (Ia) afferents is a predominant oligosynaptic inhibition of homonymous and synergist motoneurones (Eccles, Eccles & Lundberg, 1957b; Jankowksa, Mackel & McCrea 1981a). During locomotion however, reflexes from group I afferents are reorganized into an excitatory system to provide weight support and forward propulsion during the weight bearing phase of locomotion (for review see Pearson, 1995). The reflex pathways that subserve this locomotor-dependent reorganization are only partially understood. Recent experiments using intracellular recording from medial gastrocnemius motoneurones have shown that instead of oligosynaptic inhibition, plantaris group I afferents evoked disynaptic excitation of medial gastrocnemius motoneurones during the extension but not flexion phase of fictive locomotion in the cat (McCrea, Shefchyk, Stephens & Pearson 1995) . Experiments presented in the first section of this thesis use the decerebrate cat preparation in which fictive locomotion is evoked by electrical stimulation of the mesencephalic locomotor region (MLR) and are designed to determine both the sources and distribution of extensor group I disynaptic excitation in many different hindlimb extensor motoneurones during fictive locomotion in the cat. The second section of this thesis uses an identical preparation to locate candidate interneurones subserving this undescribed pathway.

Results from the first portion of this thesis will show that during the extension, but not flexion phase of fictive locomotion, stimulation of group I afferents from any of the ankle extensor nerves evokes disynaptic EPSPs in ankle, knee and hip extensor motoneurones. Motoneurones innervating the bifunctional posterior-biceps & semitendinosus muscle also receive ankle extensor group I disynaptic EPSPs during locomotor extension. Hip extensor group I afferents evoked homonymous disynaptic EPSPs but were ineffective in depolarizing ankle extensor motoneurones. It is argued that these effects are due to cyclic disinhibition of a population of previously undescribed excitatory interneurones.

The second portion of this thesis describes experiments dedicated to locating

candidate interneurones in this group I disynaptic locomotor-related pathway. From the pattern of locomotor-related disynaptic EPSPs described in the first portion, a set of criteria was used to identify candidate interneurones in the novel disynaptic pathway. Extracellular recording of single interneurones was used to show that candidate interneurones were 1) weakly responsive or unresponsive to extensor group I afferents at rest, 2) antidromically activated from extensor motor nuclei known to receive disynaptic EPSPs during extension, 3) activated by ankle extensor group I afferents during fictive locomotion with maximal excitability occurring during extension and 4) activated at monosynaptic latencies measured from the group I afferent volley. Interneurones fulfilling these criteria were located in the intermediate nucleus (1.8 - 2.67 mm below the dorsal surface) of the lumbosacral spinal cord. None of the candidate excitatory interneurone were activated by cutaneous afferents and none had ascending axonal projections along the dorsolateral funiculus or thoracic dorsal columns.

It is proposed that during real locomotion, feedback from primary muscle spindle and Golgi tendon organ afferents facilitate weight support and forward propulsion in part via disynaptic excitation of extensor motoneurones. The control of excitability of candidate interneurones by supralumbar neurones and the central pattern generator is discussed.

Acknowledgements

Dave, just about everything that has made me a better scientist, thinker and problem solver (both scientific and non-scientific) has been the direct result of your teaching and guidance. You have a remarkable gift as a mentor and I feel so very fortunate to have worked with you throughout my entire graduate experience. For these reasons I dedicate this thesis to you... after all, you *are* Bwana Dik.

To my colleagues and former office-mates Pierre Guertin and Marie-Claude Perreault, I will not soon forget our time together in the McCrea lab. Thanks for your friendship, for your lively scientific debates and for forcing me to defend (to near death sometimes) the importance of the holy disynaptic EPSP. To Sharon McCarteny, technician extraordinaire, thanks for the million things you've done to make my life easier.

I cannot thank my committee members Drs., Susan Shefchyk, Brian Schmidt and Larry Jordan enough for their unfailing support, guidance, senses of humour, and most importantly, tolerance.

To Dr. Ismael Jimenez, thanks for convincing us that the sucrose gap is too difficult to be a required portion of my thesis experiments. Thank you also for all you help with the 'disynaptic' study.

Jason MacLean, the blind-patch wonder and the little bro I never had, thank you for everything, especially your friendship. I shudder to think what my non-scientific life would have been like had you not made the wise choice coming to Winnipeg. I expect only great things from you in the years to come, homey.

To Kris Cowley and Matt Ellis (the departmental romance that has hitherto gone unnoticed) thank you for your unfailing interruptions, persistent pestering and for keeping me updated on the lamest jokes you found on the net.

I also wish to acknowledge Professor Elzbieta Jankowska with whom I had the great honour and privilege of collaborating. Thank you Elzbieta for helping us find our interneurones!

I would like to thank my entire family Aubie, Esther-Rose, Jennifer, Jonathan, Suzanne and Steven and friend Wes Schyngera for all their love and support.

Finally, thank you Sara Borins for always believing in me and helping me find my way.

The generous financial support from the Rick Hansen Man in Motion Legacy Studentship Fund for Spinal Cord Research is gratefully acknowledged.

--Michael

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General Introduction

Overview

This introduction is aimed at giving a brief historical perspective (i.e. a time line) of investigations of spinal reflexes evoked by Ib afferents from Golgi tendon organs. Works cited represent major advances in the understanding of the control and operation of tendon organ reflexes and their role in locomotion. These will commence with Sherrington's lengthening reaction-- the first description of autogenic negative force feedback-- and will be followed by the classical electrophysiological studies on reflexes which used ventral root recording of conditioned monosynaptic reflexes, and intracellular recording from motoneurones and interneurones. The original proposed roles of reflexes from tendon organs and primary muscle spindle afferents during motor behaviour derived from non-locomoting preparations will serve as a contrast to the dramatic group I reflex reversal (i.e., reflex inhibition to excitation) reported from present day investigations during treadmill and fictive locomotion. In particular, the latter portion of this General Introduction will focus on the discovery of a group I (Ia & Ib) disynaptic excitatory reflex pathway that is activated during fictive locomotion. The examination of the distribution of this locomotor-dependent group I excitatory pathway, and preliminary characterization of the candidate interneurones mediating this new reflex will be the central focus of the present thesis. The description of Ib reflex pathways leading up to the locomotor-dependent group I reflex reversal will focus on, but will not be restricted to, the classical auto- and heterogenetic inhibition of extensor motoneurones in the cat hindlimb.

The Lengthening Reaction and The Silent Period

It is a humbling experience to read Sir Charles Sherrington's original manuscripts on the organization of spinal reflexes and their relationship to motor behaviour, as many of the details of spinal segmental circuitry revealed in the twentieth century may be considered as extensions of Sherrington's work wherein he used only the most rudimentary forms of myography. Sherrington's animal model of choice for investigations of spinal

reflexes was the decerebrate cat or dog because of the persistent tone of their extensor muscles, coined by Sherrington as decerebrate rigidity. Decerebrate rigidity was first described by Sherrington in 1897 and is characterised by an exaggerated extensor tone throughout the body's musculature that is produced by removal of the cerebral hemispheres. Sherrington showed that a tonic stretch reflex originating from muscle afferent receptors in the fleshy belly of extensor muscles (Liddell & Sherrington 1924; Liddell, Oxon & Sherrington, 1925) was the source of the extensor excitation, and it was later shown that the fast conducting, large diameter afferent fibres relayed these sensory signals into the spinal cord (Lloyd, 1943a). One of Sherrington's many important observations-- and central to the first part of the introduction-- was that the process of central inhibition was demonstrable in this preparation by reflexly evoking transient reductions of the otherwise persistent extensor tone. Sherrington thus showed that the tonic stretch reflex could be inhibited, or display 'plasticity' by activation of other proprioceptors.

One of the 'plastic reflexes' described by Sherrington in the decerebrate dog and cat, is the lengthening reaction (Sherrington 1909). The lengthening reaction is characterized as follows. When the investigator imposes flexion upon a decerebrate dog's knee by immobilising the thigh and exerting a force on the shank to flex the knee, the limb initially resists the movement i.e. the shortening reaction. Sherrington showed that as he increased the imposed force, eventually the extensor [vastus] muscle gives way like a clasp knife and the limb is easily flexed to a new position. In this new position, tonic activity in the extensor muscle returns to its original state and the tension of the muscle is restored, however the limb remains in a stiff flexed position. Sherrington called this 'the lengthening reaction' and showed it to be proprioceptive in origin caused by impulses set up in the sensory end-organs of the stretched muscle, i.e., the vastus crureus muscle.

The proprioceptive end-organs responsible for the lengthening reaction were unknown. However, in the decerebrate rigid dog, Sherrington noticed that stroking near the exposed vastus tendon with a glass rod produced a marked lengthening of the vastus muscle. Sherrington therefore proposed that the tendon organs may be responsible for the

lengthening reaction (Sherrington, 1909).

An important observation made by Fulton & Pi-Suner (1928)-- using a string galvanometer to measure action-currents in muscle (early electromyogram)-- was that a muscle twitch produced by a patellar tendon tap during a tonic stretch reflex of the vastus crureus muscle in the decerebrate cat produced a silencing of muscle action currents during the evoked rise in muscle tension. Denny-Brown (1928) coined this effect the 'silent period' and showed that its expression required initial efferent-induced muscle contraction and subsequent afferent discharge in response to the activated muscle. This sequence of muscle activation then inhibition corresponded to the shortening and lengthening reactions previously described by Sherrington. Denny-Brown suggested the 'silent period' was an electrophysiological representation of the lengthening reaction. Most interesting was that in addition to the vastus twitch-induced silent period in the vastus muscle, there was a simultaneous reduction of action currents in the gastrocnemious electromyogram. Denny-Brown concluded that muscular contraction was the adequate stimulus for muscle spindles, and activation of their afferent fibres produced centrally mediated inhibition of homonymous and synergist motoneurones. Denny-Brown was the first to suggest that proprioceptors have a self-regulatory role of their parent muscle's activity. Furthermore, Denny-Brown demonstrated that an afferent barrage from a contracting muscle could affect the excitability of motoneurones whose target muscle were not engaged in the contraction.

Two opposing theories existed for the mechanism of the origin of the silent period. Fulton & Pi-Suner (1928) suggested the silent period was a peripheral phenomenon. By virtue of muscle spindles' *in parallel,* and Golgi tendon organs' *in series* orientation relative to the contractile extrafusal elements (terminology put forth by these authors) together with the evidence suggesting that muscle spindles are the end-organ of the stretch reflex (Liddell & Sherrington, 1924; Fulton & Pi-Suner, 1928), Fulton & Pi-Suner suggested that shortening of the muscle would slacken the spindle and cause by a transient pause in the tonic muscle spindle discharge. Thus the silent period was not central inhibition *per se* but a phasic absence of excitation. However, they did not rule out the possibility that impulses arising from the in-series Golgi tendon organs produce central

inhibition. Denny-Brown (1928) on the other hand, believed that the twitch produced by tendon-tap is evoked by activation of tendon organs, and muscle spindles are activated by muscle contraction. Thus he proposed muscle spindles evoked central inhibition of the homonymous and some synergist motoneurones. In view of these conflicting theories it was clear that the adequate stimuli of muscle spindles and tendon organs needed to be determined in order to sort out the possible mechanism of the silent period.

Classification of Stretch and Tension Sensitive End-organs

The seminal work of Matthews (1933) provided important insights into the adequate stimuli of fibres innervating proprioceptive end organs in the cat. Matthews examined the responses of individual fibres innervating proprioceptive end-organs to muscle stretch and contraction and found several distinct response patterns: with an initial tension on the muscle, a group of fibres Matthews termed "A" had a resting discharge which accelerated during passive stretch. On the other hand, during contraction A-fibres slowed their firing, or paused completely during the first portion of the twitch, and resumed their baseline rate of activity during the falling edge of the myogram. Those which Matthews called "B fibres" discharged during muscle contraction as well as during stretch, however their threshold for firing during stretch was higher than that of A fibres. The activity of Matthews' A-fibres in response to muscle stretch and contraction was the same as the identified muscle spindle afferents in the frog. Thus Matthews concluded that A-fibres arise from muscle spindles. Adopting the phraseology of Fulton & Pi-Suner (1928) Matthews argues that B-fibres arise from tension sensitive end-organs which lie *in-series* with the contractile elements of the muscle and thus arise from Golgi tendon organs. Although this study did not address reflex effects of A and B fibres, Matthews suggested-- based on their responses to stretch and contraction-- A fibres probably elicit the stretch reflex and B fibres, owing to their exquisite sensitivity to muscle tension, likely evoke the silent period. Matthews' result do not, however, rule out silencing of A fibres as a contributing factor to the silent period. Silencing of A fibres following muscle twitch could not be the sole mechanism of the silent period since muscle twitch evoked a silent period

in a heteronymous electromyogram (Denny-Brown, 1928) and quadriceps A fibres do not contribute to tonic the stretch reflex activation of the gastrocnemeous muscle.

Contraction-Induced Autogenetic Inhibition

If the silent period was indeed the result of central inhibition of the stretch reflex (Denny-Brown, 1928) a novel approach which can differentiate between centrally and peripherally-induced decreases in motoneurone activation following muscle contraction would be of critical importance. The technique of ventral root recording was fully developed by Lloyd in the 1940s who used it to assess the patterns of the group I monosynaptic reflexes, and patterns of 'direct inhibition' by conditioning test monosynaptic reflexes with stimuli to homonymous or antagonist group I afferents (Lloyd 1943a,b). The rationale of this technique was that if a pool of motoneurones receives monosynaptic excitation (Eccles & Pritchard, 1937) from group I afferents, then any increase or decrease in the amplitude of the test reflex evoked by conditioning another nerve at an appropriate interval will be directly attributed to either excitation or inhibition, respectively, of the target motoneurones. Granit showed that if a test (T) homonymous monosynaptic reflex was preceded by a conditioning (C) homonymous muscle contraction (10ms conduction-test or C-T interval), the test monosynaptic reflex was diminished and thus the target motoneurones were inhibited (Granit 1950). By recording the probability of firing of single motoneurones, Granit & Strom (1951) showed that the reduction in monosynaptic reflex initially described by Granit (1950) was a consequence of afferent-evoked inhibition and not simply due to motoneuronal refractoriness since motoneurones in the subliminal fringe are further inhibited by homonymous muscle contraction. Granit called this autogenetic inhibition.

Although it was later shown that the twitch-evoked 'silent period' of homonymous muscles can be predominantly attributed to the slackening of muscle spindles producing a temporary reduction of tonic excitation and a pause in the electromyogram (as originally proposed by Fulton & Pi-Suner 1928, and confirmed by Jansen & Rudjord 1964) Denny-Brown's study was the first demonstration

that central inhibition of synergists could be evoked by afferents whose adequate stimulation is muscle contraction.

Autogenetic inhibition was considered an inhibitory process mediated by afferents whose end-organs are sensitive to muscle contraction. Not surprisingly, muscle stretch facilitated the contraction-induced autogenetic inhibition in gastrocnemious motoneurones (Haegbarth & Naess, 1950). This is consistent with Matthew's observations that B fibres were also somewhat responsive to stretch. However, following the demonstration that small efferent stimulation (gamma motoneurones) produced contraction of the muscle spindle-- hence increasing the activity of A-fibres but not B-fibres (Kuffler, Hunt & Quillam, 1951; Hunt & Kuffler, 1951)-- Hunt (1952) showed that selective activation of small efferents, on occasion, evoked autogenetic inhibition. This pure spindle-evoked inhibition may have been one of the mechanisms underlying the facilitation by stretch of the autogenetic inhibition observed by Haegbarth & Naess, (1950, see later).

Ib Afferents Evoke Interneuronally Mediated Inhibition

Laporte & Lloyd (1952) readdressed patterns of inhibition and facilitation of the monosynaptic reflex using single electrical conditioning stimuli of peripheral nerves. In their landmark paper in 1952, Laporte & Lloyd demonstrated that by increasing the condition-test interval of a synergist nerve to 0.5 ms, they observed a dramatic decline in the curve of facilitation of test monosynaptic reflexes which reached a maximum well below the amplitude of control monosynaptic reflexes. Two striking characteristics of this inhibition were: its consistent sensitivity to condition-test intervals, and its widespread distribution among different combinations of nerves. Laporte & Lloyd reported that inhibition was demonstrable:

> ...utilizing the following couplings of conditioning and test volleys named in reference to the muscles of origin: quadriceps and triceps surae; quadriceps and plantaris; knee flexors and triceps surae; extensor brevis and triceps surae; triceps

surae and a) quadriceps b) plantaris c) flexor brevis; flexor longus and a) quadriceps b) the knee flexors c) triceps surae; plantaris and quadriceps; anterior hamstrings and pretibial flexors (Laporte & Lloyd, 1952, p 615).

By gauging the strength of the conditioning stimuli it was shown that the threshold for evoking this inhibition was similar to that which evoked a facilitation of the monosynaptic reflex, i.e., it was evoked via the largest, fastest 'group I' muscle afferents (Lloyd 1943a). However it was also shown that the degree of inhibition often became more robust with increasing strength of the conditioning stimulus (see Laporte & Lloyd 1952, fig 6). Two possible explanations might have accounted for these observations: 1) the inhibition might be mediated monosynaptically by afferent fibres having slower conduction velocities or 2) inhibition is mediated by afferents with similar conduction velocity, however the latency of inhibition is due to synaptic delay via an interposed inhibitory interneurone. Because a C-T interval of 0.5-0.6 ms consistently evoked inhibition when utilizing either proximal or distal muscle-nerves for conditioning, it was concluded that the inhibitory deviation from the monosynaptic reflex facilitation curve at C-T intervals of 0.5-0.6 ms was due to synaptic delay produced by one inhibitory interneurone interposed between afferent and motoneurone i.e. an addition synaptic delay (Lorente de No, 1935).

There was strong circumstantial evidence that the inhibition reported by Laporte & Lloyd was evoked by impulses arising from Golgi tendon organs. Hunt & Kuffler (1951) had shown that fibres conducting impulses from contraction sensitive end-organs are of the large group I range, and Hunt (1952) had provided the most convincing evidence that inhibition of homonymous and synergist motoneurones was mediated by the selective activation of Golgi tendon organs. Results from Laporte & Lloyd (1952) were in keeping with these two important observations, and with the advantages of using electrical stimulation of many nerves it was possible to disclose the widespread pattern of this Golgi tendon organ, short latency inhibition between synergists at the same joint and across several joints.

In addition to this widespread inhibition Laporte & Lloyd (1952) reported the

existence of disynaptic excitation of antagonists mediated by the same large diameter afferents. This disynaptic pathway was termed the *inverse myotactic reflex* since group I afferents from like muscles inhibited synergist motoneurones and group I afferents from antagonist muscle produced excitation of the antagonist motoneurones.

Intracellular Recording of Ib Reflexes

The year 1952 proved to be a pivotal one in the investigation of spinal reflexes as the introduction of the technique of intracellular recording from single motoneurones (Brock, Coombs & Eccles, 1952) revealed the subthreshold electrical events that occur at the level of the motoneurone membrane. Using intracellular recording from single motoneurones, Eccles, Eccles & Lundberg (1957a) examined the synaptic effects attributable to afferents which evoke the late component of the group I afferent volley. When the early and late components of the group I volleys were separable (Bradely & Eccles, 1953), it was shown that early volley evoked monosynaptic excitatory postsynaptic potentials (EPSPs) in homonymous motoneurones, and fibres conducting the late group I volley evoked non-monosynaptic inhibitory postsynaptic potentials (IPSPs) in synergist motoneurones. By deduction it was presumed that afferents from Golgi tendon organ were responsible for the late volley. The use of intracellular recording was significant as it revealed several shortcomings of the excellent work provided by Lloyd's group. Three of Lloyd's conclusions were that 1) inhibition of synergist motoneurones by large diameter afferents was always disynaptic, 2) flexors evoked autogenetic inhibition similar to that observed in extensors, and 3) the same afferents that evoked disynaptic inhibition of synergist, also evoke disynaptic excitation of antagonist, i.e. Lloyd's inverse myotactic reflex. Using intracellular recording Eccles, Eccles & Lundberg (1957b) showed two peaks in the frequency distribution of latencies of Ib IPSPs; one peak occurred at 1.5-1.6 ms and another at 2.7-2.8 ms. These are consistent with disynaptic and trisynaptic pathways respectively. Eccles *et al.* (1957b) only rarely observed auto- and heterogenetic inhibition of flexor motoneurones compared to that found in extensor motoneurones (see tables 1-3 in Eccles *et al.* 1957b). Finally, Eccles *et al.* (1957b) found little evidence for a strict

reciprocal disynaptic excitation of antagonists. For example, flexor digitorum longus Ib afferents evoke Ib EPSPs in posterior biceps & semitendinosus motoneurones but not in their antagonist extensor digitorum longus motoneurones; posterior biceps & semitendinosus Ib afferents rarely evoked Ib excitation of quadriceps motoneurones but did evoke Ib inhibition in about 20% of pretibial flexor motoneurones; Ib afferents from triceps surae effectively evoked disynaptic EPSP in the posterior biceps & semitendinosus motoneurones however disynaptic Ib EPSP were rarely evoked in tibialis anterior motoneurones. Finally peroneal motoneurones which received Ia reciprocal inhibition from triceps surae could also receive oligosynaptic Ib inhibition (Eccles *et al.* 1957). Thus not only did the pattern of inhibition of motoneurones by Ib afferents not resemble an inverse myotactic reflex i.e. disynaptic excitation of strict antagonists, but some antagonist motoneurones had overlapping Ia reciprocal and Ib inhibition. Thus there appears no predicted reciprocity between Ib afferents and motoneurones based on the motoneurones' "Ia receptiveness"

Descending and Multisensory Control of Ib Reflexes

The system of oligosynaptic (di- and trisynaptic) inhibition of hindlimb motoneurones from Ib afferents is widespread and consistent in a number of experimental preparations. For example, in anaesthetized spinal (Jankowksa, McCrea & Mackel 1981a), decerebrate intact (Eccles & Lundberg, 1959), decerebrate spinal cats (Eccles *et al.* 1957) Ib afferents from triceps surae routinely evoke oligosynaptic inhibition of triceps surae, plantaris, flexor digitorum hallucis and longus, quadriceps, semimembranosus & anterior biceps motoneurones. Sensory information carried by Ib afferents from ankle extensors can therefore have an inhibitory influence on motoneurones innervating synergist muscles at the toes, ankle, knee and hip. Because this pathway is mediated by interneurones, the opportunity exists that the interneurones involved may be shared by other systems. The pioneering work of Lundberg and his group in Goteborg disclosed the extent to which inhibition evoked by Ib afferents was shared by other segmental and descending systems.

By conditioning monosynaptic reflexes from extensors with stimuli applied at group

Ib strength Eccles & Lundberg (1959) showed that the magnitude of Ib inhibition was greater in the spinal cat than in the decerebrate spinal intact preparation. Low pontine lesions in the decerebrate cat released a tonic descending inhibition of the interneurones mediating Ib inhibition (Eccles & Lundberg, 1959; Holmqvist & Lundberg 1959) which was later shown to be mediated by the noradrenergic reticulospinal system (Anden, Jukes & Lundberg, 1966). Activation of neurones in the pyramidal tract by stimulation of the motor cortex facilitated Ib inhibition from flexor digitorum longus in gastrocnemius-soleus and plantaris motoneurones (Lundberg & Voorhove, 1962). Engberg, Lundberg & Ryall (1968a, b) showed that selective activation of dorsal reticulospinal fibres reduced the quadriceps-evoked Ib inhibition in gastrocnemius-soleus motoneurones. The rubrospinal tract also facilitates Ib inhibition from extensors to extensors, and extensors to flexors (Hongo, Jankowska & Lundberg, 1969). The effects produced by activation of the rubrospinal tract are widespread as it facilitates the inhibition of motoneurones innervating muscles at different joints, and can disclose Ib inhibitory connections which--without the conditioning rubrospinal stimuli-- are very weak or absent (Hongo *et al.* 1969). The tonic suppression of Ib reflexes in the decerebrate state that is released by low pontine lesions, the inhibition by dorsal reticulospinal neurones, together with the routine appearance of spatial facilitation of segmentally-evoked Ib inhibition by activation of descending corticospinal and rubrospinal systems, suggest a massive convergence of descending systems onto interneurones mediating inhibition from Ib afferents. The contribution of these descending systems onto this oligosynaptic pathway suggests that higher motor centres probably influence motoneurone excitability via the Ib interneurone during purposeful movements.

Although Ib afferents represent the most dominant input onto this reflex pathway, the pattern of convergence from sources other than Ib afferents extends to afferents whose receptors have different adequate stimuli than tendon organs. Lundberg & Malmgren (1975; 1977) demonstrated spatial facilitation of Ib inhibition by conditioning cutaneous or joint afferents. As originally demonstrated by Granit (1950) and Hunt (1952), activation of fibres innervating muscle spindles by natural stimulation could evoke autogenetic

inhibition. The possible contribution of Ia afferents to autogenetic inhibition was largely ignored until Fetz, Jankowska, Johannisson & Lipski (1979), using intracellular recording from motoneurones, reexamined the effect on motoneurones of selectively activating Ia afferents. Indeed, small, brief stretches to triceps surae and plantaris muscles evoked small amplitude oligosynaptic IPSPs in ankle and knee extensor motoneurones with latencies consistent with di- and trisynaptic linkages. Furthermore it was shown shortly after that the pattern of Ia-evoked inhibition of synergists overlapped with the pattern of inhibition evoked by Ib afferents (Jankowska, McCrea & Mackel 1981) and using the spatial facilitation technique, Ia and Ib afferents were shown to evoke inhibition via common inhibitory interneurones (Jankowska & McCrea 1983). The similar effects of Ia and Ib afferents lead to the replacement of the term "Ib inhibition" to a more appropriate "group I non-reciprocal inhibition," (Jankowska *et al.* 1981). The interneurones mediating non-reciprocal inhibition are thus subject to excitatory and inhibitory control from numerous segmental and descending systems.

The description of pathways that facilitate Ib effects set the stage for investigations aimed at locating the interneurones subserving group I non-reciprocal inhibition. Based on the sources that facilitated Ib reflexes, it was possible to predict the response patterns of candidate inhibitory interneurones in this pathway.

Interneurones mediating group I non-reciprocal inhibition in the cat hindlimb

Eccles, Fatt, Langren & Windsbury (1954) recorded intraspinal field potentials evoked by group I stimulation in an attempt to locate interneurones mediating Ia reciprocal inhibition of antagonist motoneurones. Their finding of a large field potential within the intermediate nucleus lead to their extracellular recording of individual intermediate nucleus interneurones with monosynaptic input from Ia afferents and non-monosynaptic input from cutaneous afferents (Eccles, Fatt & Langren, 1956). Although they reported that these interneurones likely produced Ia inhibition of antagonists, we have the luxury of hindsight to point out that, unbeknownst to Eccles *et al.* (1956), the interneurones in that study represented the first recordings of interneurones mediating group I non-reciprocal

inhibition. The full identification of interneurones mediating group I non-reciprocal inhibition in the cat hindlimb was performed to a great extent by Jankowska's laboratory in Sweden. Armed with similar electrophysiological techniques used to identify the Ia inhibitory interneurone (Jankowska & Roberts, 1972) Jankowska's group located interneurones in Rexed lamina V-VI in the lumbosacral cord (with the highest number in L6) which project monosynaptically to the motor nuclei and had mono- or disynaptic input from group Ib as well as Ia and cutaneous afferents. An axon collateral of these interneurones ascends the dorsolateral funiculus to synapse on the cells of the dorsal spinocerebellar tract (DSCT). Using the technique of sucrose gap recording of ventral root potentials, it was shown that activation of these interneurones produced IPSPs in extensor motoneurones (Brink, Harrison, Jankowska, McCrea & Skoog, 1983; Hongo, Jankowska, Ohno, Sasaki, Yamashita & Yoshida, 1983). Interneurones mediating non-reciprocal inhibition are also subdivided into discrete subpopulations based on their axon projections (see Jankowska, 1992). Subdivision of interneurones based on synaptic input is difficult since many interneurones in the sampled population examined by Harrison & Jankowska (1985) that receive Ib input could also receive mono- or disynaptic EPSPs from Ib afferents from other muscles, as well as mono- or disynaptic excitation from triceps surae and plantaris Ia afferents, and any combination of cutaneous and descending pyramidal and rubrospinal input. Another feature of interneurones mediating non-reciprocal inhibition is their mutual inhibition. Thus stimulation of group I afferents could evoke EPSPs and non-monosynaptic IPSPs in the same interneurones (for summary diagram see Harrison & Jankowska, 1985).

Although Ib input to these interneurones was used for their initial identification, and indeed represents their predominant synaptic input, the contribution from multisensory sources makes the term "Ib inhibition" incorrect since during natural movements Ib afferents will never be activated without coincident activation of cutaneous and joint afferents as well as perhaps a contribution from a variety of descending systems. In fact, interneurones mediating group I non-reciprocal inhibition of motoneurones can hardly be called a simple reflex pathway; the presence of mutual inhibition between interneurones

and the massive convergence of segmental, propriospinal and descending input onto these interneurones suggests a highly organized system for sensorimotor integration that serve to coordinate the activity of motoneurones throughout the limb (see below).

Role of Ib System During Motor Behaviour

The theory that Ia and Ib only provide opposing synaptic effects, i.e. excitation and inhibition respectively of the same motoneurone, which balance each other to produce a constant muscle stiffness (for review, Houk 1979), should have been in question from observations by Granit (1950) and Hunt (1952), who demonstrated that activation of muscle spindles could also evoke autogenetic inhibition. However not until evidence for shared inhibitory pathways of Ib and Ia afferents was provided could Houk's theory of constant stiffness be dismissed. The Ia contribution to group I non-reciprocal inhibition is a difficult matter to reconcile since Ia afferents also produce monosynaptic excitation of homonymous and some close synergist motoneurones. Lundberg & Malmgren (1988) proposed that Ia input to Ib interneurones provided dynamic sensitivity to Ib reflexes which would allow the excitability of the interneurones to parallel the dynamic length changes in the muscle. Based on the relatively weaker Ia input to these interneurones (Fetz *et al.* 1979), it was proposed that the actions of Ia afferents alone would not be to fire the Ib interneurones but would to add to the Ib input during length changes and allow continuous tension regulation as long as the spindles are activated thus giving Ib reflexes dynamic sensitivity across various muscle length changes.

The multisensory and descending convergence onto the Ib inhibitory pathway lead Lundberg et al. (1974, 1977) to suggest that the Ib pathways may be important for the termination of exploratory movements; the contribution of cutaneous, joint and descending inputs onto common interneurones would facilitate and coordinate the regulation of muscular tension of muscles at different joints.

Role of Group I Afferents During Locomotion

Sherrington's early experiments on the reflex control of stepping in the cat revealed important concepts in the peripheral regulation of centrally programmed movements. From observations in the decerebrate spinal cat, Sherrington (1910) proposed that sequential activation of the flexor reflexes and rebound extension reflexes were the predominant source of excitatory drive to a centrally generated rhythm. Observing the locomotor effects produced by stimulating the cut surface of the bulb, Sherrington concludes:

> The seat of the rhythm is obviously not peripheral. It is not in the muscles or their motor nerves for they do not when thrown into activity in other ways show any trace of rhythm of this frequency. Nor can it lie in the receptive organs of the skin or their afferent nerve trunks for direct stimulation of the cross section of the spinal axis itself provokes the rhythmic reply. The rhythm is therefore central in its seat. Remembering that proprioceptive stimuli aroused in the limb itself can excite the reflex the possibility naturally suggests itself that the reflex movement of the limb initiated by a distant stimulus will arouse in the limb stimuli capable of modifying the influence there of the original stimulus. (Sherrington, 1910 pg 86-87).

After reviewing Phillipson's early gait analysis (1905), Liddell & Sherrington (1924) noticed that during locomotion, the stretching that occurs during the angular excursions of the joints is sufficient to elicit a myotactic reflex. The myotactic reflex was initially postulated to contribute to the switching between phases of locomotion i.e. from flexion to extension. It was suggested that flexion of a joint would cause a lengthening of the extensor muscle acting at that joint resulting in a myotactic reflex which would help in the switch from flexion to extension. On the relation of the stretch reflex to the flexion reflex during the switch from knee flexion to extension during locomotion, Liddell & Sherrington state:

> Reciprocal inhibition [flexion reflex inhibition of knee extensors] may have its

raison d'etre in preventing the stretch-reflex of the antagonist extensor and thus avoiding unnecessary work spent against opposed muscular contractions. But on diminution and subsidence of the reflex flexion the reciprocal inhibition of the extensor likewise diminishes and subsides; there then remains the stretched posture imposed on the extensor by the flexed position given to the knee. This stretched posture of the extensor excites, as the inhibition of the extensor muscle's proprioceptive arc subsides, a reflex contraction...in the stretched muscles. This reflex contraction will in its turn, by opening the knee-angle and relieving the muscle of its stretch, diminish or abrogate the reflex extensor's contraction, lessening the stimulus which excites it, undermining its contraction...and undoing the peripheral augmentation of contraction which lengthened condition of the muscle carries with it, especially markedly in the case of this [vastus] muscle" (Liddell & Sherrington, 1924, p).

Liddell & Sherrington thus supposed that Ia afferents exert their effects during locomotion when their parent muscle is passively stretched. Severin, Orlovskii & Shik (1967) made an important advance in the understanding of the role of Ia afferent in locomotion with their afferent recordings during mesencephalic treadmill locomotion (see later). Severin *et al.* showed that Ia afferents fired coincidently with their parent alpha-motoneurones. Thus gamma innervation of muscle spindles permitted Ia afferents to fire during extrafusal muscle contraction. Furthermore, it was later shown that selective inactivation of cutting gamma motoneurones reduced the amplitude of alpha motoneurone activity during treadmill locomotion (Severin, 1970). In view of the activity pattern of hindlimb muscles during normal unrestrained locomotion, Lundberg (1969) concluded that with the aid of alpha-gamma coactivation (Severin *et al.* 1967) the organization of monosynaptic excitation among hindlimb muscles would be ideal for re-enforcing extension and facilitating forward propulsion of the centrally programmed rhythm of locomotion. In accordance with Lundberg's hypothesis, Dietz, Schmidtbleicher & Noth (1979) showed that in the running human, much of the force generated in ankle extensor muscles results

from the lengthening contraction that occurs during the yield phase of extension and is mediated by Ia afferents. Another interesting characteristic of the pattern of Ia monosynaptic connections that further links its organization with a functional role in locomotion is their consistent reconfiguration throughout evolution from digitigrade walking in cats, to plantigrade walking in humans: monosynaptic excitation from medial gastrocnemius to soleus is strong in the cat, weaker in the baboon (Hongo, Lundberg & Phillips 1984) and absent in humans (Mao, Ashby, Wang & McCrea 1984).

The role of the interneuronally mediated Ib reflexes during locomotion is less clear. As previously mentioned, non-reciprocal inhibition can be evoked in synergist motoneurones acting at several joints and also in some flexors. Taken together with its multisensory and descending control it seems that Ib reflex pathways may be more important for coordinating and adjusting muscle tension in muscles of different joints rather than simply being a negative force feedback system for its parent muscle. In the case of extensor muscles in the cat this organization might allow adequate yield of the limb during extension and permit a smooth, bouncing gait during walking, trotting and galloping (Lundberg 1969; Goslow, Reinking & Stuart, 1973).

Although all theories concerning the roles of interneuronally mediated group I reflex pathways during locomotion were based on rigorous electrophysiological tests and scrutinized by the leaders in the field, they were acquired from non-locomoting preparations. The hypothesis was that during locomotion, the central nervous system utilizes the interneurones interposed in reflex pathways identified under experimental conditions (non-locomoting preparations) to modify the centrally generated locomotor rhythm. The tacit assumption was that reflex pathways operational during locomotion are the same as those activated at rest, however under tight control of spinal and supraspinal systems. Thus since the earliest reports of Ib reflexes in homonymous and synergist motoneurones using ventral roots recording (Granit 1950; Hunt 1952; Laporte & Lloyd, 1952) and from intracellular recording in homonymous and synergist motoneurones (Eccles *et al.* 1957b,c) in decerebrate, decerebrate spinal, spinal anaesthetized, or anaesthetised spinal intact cats, the effects produced by Ib afferents has been inhibitory. There appeared

in the literature an agreement that activation of Golgi tendon organ afferents during locomotion evoked oligosynaptic inhibition of homonymous and synergist motoneurones and oligosynaptic excitation of some antagonist motoneurones, and that Ia convergence onto this pathway offered a dynamic sensitivity (Lundberg & Malmgren, 1988; however see Powers & Binder, 1985).

So far this General Introduction has briefly described the origins and organization of group I non-reciprocal inhibition and the supposed roles of group I reflexes during locomotion. A group of laminae V-VI interneurones mediating non-reciprocal inhibition have also been outlined. The remainder of this Introduction will focus on the dramatic reorganization of group I reflexes from the inhibitory system (described above) to an excitatory reflex system during locomotion. Special attention will be paid to the identification of a new locomotor-dependent disynaptic excitatory pathway and its inference of a previously undescribed population of excitatory spinal interneurones.

Locomotor Preparations

The examination of reflex pathways using locomotor preparations has revolutionized our current understanding of the possible roles of group I (Ia and Ib) afferents during locomotion. Two ways in which locomotor activity in limb motoneurones can be activated in controlled experimental conditions are via electrical stimulation of the mesencephalic locomotor region (MLR), or by intravenous administration of certain drugs. In the former, it was shown by Shik, Severin & Orlovski (1966) that monopolar electrical stimulation (50-200μA, posterior 1-2 mm, lateral, 4 mm, 3-6 mm below the surface of the brainstem) in the midbrain corresponding to the cuneiform nucleus can induce a cat to walk on a treadmill. Cells in the medial reticular formation (MRF) receive input from the MLR and descend uni- and bilaterally via the ventrolateral funiculus (VLF) and activate the spinal central pattern generator for locomotion (Jordan, 1983). With the use of neuromuscular blocking agents and artificial ventilation it is also possible to produce rhythmic locomotor depolarization and hyperpolarizations of motoneurones (Jordan, 1983) that have similar frequencies, durations and alternating pattern to that seen in a real

walking cat (Engberg & Lundberg 1969) except the cat remains motionless and has no phasic muscular contractions or afferent feedback i.e. fictive locomotion. Locomotion can also be evoked pharmacologically. Intravenous application of either L-DOPA and the monoamine oxidase inhibitor nialamide (Grillner & Zangger, 1979), or the alpha-2 adrenergic agonist clonidine and opioid antagonist naloxone in the spinal cat can activate the spinal locomotor circuitry (Pearson & Rossignol, 1991). MLR-evoked locomotion is more advantageous over pharmacologically-induced locomotion when studying locomotor-related reflexes since the experimenter has an easy control-switch to "turn on" and "turn off" the locomotor circuitry thus providing control [absence of locomotion] and locomotor states throughout the experiment.

Reversal of Ib Reflexes During Locomotion

The landmark study by Duysens & Pearson (1980) provided the first evidence that during locomotion new Ib reflexes are revealed which cannot be explained by the pattern of Ib reflexes previously recorded at rest (Eccles *et al.* 1957b). During spontaneous treadmill locomotion in the decerebrate cat, with three moving and one fixed limb, Duysens and Pearson (1980) showed that selective activation of triceps surae and plantaris Ib afferents by ventral root stimulation during flexion terminated flexor activity in the same limb, as recorded by electromyogram (EMG), and the same stimulus prolonged the extensor EMG activity. In addition, muscle stretches that increase the force on the triceps tendon beyond 4 kg produced similar effects. In other experiments clamping the intact ankle at 45 degree, caused the cat to maintain a tonically extended ipsilateral limb and prevented limb flexion. The positive evidence for a contribution by Ib afferents (ventral root-evoked muscular contraction) and the negligible effect of 6-10μm vibrations, taken together with the effects produced by clamping the ankle in a flexed position, lead Duysens & Pearson (1980) to conclude that Ib and not Ia afferents were responsible for these effects. It was postulated that removal of sensory information conducted by Ib afferents was necessary for the switch from stance to swing and the prolongation of extension was mediated via alternate Ib pathways. Two important observations from this study include

1) new excitatory Ib reflex pathways are expressed during locomotion and 2) Ib reflexes can perturb, or *reset* the locomotor pattern.

The details of the synaptic connectivity underlying this new Ib reflex remained poorly understood until they were reexamined in the fictive locomotor preparation wherein locomotor discharges in motoneurones was evoked by intravenous administration of L-DOPA and inhibitor nialamide (Conway, Hultborn & Kiehn, 1987). Using intracellular recording from flexor motoneurones it was shown that stimulation of the plantaris nerve at group I strength terminated flexor activity and initiated a phase advanced extension, i.e. reset the step-cycle to extension. ENG recordings revealed that when the same stimulus was delivered during the extension phase of fictive locomotion, extensor activity was prolonged and the subsequent flexion delayed. As in Duysens & Pearson (1980), it was shown that ventral root-evoked contraction of the triceps muscle reset the locomotor cycle to extension, but vibration of the lateral gastrocnemius-soleus muscle was ineffective. Further evidence that the effects produced by Ib afferents are mediated via interneurones of the spinal central pattern generator include the ability of sinusoidal stretches of triceps surae to entrain the endogenous rhythm of locomotion (Conway *et al.* 1987; Pearson, Ramirez, Jiang, 1992). Conway *et al.* (1987) put forth the hypothesis that during locomotion, Ib afferents evoke their effects on motoneurones by activating the excitatory interneurones that produce the "late DOPA reflexes" (cf. Conway *et al.* 1987, figs. 3 & 4; see also Gossard, Brownstone, Bajaron & Hultborn, 1994) which have been implicated as components of the spinal central pattern generator for locomotion (Jankowska, Jukes, Lund & Lundberg 1967 a, b). This polysynaptic pathway has a central latency of 4ms and the EPSPs recorded in extensor motoneurones are voltage dependent (Brownstone, Gossard & Hultborn 1994) and are larger during the flexion phase of fictive locomotion (Gossard *et al.* 1994).

Pearson & Collins (1993) showed that in the chronic and acute spinalized (T_{13}) cat, the inhibition of MG EMG evoked by plantaris group I stimulation during tonic activity is replaced by excitation during the extensor phase of clonidine-evoked treadmill locomotion. The inability of vibration of the single plantaris muscle (amplitudes sufficient

to activate all Ia afferents) is evidence that the effects on the motor pattern are mediated by Ib afferents although the authors submit that Ia afferents could have some kind of facilitatory effect. The importance of this finding was that the reversal of Ib reflexes is present during locomotion that more closely resembles real walking (see also Whelan, Hiebert & Pearson, 1995).

The evidence that the triceps Ib-evoked inhibition of ankle extensor motoneurones is replaced by excitation begs the question of whether group Ib reflexes to extensor motoneurones of other joints are also excitatory during locomotion. Furthermore it remained unknown if group I afferents from muscles at other joints had the same effects on the locomotor pattern as do group I afferents from ankle extensors. These questions were addressed in the MLR-evoked fictive locomotor preparation (Guertin, Angel, Perreault & McCrea 1995). Trains of electrical stimulation of any of the ankle extensor nerves at group I strength were shown to prolong the extension phase of locomotion at the ankle, knee and hip joints as recorded in peripheral nerve ENGs. Although group I afferents from more proximal extensor muscles (i.e. SmAB and Q) could enhance homonymous and some synergist locomotor activity, the widest pattern of enhanced extension was evoked by group I afferents of the ankle extensors. The observation that trains of stimuli at very low intensity (i.e. 1.16T x 20 shocks) could evoke extension enhancement suggested that a contribution of Ia afferents remained a viable possibility!

Ia Afferents and Locomotion: Resetting the Record

There has been no conclusive evidence to refute the hypothesis that, like Ib afferents, Ia afferents impinge on interneurones of the central pattern generator and perturb the locomotor cycle. Even the earliest investigations (Duysens & Pearson 1980; Conway *et al.* 1987) although not articulated, indicated an existence for interneuronally mediated Ia excitation of motoneurones via the CPG. For example, Duysens & Pearson (1980) report, "When small amplitude vibration was applied, in the range of 6-10 μm (f=64Hz) the effect was to decrease the duration of the TA bursts (by about 40%) but not to produce TA burst failures. TA burst failures did appear when the vibration amplitude was increased

to beyond 30 μm." Fetz *et al.* (1979) previously demonstrated that stretches up to 40μm of the triceps with an initial tension of 5N will selectively activate all Ia afferents without activating Ib afferents. The absence of a systematic examination of the threshold amplitude of vibration that evoked resetting undermines the conclusion that the positive results evoked by vibration (i.e. resetting at stretch amplitudes $>30\mu$m) are "Ib effects". Experiments reported by Conway *et al.* (1987) repeated the protocol of Fetz *et al.* (1979) for selective activation of Ia afferents but they only stretched the lateral gastrocnemius-soleus muscle. The effect of activating Ia afferents from all the ankle extensors was not tested as was the case in Fetz *et al.* (1979). Nevertheless, the reader is referred to their fig. 7b (Conway *et al.* 1987) in which selective activation of lateral gastrocnemius-soleus Ia afferents prolongs the contralateral PBSt (coPBSt) ENG beyond the duration of the stimuli. CoPBSt motoneurones do not receive monosynaptic Ia connections from ipsilateral ankle extensors. Guertin *et al.* (1995) and Angel, Guertin & McCrea (1995) examined the effects of selective activation of all four ankle extensor [triceps surae and plantaris] group Ia afferents during MLR-evoked fictive locomotion. It was shown that Ia afferents significantly enhanced the extension phase in the ankle, knee and hip motoneurones and delayed the onset of flexion. Thus although slightly weaker than Ib afferents, Ia afferents can enhance extension of ankle, knee and hip motoneurones by interacting with interneurones of the central pattern generator for locomotion. These results are of great importance for future identification of the interneurones mediating resetting during locomotion.

The widespread excitation from ankle extensor group I (Ia and Ib) afferents to homonymous and synergist extensor nerves throughout the limb is in stark contrast to the pattern of interneuronally mediated group I reflexes at rest (Eccles *et al.* 1957b; Jankowska *et al.* 1981a,b). The results of Duysens & Pearson (1980), Conway *et al.* 1987, Pearson & Collins (1993) and Guertin *et al.* (1995) indicate a functional reorganization of group I reflex pathways during locomotion. The synaptic connectivity underlying these locomotor-related effects remains poorly understood, and a reassessment using intracellular recording from motoneurones during fictive locomotion is required to better understand

the new organisation of reflexes from extensor group I afferents to extensor motoneurones. Recently McCrea, Shefchyk, Stephens & Pearson, (1995) have demonstrated a group I afferent-evoked disynaptic excitatory pathway from plantaris to medial gastrocnemius motoneurones that is expressed during the extension but not flexion phase of MLR-evoked fictive locomotion, and is mediated by an unknown population of excitatory interneurones. Thus one of the reflexes underlying the medial gastrocnemius enhanced ENG activity following plantaris group I stimulation is disynaptic excitation. It remains unknown, however, if motoneurones other than medial gastrocnemius receive locomotor-related group I disynaptic EPSPs. Furthermore it is not known if group I afferents from muscles other than plantaris evoked similar disynaptic EPSP. The extent to which the pattern of disynaptic EPSP from group I afferents overlaps the pattern of enhanced extension is unknown.

The study by McCrea *et al.* (1995) brings the reader to the most recent point in the history of published intracellular investigations of group I reflexes during locomotion. It is at this point that the present thesis takes over to more fully examine the control and operation of extensor group I short latency reflexes during fictive locomotion. This thesis is divided into two sections. The first section tests the hypothesis that disynaptic EPSPs from plantaris group I afferents can be evoked in extensor motoneurones of the ankle, knee and hip during the extension phase of MLR-evoked fictive locomotion in the cat. In addition it is proposed that group I afferents from other extensor muscles have common actions as those of plantaris. Standard intracellular recording from single motoneurones will be used in paralysed, decerebrate cats. These data are published in *The Journal of Physiology* **494.3**, 581-591. The second part of this thesis tests the hypothesis that a population of previously undescribed interneurones exists in the lumbosacral spinal cord which can be identified by a set of criteria based on their response pattern to group I afferent stimulation in the absence, and during fictive locomotion. Those interneurones which fulfill the identification criteria, based on extracellular recording of their activity, are deemed candidate excitatory interneurones mediating group I disynaptic EPSPs. The second part of the thesis will also provide an initial description of the control by the CPG

of these interneurones. For a description of longer latency group I reflexes activated during locomotion and the possibility of expression of intrinsic membrane properties of motoneurones during group I-evoked enhanced extension, the reader is referred to the PhD Thesis of Pierre Guertin.

Section I

GROUP I EXTENSOR AFFERENTS EVOKE DISYNAPTIC EPSPs IN CAT HINDLIMB EXTENSOR MOTONEURONES DURING FICTIVE LOCOMOTION

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Journal of Physiology 494.3, 581-591

Summary

1. Intracellular recording from extensor motoneurones in paralysed, decerebrate cats was used to examine the distribution of short-latency, non-monosynaptic excitation by group I afferents during fictive locomotion produced by stimulation of the mesencephalic locomotor region (MLR).

2. During the extension but not the flexion phase of fictive locomotion, stimulation of ankle extensor nerves at 1.2 -2T evoked excitatory postsynaptic potentials (EPSPs) in motoneurones innervating hip, knee and ankle extensors. Disynaptic EPSPs were also evoked by selective activation of group Ia muscle spindle afferents by muscle stretch.

3. The central latencies of these group-I evoked EPSPs (mean 1.55 ms) suggest their mediation by a disynaptic pathway with a single interneurone interposed between extensor group I afferents and extensor motoneurones. Disynaptic EPSPs were also evoked during periods of spontaneous locomotion following the cessation of MLR stimulation.

4. Hip extensor motoneurones received disynaptic EPSPs during extension following stimulation of both homonymous and ankle extensor nerves. Stimulation of hip extensor nerves did not evoke disynaptic EPSPs in ankle extensor motoneurones.

5. The appearance of disynaptic EPSPs during extension appears to result from cyclic disinhibition of an unidentified population of excitatory spinal interneurones and not postsynaptic, voltage-dependent conductances in motoneurones or phasic presynaptic inhibition of group I afferents during flexion.

6. The reorganization of group I reflexes during fictive locomotion includes the appearance of disynaptic excitation of hip, knee and ankle extensor motoneurones.

This excitatory reflex is one of the mechanisms by which group I afferents can enhance extensor activity and increase force production during stance.

Introduction

During locomotion, reflexes evoked from extensor group I muscle afferents are reorganized into a system that can increase extensor activity and thus promote weight support and forward propulsion during extension (Conway, Hultborn & Kiehn, 1987; Pearson & Collins, 1993; Whelan, Hiebert, & Pearson, 1995; Guertin, Angel, Perreault & McCrea, 1995; McCrea, Shefchyk, Stephens & Pearson, 1995). It was originally believed that only afferents from Golgi tendon organs were capable of non-monosynaptic extensor excitation during locomotion (Conway et al. 1987; Pearson & Collins, 1993), but it is now clear that both extensor muscle spindle primary (Ia) and Golgi tendon organ (Ib) afferents can evoke what has been termed "group I-evoked extension enhancement" (Guertin *et al.* 1995). This extension enhancement is characterised by increased amplitude or duration of extensor electroneurogram (ENG) activity following short duration stimulus trains activating extensor group I afferents during fictive locomotion. The reflex pathways that produce this increased recruitment of extensor motoneurones during fictive locomotion are presently poorly characterised. Although monosynaptic group Ia EPSPs would contribute to motoneurone excitation in locomoting preparations, there are no monosynaptic EPSPs in some of the motor nuclei in which extension enhancement occurs (Guertin *et al.* 1995). The oligosynaptic group I-evoked excitation seen in non-locomoting preparations cannot be responsible for extension enhancement because oligosynaptic reflexes evoked from extensor nerves to extensor motoneurones are predominantly inhibitory (Eccles, Eccles & Lundberg, 1957; Jankowska, McCrea & Mackel, 1981a, b). Thus extension enhancement is evoked through excitatory group I reflex pathways that are not operational in the non-locomoting preparation.

There is now evidence for short latency, group I-evoked excitatory reflex pathways that become operational only during fictive locomotion. Homonymous oligosynaptic excitation of extensor motoneurones during DOPA-induced fictive locomotion (Schomburg & Behrends, 1978) and occasionally during MLR-evoked fictive locomotion (Shefchyk, Stein & Jordan, 1984) has been reported. Recently an excitatory pathway activated by

plantaris (Pl) group I afferents has been identified that, during the extension phase of MLR-evoked fictive locomotion, produces a disynaptic excitation of medial gastrocnemius (MG) motoneurones (McCrea *et al.* 1995). It is unknown whether this locomotor-related disynaptic excitation is more widespread and could contribute to the enhancement of extensor activity of motoneurones acting at the ankle, knee and hip joints. The present investigation was undertaken, therefore, to determine which hindlimb extensor motoneurones receive excitation following Pl nerve stimulation and to determine whether extensor group I afferents other than those from Pl can produce disynaptic excitation during fictive locomotion. Results will show that stimulation of any of the ankle extensor nerves evokes disynaptic EPSPs simultaneously in ankle, knee and hip extensor motoneurones during extension and that this is due to the recruitment of an unidentified population of spinal interneurones. Some of these observations have been presented in abstract form (Angel, Guertin, Jiménez & McCrea, 1994a, b).

Methods

Data were collected from paralysed, decerebrate cats in which fictive locomotion was evoked by electrical stimulation of the mesencephalic locomotor region (MLR). Animals were anaesthetised with halothane delivered in a mixture of oxygen and 70% nitrous oxide. Selected hindlimb nerves were dissected and cut for placement on conventional bipolar hook electrodes for either stimulation or recording. These included medial gastrocnemius (MG), lateral gastrocnemius and soleus (LGS), plantaris (Pl), semimembranosus and anterior biceps taken together (SmAB), tibialis anterior (TA), posterior biceps with semitendinosus (PBSt) and the cutaneous superficial peroneal (SP) nerves. The nerve abbreviated as FDHL is the branch of the popliteal nerve which includes the innervation of flexor digitorum and hallucis longus muscles as well as branches to interosseous, tibialis posterior and popliteal muscles. Quadriceps (Q, vastii and rectus femoris) and sartorius (Sart, medial and lateral branches) were placed in buried, bipolar, cuff electrodes. Selected contralateral nerves were dissected and used to provide a monitor of contralateral fictive locomotor activity. The obturator nerves and all remaining branches of the sciatic and femoral nerves were cut bilaterally and tendons around the hip severed. In one experiment ipsilateral ankle extensor nerves were left attached to their muscles to permit activation of triceps surae and Pl Ia afferents by brief (2 ms) 35 μm stretches of the Achilles tendon (for details see, Guertin *et al.* 1995; McCrea *et al.* 1995). Intensity of nerve stimulation is expressed in multiples of the threshold for the most excitable fibres (T).

Following an L4-L7 laminectomy, the brain was exposed by craniotomy and a precollicular-postmammillary decerebration performed using blunt transection. Both cortices and all tissue rostral to the transection were removed. Anaesthesia was then discontinued and the cat paralysed with gallamine triethiodide (2-3 mg kg^{-1} hr^{-1}) and artificially ventilated. Fictive locomotion was evoked by unilateral or bilateral stimulation of the MLR (80μA-200μA, 1 ms pulses at 12-18 Hz; see Guertin *et al.* 1995). In one

experiment clonidine (100 μg/kg, Sigma Chemicals) was administered intravenously to aid in the generation of MLR-evoked fictive locomotion. Antidromically identified hindlimb extensor motoneurones were impaled with glass microelectrodes (1.6 to 2.0μm diameter) filled with 50 mM QX-314 (Alamone Laboratories, Jerusalem, Israel) in 2M potassium acetate. Passive diffusion of QX-314 from the microelectrode prevented the generation of action potentials in the impaled motoneurones during fictive locomotion and facilitated analysis of locomotor-related postsynaptic potentials.

Single shocks or occasionally brief trains of stimuli to peripheral nerves were delivered at 4-5 Hz and independently of MLR stimulation and fictive locomotor ENG activity. Postsynaptic potentials (PSPs) evoked by stimulating extensor group I afferents were recorded before and during locomotion. The presence of EPSPs during locomotion was determined from averaged records. All reported EPSPs were larger than 250 μV and had a sharply defined onset. In order to analyse the modulation of PSPs potentials, the records of an integrated rectified ENG were used to divide the fictive locomotor step cycle into flexion and extension phases. The analysis software was then used to assign the trials of peripheral nerve stimulation to either the flexion or extension phase and calculate the average of the intracellular and cord dorsum potentials. Data capture and analysis was accomplished with software developed within the Winnipeg Spinal Cord Research Centre (a Concurrent computer running real time Unix or a Pentium PC running Linux). Further details of the recording and analysis techniques are provided in McCrea *et al.* (1995).

Results

The present results were obtained in preparations in which there was both coordinated fictive locomotion as evidenced by bilateral alternating flexor and extensor peripheral nerve ENG activity, and in which short latency (< 2 ms) group I evoked EPSPs were recorded in at least some motoneurones. Eleven of 14 experiments in this series met these criteria and intracellular recordings were made from 57 extensor and 9 PBSt motoneurones. During the extension phase of fictive locomotion, stimulation of hindlimb extensor afferents at group I strength evoked distinct short latency EPSPs in 50 motoneurones, IPSPs in 10 motoneurones (6 Pl, 1 MG 1, LGS, and 2 SmAB) and a mixture of hyperpolarization and depolarization in 6 cells. The latencies of IPSPs encountered were 1.4 to 2.5 ms and within those expected for group I non-reciprocal inhibition described in anaesthetised preparations (see Jankowska *et al.* 1981a) and are not discussed further. The effects of stimulating two or more extensor nerves were tested in most motoneurones during fictive locomotion.

Group I evoked disynaptic EPSPs during spontaneous locomotion.

---Figure 1 here---

The integrated and rectified ENGs in Fig. 1A show alternating flexor and extensor activity during MLR-evoked fictive locomotion as well as during a brief period of spontaneous locomotion after termination of MLR stimulation. The period of brainstem stimulation is shown by the horizontal bar above the Q ENG. Panels B-E are averaged intracellular (upper) and cord dorsum (lower) records of the effects of Pl stimulation at 1.8T in this MG motoneurone. From the left, the panels show effects recorded in the absence of locomotion (B; first control), during the extensor and flexor phases of MLR-evoked locomotion (C), during the silent period following MLR stimulation (D; second control) and during spontaneous locomotion (E). The IPSPs evoked in the absence of

locomotion (B) were replaced by EPSPs during extension but not flexion (top trace, C and E). The latency of these EPSPs is 1.7 ms and as discussed by McCrea et al. (1995), is evidence for a disynaptic pathway from extensor group I afferents to extensors motoneurones. The excitation of MG motoneurones following Pl nerve stimulation has been reported previously (McCrea *et al.* 1995). New observations are presented in panels A, D, and E of Fig. 1. Panel D shows that when fictive locomotion ceased following the termination of MLR stimulation (period of ENG inactivity in panel A labelled with the bracketed D) there was absence of disynaptic excitation. After this brief period of inactivity, a few cycles of spontaneous fictive locomotion ensued. The averaged PSPs during spontaneous locomotion in panel E show a disynaptic excitation during extension similar to that occurring during MLR stimulation. The presence of short latency group I-evoked EPSPs during spontaneous fictive locomotion was recorded in 6 motoneurones (1 MG, 1 LGS, 3 SmAB and 1 Q) in 3 experiments.

Disynaptic EPSPs evoked from group I strength stimulation of ankle extensor nerves.

--- Figure 2 here ---

Figure 2 A-C shows averaged intracellular recordings from 3 ankle extensor motoneurones obtained during the extension (upper solid traces) and flexion phases (dotted traces) of the fictive locomotor step cycle following stimulation of ankle extensor group I afferents. During extension but not flexion, prominent depolarizations follow the heteronymous (panel A) and homonymous (B) Ia monosynaptic EPSPs with latencies of 1.5 and 1.7 ms respectively. Figure 2A shows that the amplitude of disynaptic EPSPs can be of the same order as that of heteronymous monosynaptic Ia EPSPs. Disynaptic EPSPs may also be evoked without preceding monosynaptic EPSPs (e. g. Fig. 1C, E; Fig. 2C-F, Fig. 4B; Fig. 6B).

Twenty-three of thirty-one motoneurones innervating ankle extensors (5 of 11 Pl, 9 of 10 MG, and 9 of 10 LGS motoneurones) received disynaptic EPSPs during the extension phase of fictive locomotion following stimulation of homonymous and/or heteronymous ankle extensor nerves at group I strength. While the minimum stimulus

intensity required to evoke disynaptic EPSPs was not systematically explored, EPSPs were evoked in 10 motoneurones by ≤ 1.4T stimulation (6 at 1.4T, 2 at 1.3T, 2 at 1.2T). Such stimulus intensities are below those required for activation of even the lowest threshold group II afferents in ankle extensor nerves (Jack, 1978). The soleus branch of the LGS nerve was dissected and stimulated separately from LG in one experiment. Soleus nerve stimulation evoked disynaptic EPSPs during extension in both the MG and LG motoneurones examined (data not shown). There was no obvious difference in the distribution of EPSPs evoked by stimulation of any of the ankle extensor nerves. The mean latency of group I, ankle extensor nerve-evoked EPSPs recorded in ankle extensor motoneurones was 1.55 ms ±0.26 S. D. (27 EPSPs in 23 motoneurones; range 1.0 - 2.0 ms with 23 EPSPs ≤ 1.8 ms). Given the small sample sizes and the preparation dependency of disynaptic EPSPs (see Discussion) the high incidence of IPSPs recorded in Pl motoneurones (6/11) may reflect a sampling bias and not a differential projection of inhibitory interneurones to Pl motoneurones.

The effects of ankle extensor nerve stimulation on knee (Fig. 2D, E) and hip (Fig. 2F, 3B-D) extensor motoneurones were also investigated. Figure 2 D (control trace) shows non-reciprocal inhibition evoked in a Q motoneurone following Pl nerve stimulation in the absence of locomotion. During the extension but not flexion phase of locomotion, stimulation of Pl evoked a sharply rising EPSP in this motoneurone with a central latency of 1.4 ms. Stimulation of ankle extensor nerves produced short latency EPSPs in 12 of 17 hip (SmAB) and 2 of 5 knee (Q) extensor motoneurones during the extension phase of locomotion. The latencies of these EPSPs were similar to those recorded in ankle extensor motoneurones (1.1 to 1.9 ms, mean=1.52 ± 0.25 S. D. ms, n=14).

In addition to the disynaptic EPSP recorded during extension, other longer-latency (> 3 ms) locomotor-related EPSPs were seen in some motoneurones following stimulation of ankle extensor nerves. Examples of longer latency depolarizations are shown in Fig. 2C and D (arrows) and in a PBSt motoneurone in Fig. 6B. These longer latency EPSPs were sometimes present during both flexion and extension and may correspond to the polysynaptic excitatory group I pathway described by Gossard, Brownstone, Barajon &

Hultborn (1994) during fictive locomotion.

Selective activation of ankle extensor Ia muscle spindle afferents

Brief stretches of the Achilles tendon were used to determine whether recruitment of ankle extensor group Ia muscle spindle afferents without Ib Golgi tendon organ afferents could produce disynaptic excitation during the extension phase of fictive locomotion in knee and hip extensor motoneurones. Figure 2E, F show averaged intracellular recordings from Q and SmAB motoneurones respectively while applying small, brief, stretches to the Achilles tendon. In both cases, activation of Ia afferents evoked prominent disynaptic EPSPs (latency 1.6 ms in the Q motoneurone and 1.8ms in the SmAB motoneurone) during the extension but not flexion phases of fictive locomotion. Records in Fig 2D & E are from the same Q motoneurone. Stretch-evoked disynaptic EPSPs were seen during extension in both Q, 1 of 2 SmAB and the single Pl motoneurone tested. Group Ia evoked disynaptic EPSPs have also been reported in MG motoneurones (McCrea *et al.* 1995).

Stretch of the Achilles tendon shows that disynaptic EPSPs can be evoked by activation of muscle spindle primaries. In order to investigate whether the removal of primary afferent depolarization (PAD) of group Ia afferents could be responsible for the gating of disynaptic EPSPs during extension, the amplitudes of monosynaptic Ia EPSPs in motoneurones were compared during flexion and extension. The ratio of EPSP amplitude (extension/flexion) ranged from .75 to 1.3 with a mean of 1.03 $\pm$ 0.13 S. D. (n=23). In 9 cases this ratio was $\geq 0.95 \leq 1.05$; in 7 cases it was > 1.05 and in 7 cases < 0.95. Figure 2A shows an example of a monosynaptic EPSP that was smaller during flexion and Fig. 2B shows an EPSP that was smaller during extension. There were no obvious differences in the modulation of homonymous and heteronymous monosynaptic EPSPs. The relatively weak modulation of monosynaptic EPSPs and the inconsistent phase in which the smaller EPSP occurred, contrasts the phase-dependent gating of disynaptic EPSPs. The expression of group Ia-evoked disynaptic EPSPs during only the extension phase (Fig 2. E, F; see McCrea *et al.* 1995) is thus unlikely due to primary afferent depolarization (PAD) of group Ia afferents during flexion or at rest.

Disynaptic EPSPs evoked by group I strength stimulation of hip and knee extensor nerves.

--- Figure 3 here ---

The records in Fig. 3 from a SmAB motoneurone illustrate disynaptic excitation

evoked from group I afferents during the extension phase of fictive locomotion. This homonymous disynaptic excitation (panel A) appears similar to that observed during DOPA-induced locomotion in acute, high-spinal cats (Schomburg & Behrends, 1978). In addition, this SmAB motoneurone received heteronymous disynaptic EPSPs during extension following Pl, LGS or MG nerve stimulation (panels B-D). This figure suggests that the simultaneous activation of group I afferents in several nerves during real locomotion could result in more depolarization of hip extensor motoneurones than shown in the present investigation using single nerve stimulation. No attempt was made, however, to examine the summation of locomotor-dependent disynaptic EPSPs from different nerves in individual motoneurones.

Because the presence of monosynaptic Ia EPSPs precluded reliable estimates of the rise times of homonymous disynaptic EPSPs, the 10-90% rise times of heteronymous disynaptic EPSPs were measured. These ranged from 0.4 ms to 1.6 ms, with a mean of 0.7 $\pm$ 0.29 S. D. ms (22 EPSPs measured in 16 SmAB, Q or ankle extensor motoneurones). A comparison of rise times of homonymous monosynaptic and heteronymous disynaptic EPSPs evoked by ankle nerve stimulation was made in 7 motoneurones (1 MG, 1 LGS, 5 SmAB). In 5 of 7 the difference between the homonymous monosynaptic and heteronymous disynaptic EPSPs rise times was $\leq$0.1 ms. The largest difference between the rise times of disynaptic and monosynaptic EPSPs was found in the motoneurone illustrated in Fig. 3 in which the rise times of disynaptic locomotor EPSPs evoked by Pl, LGS and MG nerve stimulation are 0.7 ms, 0.6 ms and 0.8 ms respectively while that of the homonymous Ia monosynaptic EPSP (also measured during fictive locomotion) is 0.4 ms.

--- Figure 4 here ---

The results presented in the previous sections show that stimulation of ankle extensor group I afferents results in disynaptic excitation of extensor motoneurones acting at hip, knee and ankle joints during the extension phase of locomotion. Figure 4 shows that stimulation of group I afferents of hip extensor nerves does not result in a similar excitation of ankle extensor motoneurones. In panel A, 4 shocks to the SmAB nerve failed

to evoke disynaptic EPSPs during extension and evoked only a small, slowly rising, polysynaptic depolarizations during flexion in this MG motoneurone. In this same cell, PI stimulation evoked large disynaptic EPSPs (panel B). SmAB stimulation failed to produce excitation in all 4 ankle extensor motoneurones examined while in the three tested, ankle extensor nerve stimulation evoked disynaptic EPSPs during extension. On the other hand, SmAB stimulation produced homonymous disynaptic excitation in 5 of 6 SmAB motoneurones and heteronymous disynaptic excitation in the single Q motoneurone tested. The records in panel B obtained with PI stimulation during flexion also show disynaptic EPSPs of increasing amplitude on top of an underlying longer latency excitation following the second, third and fourth shock. This example is one of the few in which single shocks to extensor nerves produced disynaptic excitation during flexion. Q nerve stimulation produced homonymous, disynaptic, group I-evoked excitation during extension in 4 of 7 Q motoneurones tested. The effects of Q stimulation in ankle and hip extensors was not tested. The mean latency of homonymous or heteronymous group I-evoked disynaptic EPSPs following stimulation of Q and SmAB nerves was 1.66 $\pm$ .18 ms (n=8; range 1.4 - 1.9 ms). In an additional Q motoneurone the homonymous group I-evoked EPSP during extension had a latency of 2.2 ms. The latency of the homonymous monosynaptic EPSP in this motoneurone was uncharacteristically long (1.0 ms).

Different reflex pathways mediate cutaneous and muscular disynaptic excitation in FDHL motoneurones.

--- Figure 5 here ---

Stimulation of the cutaneous SP nerve can produced a disynaptic excitation of FDL motoneurones at rest (Fleshman, Lev-Tov & Burke, 1984) that are modulated during fictive locomotion (Schmidt, Meyers, Fleshman, Tokuriki & Burke, 1988). The left portion of Fig. 5 shows an averaged SP-evoked EPSP in a motoneurone antidromically activated by stimulation of the FDHL nerve (latency 1.8 ms on "selected" trace). The SP-evoked EPSP was largest during the flexion phase of the locomotor cycle (Fig. 5 arrow; also see Schmidt *et al.* 1988). The right portion of Fig. 5 shows that stimulation of the

FDHL nerve produced a disynaptic EPSP (latency 1.8 ms) following the monosynaptic EPSP during extension (top trace) but not flexion. This disynaptic group I EPSP is thus similar to those recorded in ankle, knee and hip extensor motoneurones. Disynaptic group I EPSPs following FDHL stimulation were found in two FDHL motoneurones and following MG stimulation in a third FDHL motoneurone tested. Although our dissection did not separate the branches of the FDHL nerves to allow unequivocal antidromic identification of the impaled motoneurones, the motoneurone in Fig. 4 received SP-evoked disynaptic EPSPs that were larger during flexion. This is consistent with its identification as an FDL motoneurone (Fleshman *et al.* 1984; Schmidt *et al.* 1988). The differential modulation of disynaptic cutaneous and muscle nerve-evoked EPSPs during fictive locomotion suggests their mediation by different reflex pathways.

Evidence against a voltage-dependent gating of disynaptic group I EPSPs.

In anaesthetised preparations, stimulation of ankle extensor group I afferents evokes oligosynaptic EPSPs in most PBSt motoneurones (e. g. Jankowska *et al.* 1981b). In accord with the general depression of group I oligosynaptic reflexes in the decerebrate preparation (Eccles & Lundberg, 1959), only 1 of 9 PBSt motoneurones in the present study were excited by group I ankle extensor stimulation at rest. During fictive locomotion, however, stimulation of MG, LGS or Pl nerves evoked disynaptic EPSPs during extension in 6 of these PBSt motoneurones.

--- Figure 6 near here ---

A PBSt motoneurone that is rhythmically depolarized during both extension and flexion phases of fictive locomotion (Perret & Cabelguen, 1980) is shown in Fig 6. The flexion phases of the first three fictive step cycles are indicated by the filled rectangles. This PBSt motoneurone is less depolarized during extension than during flexion. Stimulation of the MG nerve (2 shocks, 2T) results in short latency (1.6 ms) excitation during extension (B, top and difference trace) but not flexion or control. This suggests that the expression of disynaptic EPSPs is dependent on the phase of the locomotor step cycle and not the level of the motoneurone depolarization. The maintained depolarization

following the second shock during extension is similar to that illustrated in other figures (2C, D) and may result from longer latency excitation following disynaptic excitation.

Discussion

During the extension phase of MLR-evoked fictive locomotion, stimulation of extensor group I afferents evokes short-latency excitation of hip, knee and ankle extensor motoneurones. This finding of a wide distribution of disynaptic, group I evoked EPSPs during extension extends the observations of McCrea *et al.* (1995) on the excitatory actions evoked by stimulation of one ankle extensor nerve on its close synergist. Stimulation of hip extensor group I afferents evokes a more restricted pattern of short latency excitation with EPSPs produced in hip and knee extensor but not ankle extensor motoneurones. As discussed previously (McCrea *et al.* 1995) the central latencies of extension-related EPSPs suggest their mediation by a disynaptic pathway with one interneurone interposed between extensor group I afferents and extensor motoneurones. The present and previous study (McCrea *et al.* 1995) show that ankle extensor group Ia afferents can recruit interneurones responsible for disynaptic excitation during extension. The likelihood that activation of Golgi tendon organ afferents can also evoke disynaptic excitation is discussed in McCrea *et al.* (1995).

Evidence that gating of disynaptic excitation is due to the modulation of interneuronal excitability

We interpret the presence of disynaptic group I excitation during extension and its absence during flexion as evidence for a modulation of the excitability of interneurones. Arguments against other mechanisms that could gate the appearance of disynaptic excitation are presented here. One such mechanism is the presence of a large conductance in extensor motoneurones during flexion that could shunt the appearance of disynaptic EPSPs. Evidence against this possibility includes the similarity of motoneurone conductance in both locomotor phases (Shefchyk & Jordan, 1985) and the lack of a consistent or substantial reduction in the size of other PSPs during flexion (e. g. monosynaptic group Ia EPSPs, Fig. 2).

Although PAD of group I afferents could modulate synaptic transmission from

group I afferents during locomotion, available evidence suggests that group I PAD does not result in the disappearance of disynaptic EPSPs during flexion. Thus synaptic transmission from group Ia afferents evokes large monosynaptic EPSPs during flexion (e. g. present results) and synaptic transmission from group Ib afferents can activate interneurones and reset the locomotor step cycle during flexion (Conway *et al.* 1987; Gossard *et al.* 1994). A PAD of only selective group I afferent terminals (see Eguibar, Quevedo, Jiménez & Rudomin, 1994; Zytnicki, LaFleur, Kouchtir & Perrier, 1995) could, however, depress disynaptic EPSPs but leave other reflexes intact. Whether such an organization exists and could be regulated to produce a suppression of disynaptic EPSPs during flexion and at rest is unknown.

Finally, if the postsynaptic currents underlying disynaptic group I EPSPs were activated only at depolarized membrane potentials, disynaptic EPSPs would appear in extensor motoneurones only as they depolarized during extension. In the present study, disynaptic EPSPs were sometimes recorded in extensor motoneurones that were only minimally depolarized during locomotion (not illustrated). Furthermore, the presence of disynaptic EPSPs in PBSt motoneurones during the less depolarized extensor phase (Fig. 6), strongly argues for a premotoneuronal mechanism for the gating of disynaptic EPSPs. Thus we propose that the appearance of the group I-evoked, disynaptic EPSP in the extension phase results from modulation of interneurone excitability.

Postulated properties of interneurones mediating disynaptic group I excitation during extension

Interneurones located in laminae V-VI of lumbar segments have been described that mediate group I oligosynaptic reflex excitation and inhibition in non-locomoting preparations. They are excited by activation of Ia, Ib, cutaneous and joint afferents, and project monosynaptically to motoneurones (see Jankowska, 1992). It is unlikely that these interneurones produce disynaptic excitation during fictive locomotion. We postulate this because the distribution of disynaptic excitation of extensors during fictive locomotion is more extensive than that seen following group I stimulation in anaesthetised preparations.

The tonic inhibition of interneurones mediating locomotor-related disynaptic EPSPs would have hindered their detection during surveys of the input to spinal interneurones in non-locomoting preparations. Other group I EPSPs recently reported during locomotion in acute spinal, DOPA-treated and MLR preparations are dissimilar to the disynaptic excitation reported here in that they are larger during flexion and of longer latency (Gossard *et al.* 1994) and their amplitudes are increased by motoneurone depolarization (Brownstone, Gossard & Hultborn, 1994). Such EPSPs may correspond to those illustrated in Fig. 2D and 4B (traces during flexion). We suggest, therefore, that disynaptic excitation of extensor motoneurones during locomotion is produced by activation of other, undescribed populations of interneurones.

Evidence suggests that the interneurones responsible for disynaptic excitation are effectively excited by group Ia muscle spindle (e. g. Fig. 2) and probably Ib tendon organ afferents (see discussion in McCrea *et al.* 1995). The present results show that group I afferents from several nerves including triceps surae, Pl, SmAB, Q and FDHL can activate interneurones producing disynaptic excitation. Identification of the responsible interneurones and subsequent intracellular recording would allow direct examination of the convergence of different segmental afferents to interneurones. This would help determine whether disynaptic EPSPs in motoneurones resulting from stimulation of several nerves (Fig. 3) are mediated by few or multiple interneuronal populations. The present results in FDHL motoneurones suggest that separate populations of interneurones are responsible for the group I and cutaneous (SP) evoked EPSPs during fictive locomotion.

The motoneuronal targets of individual group I excitatory interneurones is also unknown. Present observations suggest that there are at least two subpopulations of excitatory interneurones; one projecting to hip, knee and ankle motoneurones with input from ankle and hip extensor group I afferents and another activated by hip extensor (SmAB) group I afferents without a projection to ankle extensor motoneurones. It is possible that interneurones mediating group I-evoked excitation of PBSt motoneurones in non-locomoting preparations (Eccles *et al.* 1957; Jankowska *et al.* 1981b) also produce disynaptic excitation of PBSt motoneurones during extension. Because group I

oligosynaptic actions are depressed in the decerebrate preparation (Eccles & Lundberg, 1959) further investigation will be needed to determine if the interneurones exciting PBSt motoneurones in anaesthetised preparations can also be activated during extension.

Disynaptic excitation was rarely evoked during flexion suggesting that operation of the extensor half-centre of the locomotor network is necessary for its expression (see also McCrea *et al.* 1995). Fig. 4B (lower trace) shows that during flexion, brief stimulus trains evoke longer latency EPSPs and only small disynaptic EPSPs that grow in amplitude with subsequent shocks (see also McCrea *et al.* 1995). Because longer duration stimulus trains to ankle extensor nerves can terminate flexion and reset the step cycle to extension (Conway *et al.* 1987; Gossard *et al.* 1994; Guertin *et al.* 1995) the effects of short-duration trains in Fig. 4B may result from a resetting that is started but prematurely terminated. Thus as the locomotor network is biased towards extension, there is a progressive recruitment of interneurones and hence disynaptic excitation of extensor motoneurones.

The ability of peripheral nerve stimulation to evoke disynaptic group I EPSPs during periods of spontaneous locomotion following the termination of MLR stimulation (Fig. 1) indicates that MLR stimulation *per se* is not required for production of disynaptic EPSPs. Their presence in high spinal (Schomburg & Behrends, 1978) but not low spinal locomotor preparations (Gossard *et al.* 1994; McCrea *et al.* 1995) may indicate that supralumbar structures are essential for the expression of disynaptic group I excitation. While other studies using the MLR preparation did not report (Gossard *et al.* 1994) or rarely found disynaptic EPSPs (Shefchyk *et al.* 1984), they were found routinely in the present and previous (McCrea *et al.* 1995) experiments. In some experiments, however, the incidence of group I EPSPs decreased as the preparation and quality of fictive locomotion began to deteriorate. Future experiments will be needed to determine the factors or systems that control the expression of extension related, disynaptic, group I-evoked EPSPs.

Functional Considerations

Group I-evoked enhancement of extension has been seen in a variety of decerebrate, fictive locomotor, cat preparations (Conway *et al.* 1987; Gossard *et al.* 1994; Guertin *et al.* 1995), decerebrate cats walking on a treadmill (Duysens & Pearson, 1980; Pearson & Collins, 1993; Whelan *et al.* 1995) and in the intact standing cat (Pratt, 1995). The present experiments used a preparation in which extension enhancement can be evoked by trains of stimuli activating group I afferents (Guertin *et al.* 1995). Since the widespread distribution of disynaptic excitation from ankle extensor group I afferents is similar to the pattern of group I-evoked extension enhancement (Guertin *et al.* 1995) disynaptic EPSPs in extensor motoneurones must enhance extensor locomotor activity. However, disynaptic excitation is not necessary for locomotor extension enhancement since it can be evoked in preparations without disynaptic EPSPs (Guertin, Angel, Jiménez & McCrea 1994).

Although the present data on disynaptic EPSPs were obtained in a reduced preparation during fictive locomotion, it is possible that these pathways are also operational during other motor behaviours. Activation of primary muscle spindle afferents by increases in muscle length or increases in the activity of motoneurones innervating intrafusal muscle fibres (i. e., gamma and beta motoneurones) could all result in a reflex disynaptic excitation if the responsible interneurones were released from inhibition. In these situations the addition of a disynaptic component to the monosynaptic Ia EPSP would cause an increase in reflex gain. For example, disynaptic Ia excitation could be one of the mechanisms contributing to the increase in soleus muscle stiffness seen during the extension phase of treadmill locomotion (Akazawa, Aldridge, Steeves & Stein, 1982). The operation of this widespread, short latency group I reflex pathway could also confound the interpretation of studies where excitatory actions of Ia afferents were assumed to be limited to monosynaptic excitation. This may be a particular problem when determining the mechanisms underlying modulation of group I reflexes recorded from the EMG.

Since both EPSP amplitude and rise time are important for motoneurone recruitment (e.g. Kirkwood, 1979; Gustafsson & McCrea, 1984) the similar rise times and often similar amplitudes of disynaptic, group I-evoked EPSPs and heteronymous group Ia

monosynaptic EPSPs (compare present results with those in Hochman & McCrea, 1994) suggest that disynaptic excitation would assist heteronymous motoneurone recruitment as effectively as monosynaptic EPSPs. Furthermore, the activity of group I afferents in multiple nerves may produce a summation of disynaptic EPSPs and more depolarization than that found in the present experiments. Given the more extensive distribution of disynaptic than monosynaptic EPSPs, muscle spindle primaries may produce more motoneuronal excitation through disynaptic than monosynaptic excitatory pathways during the extension phase of locomotion. The wide distribution of disynaptic excitation would also help synchronize the activity of extensors acting at ankle, knee and hip joints during real locomotion particularly when there is co-activation of ankle muscle spindle and tendon organ afferents (Prochaska, Trend, Hulliger & Vincent 1989).

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Figures and Figure Legends

Figure 1. *Pl group I afferents evoke disynaptic excitation during MLR-evoked and spontaneous locomotion.*

The five traces in *A* are integrated and rectified ENGs from extensor (Q, SmAB, LGS) and flexor (Sart, TA) nerves during fictive locomotion. The horizontal bar above the Q ENG indicates the duration of MLR stimulation. Following MLR stimulation, locomotion stops for approximately 2 s after which spontaneous locomotion commences. Panels *B-E* show averaged, high gain intracellular recordings from a MG motoneurone (upper trace). The bottom trace in *B-E* is the averaged cord dorsum recording with the vertical dashed lines indicating the time of arrival of the afferent volley from which central latencies were measured. *B* & *D* show the effects of Pl nerve stimulation in the absence of locomotion and during the pause after MLR stimulation respectively. *C* & *E* show disynaptic EPSPs evoked during extension by Pl stimulation during MLR-evoked and spontaneous locomotion respectively. Averages in *B*,*C* & *E* were calculated from a minimum of 29 sweeps and in *D* from eight sweeps.

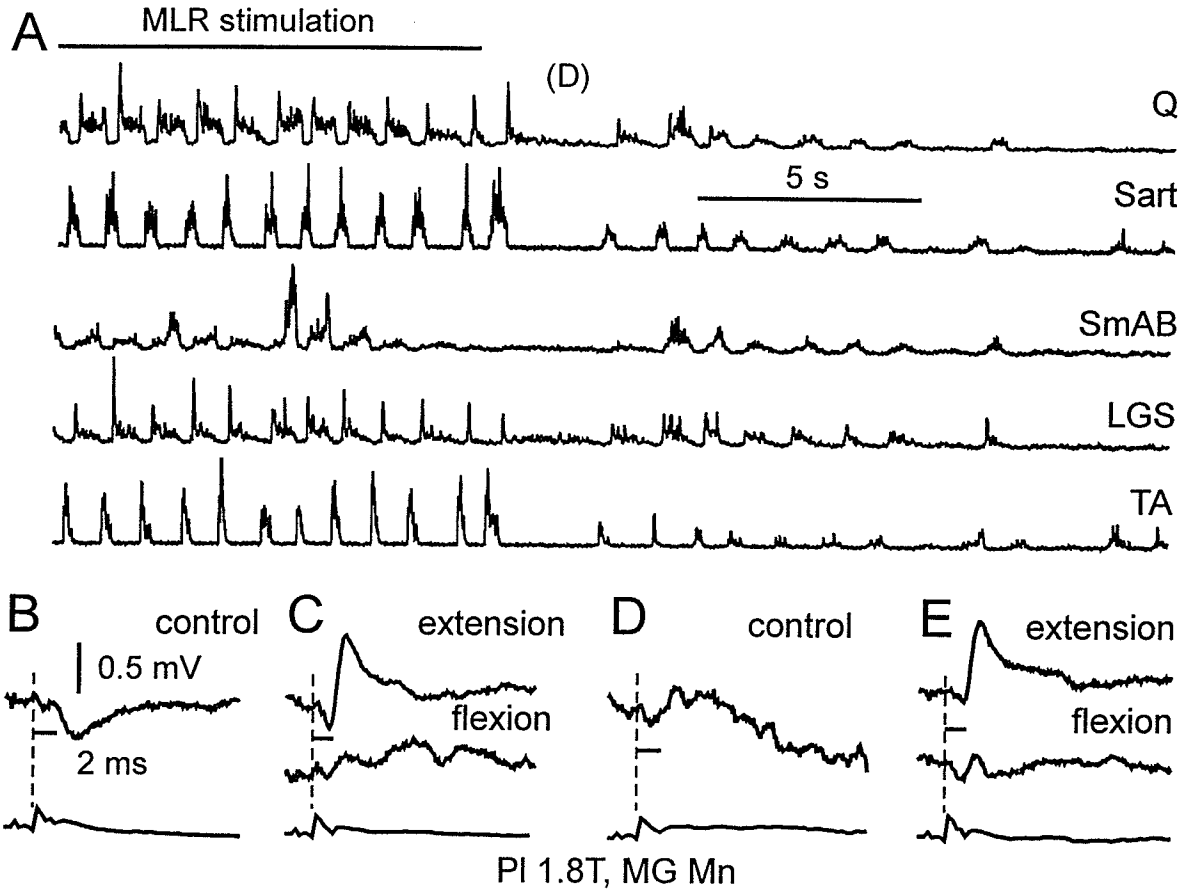

A
MLR stimulation
(D)
Q
5 s
Sart
SmAB
LGS
TA
B
control
0.5 mV
2 ms
C
extension
flexion
D
control
E
extension
flexion
Pl 1.8T, MG Mn

Figure. 2 *Ankle extensor group I afferents evoke extension-related disynaptic EPSPs in ankle, knee and hip extensor motoneurones during fictive locomotion.*

The top two traces in each panel are averaged intracellular recordings showing the response during extension (solid) and flexion (dotted) to single shock stimulation (*A-D*) or Achilles tendon 35 μm stretch (*E-F*) in ankle, knee or hip extensor motoneurones. In *A-C* the arithmetic differences between PSPs obtained during extension and flexion are indicated by the 'difference' trace. In all panels the bottom trace is the averaged cord dorsum recording with the time of arrival of the afferent volley indicated by the vertical dashed lines. The nerve and intensity of stimulation are indicated above panels *A-D*. *A & B*, during extension but not flexion group I strength stimulation evokes disynaptic EPSPs on top of the monosynaptic EPSPs. *C*, a heteronymous, disynaptic EPSP is evoked during extension in this LGS motoneurone following Pl nerve stimulation without a preceding heteronymous monosynaptic EPSP. *D*, stimulation of the Pl nerve evokes an IPSP in the absence of locomotion (control) in this Q motoneurone, that is replaced by a disynaptic EPSP during extension. *E*, in the same motoneurone, selective activation of triceps surae and Pl Ia afferents by muscle stretch also evokes disynaptic EPSPs during extension but not flexion. F, stretch-evoked disynaptic EPSP in a SmAB motoneurone during extension. The arrows in *C & D* indicate longer latency depolarizations. Each average was calculated from a minimum of 32 sweeps. Calibration bars are 2 mV (*A, C, D-F*), 4 mV (*B*) and 2 ms.

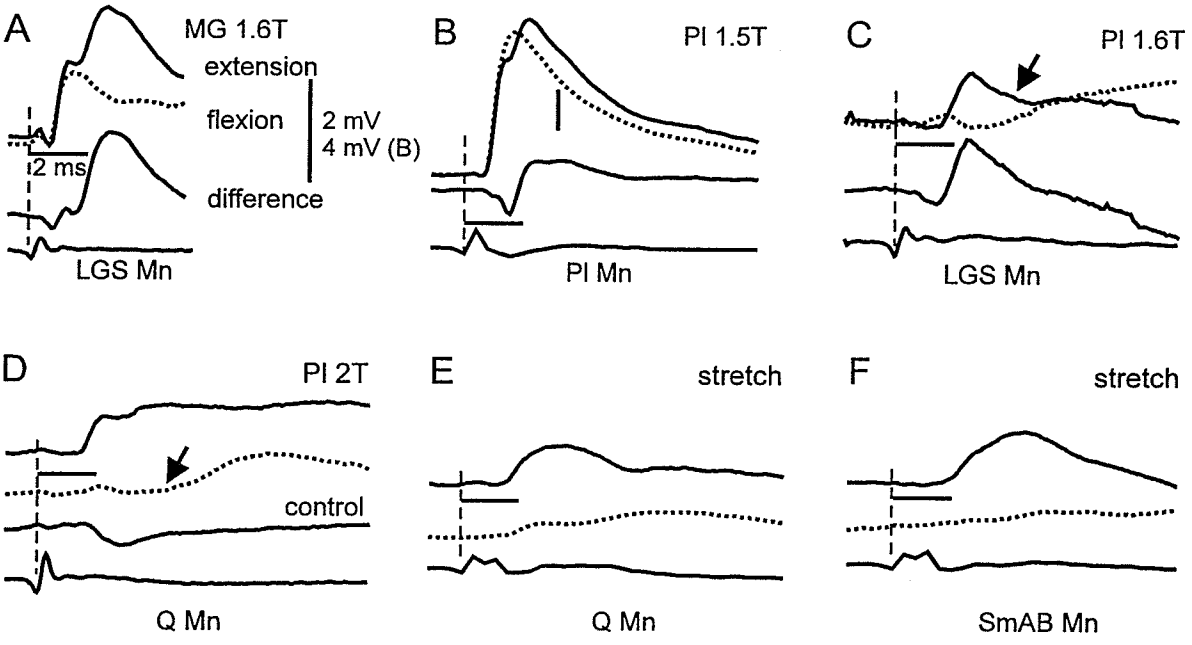

A
MG 1.6T
extension
flexion
2 mV
4 mV (B)
2 ms
difference
LGS Mn
B
PI 1.5T
PI Mn
C
PI 1.6T
LGS Mn
D
PI 2T
control
Q Mn
E
stretch
Q Mn
F
stretch
SmAB Mn

Figure 3. *SmAB motoneurones receive disynaptic EPSPs during extension following stimulation of hip and ankle extensor group I afferents.*

The top solid traces in *A-D* are averaged intracellular recordings from a SmAB motoneurone obtained during the extension phase of fictive locomotion; the dotted traces indicate records obtained during flexion. In *A*, a prominent, disynaptic EPSP is seen during extension following the homonymous EPSP, and in panels *B-D*, EPSPs with similar latencies are evoked from ankle extensor nerve stimulation. Calibration bars are 1 mV and 2 ms.

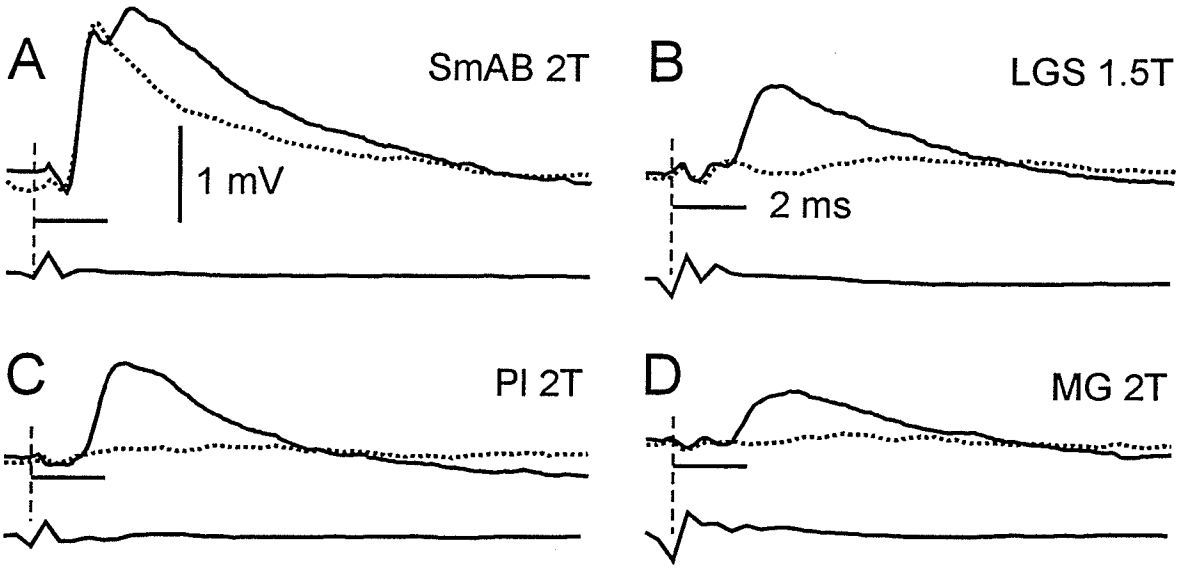

A
SmAB 2T
1 mV
B
LGS 1.5T
2 ms
C
PI 2T
D
MG 2T
SmAB Mn

Figure 4. *Stimulation of hip extensor nerves does not evoke disynaptic EPSPs during extension in ankle extensor motoneurones.*

The averaged effects of stimulation of hip extensor group I afferents (SmAB; panel *A*) and heteronymous ankle extensor (Pl; panel *B*) with four shocks (one shock followed by a train of three shocks at 200 Hz) during fictive locomotion are shown in this MG motoneurone. The arrows indicate the arrival of the volley at the cord dorsum. During extension, SmAB stimulation is without effect, while Pl stimulation evokes large, short-latency EPSPs (1.8 ms). During flexion, Pl stimulation produces small disynaptic EPSPs that grow with each subsequent shock and are superimposed on a slowly rising depolarization. Averages consist of a minimum of 49 sweeps. Calibration bars are 2 mV, 2 ms.

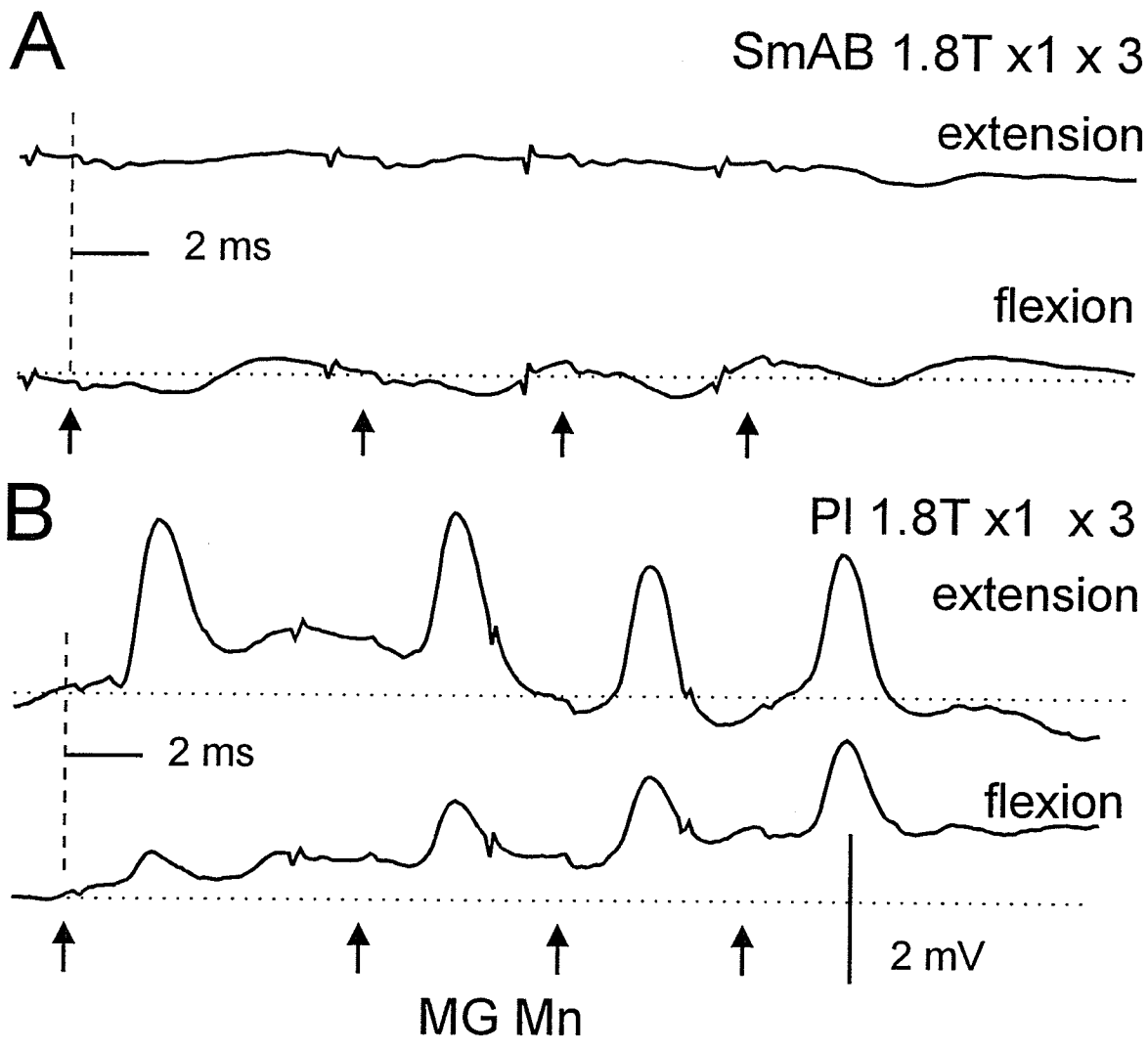

A
SmAB 1.8T x1 x 3
extension
2 ms
flexion
B
PI 1.8T x1 x 3
extension
2 ms
flexion
2 mV
MG Mn

Figure 5. *Disynaptic cutaneous and group I EPSPs in a FDL motoneurone are differentially modulated during locomotion.*

The SP and FDHL nerves were stimulated about 10 ms apart while recording in this FDL motoneurone. The top 4 traces are intracellular records with the vertical dashed lines indicating the arrival of the afferent volleys as judged from the cord dorsum recording (bottom trace, truncated). All records are averages (from top to bottom n= 40, 43, 59, 15, 15). The amplitude of the SP-evoked EPSP was largest during the early portion of the flexion phase of fictive locomotion. The trace labelled "selected" is an average calculated from 15 of the shortest-latency, SP-evoked EPSPs selected by visual inspection without regard for the locomotor step cycle and shows an EPSP with a latency of 1.8 ms (arrow). Subsequent examination showed that all of these 15 records occurred during the flexion phase of fictive locomotion. In contrast to the occurrence of the largest SP-evoked EPSP during flexion, disynaptic EPSPs evoked by FDHL stimulation were present only during extension (top trace).

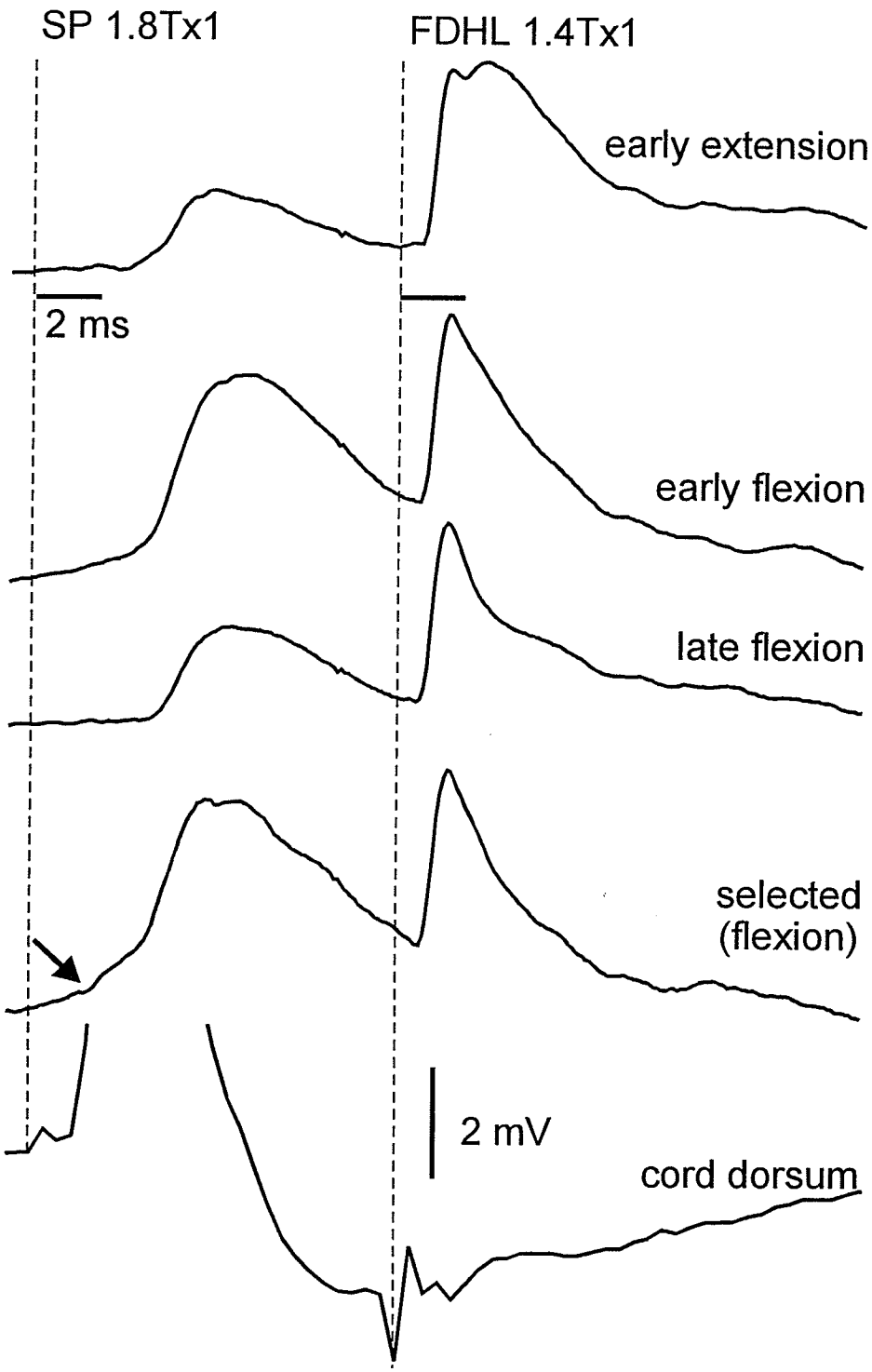
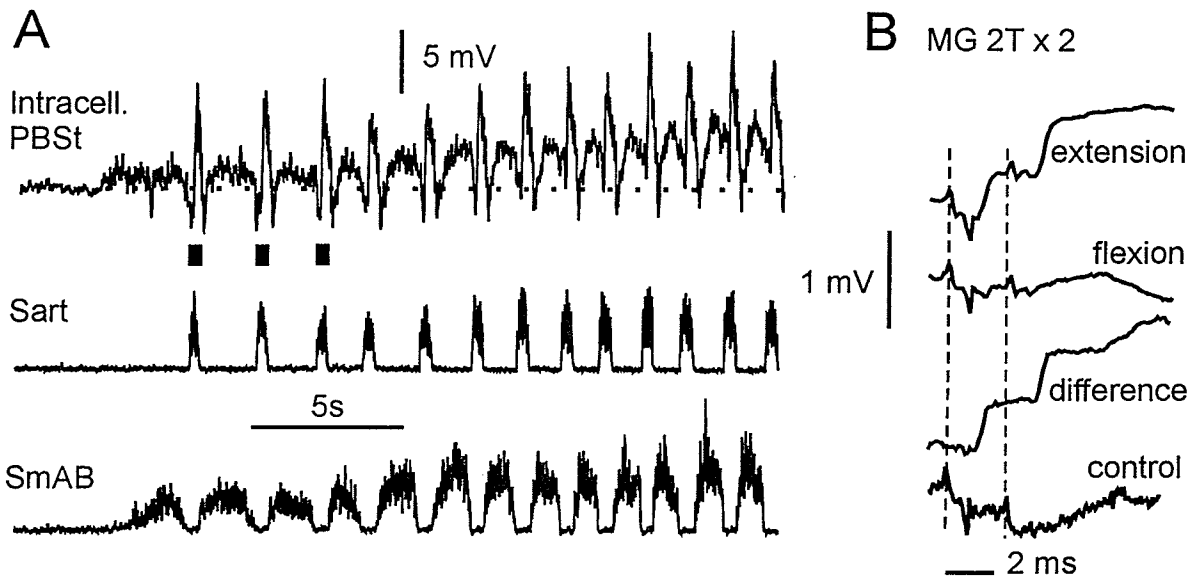

SP 1.8Tx1
FDHL 1.4Tx1
early extension
2 ms
early flexion
late flexion
selected
(flexion)
2 mV
cord dorsum

Figure 6. *Disynaptic EPSPs are evoked in a PBSt motoneurone during the extension but not the flexion phase of locomotion.*

A, the top trace is a low gain intracellular recording (DC coupled, high pass filtered at 15Hz) of a PBSt motoneurone showing a biphasic depolarization during locomotion. The middle and bottom panels are integrated, rectified, ENG recordings from Sart (flexor), and SmAB (extensor) nerves respectively. The filled rectangles indicate the duration of Sart ENG activity and hence the duration of the flexion phase of first three fictive locomotor steps. The membrane potential before fictive locomotion is plotted as the dotted horizontal line (-60 mV). *B*, averaged, high gain, intracellular recording (10 Khz) from this motoneurone following MG nerve stimulation (2T, 2 shocks, 400 Hz) during extension (n=201), flexion (n=63) and in the absence of locomotion (control; n=5). Subtraction of the flexion and extension traces shows the disynaptic EPSPs more clearly ('difference' trace). The averages in *B* were calculated from a longer bout of locomotion which includes those steps illustrated in *A*. The vertical dashed lines indicate the arrival of the afferent volleys at the cord dorsum. Calibration bars are 5 mv, 5 s in *A*, and 1 mV, 2 ms in *B*.


A
5 mV
Intracell.
PBSt
Sart
5s
SmAB
B
MG 2T x 2
extension
flexion
1 mV
difference
control
2 ms

Section II

FICTIVE LOCOMOTOR ACTIVITY OF CANDIDATE INTERNEURONES MEDIATING GROUP I DISYNAPTIC EPSPs IN EXTENSOR MOTONEURONES IN THE CAT

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Summary

1. During the extensor phase of mesencephalic locomotor region (MLR)-evoked fictive locomotion there is a reversal of non-monosynaptic, group I-evoked reflexes from non-reciprocal inhibition to disynaptic excitation of homonymous and synergist extensor motoneurones. The present study used extracellular recording from lumbosacral interneurones in decerebrate paralysed cats during MLR-evoked locomotion to describe the activity of interneurones that may mediate the locomotor-dependent disynaptic excitation of extensor motoneurones evoked from ankle extensor group I afferents.

2. Data were obtained from cats in which group I-evoked disynaptic excitatory postsynaptic potentials (EPSPs) could be evoked in extensor motoneurones during extension. Interneurones mediating these reflexes should have the properties of being antidromically activated from extensor motor nuclei, weakly responsive to stimulation of extensor muscle group I afferents in the absence of locomotion, and strongly activated from group I afferents at monosynaptic latencies during locomotion.

3. Eight interneurones fulfilled at least some of these criteria for their consideration as candidate excitatory interneurones. All but one was located in mid to caudal L7 segments between 1.8 mm and 2.67 mm below the dorsal surface of the spinal cord, and most were within 2 mm of their target motoneurones in the rostrocaudal plane. Seven of the eight interneurones were weakly responsive or unresponsive to group I input at rest but activated by ankle extensor group I afferents at monosynaptic latencies during the extensor phase of fictive locomotion. Four of these seven interneurones were antidromically activated from the ankle extensor motor nucleus and thus met all the necessary criteria for candidate interneurones mediating disynaptic EPSPs from ankle extensor group I afferents in this study. The remaining interneurone was antidromically activated from the motor nucleus and unresponsive to group I stimulation before and during fictive locomotion. Interneurones responsible for the non-reciprocal inhibition of extensors in non-locomoting

conditions were found to be inhibited throughout fictive locomotor trials.

4. In the absence of peripheral nerve stimulation, 12 interneurones were found to be rhythmically active with maximal activation during the extension phase of fictive locomotion. Six were weakly responsive or unresponsive to group I input at rest but activated by group I input during extension. Three of these six interneurones projected to the motor nucleus and are considered as candidate excitatory cells. The fourth interneurone fulfilling all criteria for candidate excitatory interneurones was not rhythmically active during locomotion. Five of the twelve interneurones active during extension could not be activated at rest or during fictive locomotion by stimulation of the tested nerves at group I strength.

5. MLR stimulation evoked action potentials in candidate excitatory interneurones during fictive locomotion with latencies suggesting a polysynaptic excitation from the MLR. Excitatory actions from the MLR onto interneurones mediating group I-evoked disynaptic excitation of extensors was also inferred from the ability of MLR stimulation to facilitate group I disynaptic EPSPs recorded in motoneurones.

6. These results show the existence of lumbosacral interneurones located in the intermediate nucleus close to their target motoneurones that are unresponsive to group I input in the absence of fictive locomotion but can be activated during extension. We suggest that they are candidates for mediating group I-evoked disynaptic excitation of excitatory interneurones during extension. Their rhythmic activity suggests that they also form part of the excitatory drive to extensor motoneurones during fictive locomotion.

Introduction

In the absence of locomotion, stimulation of ankle extensor group I afferents evokes a complex pattern of oligosynaptic excitation and inhibition of motoneurones, with the dominant effect in extensors being inhibition (Eccles, Eccles & Lundberg 1957; Jankowska, Mackel & McCrea 1981a, b; for review see Jankowska 1992). The inhibitory effects observed in homonymous and synergist extensor motoneurones of the toe, ankle, knee and hip have been termed 'non-reciprocal inhibition' (Jankowska *et al.* 1981a). This inhibition is mediated by interneurones located in the intermediate nucleus (laminae V-VI) of the lumbosacral cord (Jankowska, Johannisson & Lipski, 1981; Brink, Harrison, Jankowska, McCrea & Skoog, 1983). Interneurones mediating group I non-reciprocal inhibition receive strong monosynaptic excitation from group I afferents and disynaptic excitation from cutaneous afferents (references in Jankowska, 1992). Axon collaterals from these interneurones ascend in the dorsolateral funiculus (DLF) and synapse on the cells of the Clarke's column (Hongo, Jankowska, Ono, Sasaki, Yamashita & Yoshida 1983). All group I excited interneurones with projections to both caudal motor nuclei and Clarke's column are believed to be inhibitory (Brink *et al.* 1983; Hongo *et al.* 1983).

During locomotion there is a reversal of non-monosynaptic, group I extensor nerve evoked reflexes from inhibitory to excitatory. This has been demonstrated by enhancing the ongoing locomotor activity recorded in extensor peripheral nerves by activating group Ia and Ib afferents with short trains of stimuli during the extensor phase of fictive (Conway, Hultborn & Kiehn 1987; Gossard, Brownstone, Bajaron & Hultborn 1994: Guertin *et al.* 1995) and treadmill locomotion (Pearson & Collins, 1993; Whelan, Hiebert & Pearson, 1995) (for review see Pearson, 1995). Termed 'group I extension enhancement,' this locomotor-dependent excitation can be evoked throughout hindlimb extensors, and is most easily demonstrated by activating ankle extensor group I afferents (Guertin *et al.* 1995).

Recently, intracellular recordings obtained during MLR-evoked fictive locomotion from extensor motoneurones showed both a reduction in group I non-reciprocal inhibition

and expression of a previously unknown excitatory pathway from extensor group I afferents (McCrea, Stephens & Pearson *et al.* 1995; Angel, Guertin, Jimenez & McCrea 1996). The mean latency of group I-evoked EPSPs in extensor motoneurones is around 1.55 ms suggesting a disynaptic pathway with a single interneurone (Angel *et al.* 1996). Group I afferents from extensor nerves can evoke disynaptic EPSPs in homonymous (Angel, *et al.* 1996), close synergist (McCrea *et al.* 1995; Angel *et al.* 1996) and extensor motoneurones operating at different joints (Angel *et al.* 1996) during the extension but not the flexion phase of fictive locomotion. Ankle extensor group I afferents have been shown to evoke the widest distribution of disynaptic excitation, evoking EPSPs in ankle, knee and hip extensor motoneurones (McCrea *et al.* 1995; Angel *et al.* 1996). Evidence has been presented that both Ia and Ib afferents evoke disynaptic EPSPs in ankle, knee and hip extensor motoneurones during locomotion. Thus one of the locomotor-dependent excitatory reflexes that likely aids in group I-evoked extension enhancement is disynaptic excitation.

Together McCrea *et al.* (1995) and Angel *et al.* (1996) have demonstrated the presence of a disynaptic reflex pathway that is inactive at rest, brought into operation during the extension phase of fictive locomotion, and mediated by a population of unidentified excitatory interneurones. Based on the occurrence and pattern of group I disynaptic EPSPs recorded in motoneurones during fictive locomotion (McCrea *et al.* 1995; Angel *et al.* 1996), criteria established for identification of candidate excitatory interneurones include i) antidromic activation by stimuli applied within extensor motor nuclei, ii) weak or no response to extensor nerve group I afferents at rest, iii) strong response to group I stimulation during MLR-evoked fictive locomotion at monosynaptic central latencies, and iv) maximal responsiveness to group I activation during extension. The location of these interneurones and their activity during fictive locomotion remain unknown.

The principle aims of this investigation are to locate candidate interneurones mediating group I disynaptic EPSPs of extensor motoneurones during locomotion and to examine their activity during fictive locomotion.

Methods

Surgery

Data on interneurones were collected from 6 decerebrate and paralysed cats in which fictive locomotion was elicited by monopolar electrical stimulation of the mesencephalic locomotor region (MLR). Details of the preparation and brainstem stimulation are provided elsewhere (Guertin *et al.* 1995). All surgery was performed on cats anaesthetised with a 1-1.6% halothane, 70% nitrous oxide 30% O_2 mixture. Data from an additional 5 cats obtained during another study and using the same preparation (Angel *et al.* 1996) were used to examine facilitation from the MLR of group I disynaptic EPSPs in motoneurones.

The following peripheral nerves were cut, dissected and placed on standard bipolar electrodes for either stimulation or recording: medial gastrocnemius (MG), lateral gastrocnemious-soleus (LGS), plantaris (Pl), semimembranosus and anterior biceps taken together (SmAB), posterior biceps and semitendinosus taken together (PBSt), tibialis anterior (TA), flexor digitorum hallucis and longus (FDHL), the cutaneous superficial peroneal nerve (SP), caudal and lateral cutaneous sural nerve (Su), and the mixed cutaneous and muscular posterior tibial nerve (Tib). Quadriceps (Q, vastii and rectus femoris) and sartorius nerves (Sart, medial and lateral branches) were placed in ventrally located bipolar cuff electrodes. Contralateral (Co) PBSt and SmAB nerves were cut and dissected and used for monitoring fictive locomotion. The remaining sciatic, femoral and obturator nerve branches were cut bilaterally and the tendons around both hips severed. Following laminectomy at T13 and L3-S1 a precollicular-postmammillary decerebration was performed by blunt transection. Both cortices and all tissue rostral to the transection were removed and anaesthesia stopped. The cats were paralysed with gallamine triethiodide (Flaxedil, 2-3 mg kg^{-1} hr^{-1}) and artificially ventilated. Tidal CO_2 levels were monitored and kept between 3-5%.

Stimulation and Recording

The search for candidate interneurones began with assessing the presence of group

I-evoked disynaptic EPSPs in extensor motoneurones during locomotion. The methods of recording disynaptic EPSPs during locomotion are detailed elsewhere (McCrea *et al.* 1995; Angel *et al.* 1996). Briefly, glass microelectrodes (1.8μ, 2-5 MΩ) filled with QX-314 to block action potentials were used to record intracellularly from antidromically identified extensor motoneurones. Averages of extensor nerve group I afferent-evoked disynaptic EPSPs were made during the extension and flexion phases of locomotion. The threshold amplitude and range of latencies for accepting EPSPs as disynaptic were approximately 250 μV and 1-1.9 ms respectively (Angel *et al.* 1996). Only after the presence of group I disynaptic EPSPs in motoneurones was established did the experiments continue. The intracellular recording microelectrode was replaced with a tungsten stimulating (tip diameter) electrode at the same location and angle. Constant current pulses (0.2 ms) were then applied along this electrode track while recording from the MG, LGS, Pl and SmAB nerve branches in order to locate extensor motor nuclei. Intraspinal stimulation (10μA-200μA) was applied at depths ranging from 1.5-2.8 mm from the dorsal lateral surface to antidromically activate interneurones that project to the extensor motor nuclei. Intraspinal stimulation of 50 μA should excite axonal branches of interneurones within about a 0.5 mm radius (see Gustafsson & Jankowska, 1976).

A second microelectrode (standard glass capillary tubing, filled with 2M sodium citrate, 2.0-2.2 μm, ~ 2MΩ) was used for extracellular recording from interneurones. This microelectrode was mounted on a second arc-styled manipulator and moved independently of the tungsten microelectrode used for antidromic activation of interneurones (see Fig 1).

Criteria for antidromic activation from the L4 and Th13 segments

Bipolar silver-chloride ball electrodes were placed on the left (ipsilateral) dorsolateral funiculus (DLF) at L_4 for antidromic activation of ascending inhibitory interneurone axons contacting cells of origin of the dorsal spinal cerebellar tract (i.e. identify lamina V-VI interneurones mediating non-reciprocal inhibition of motoneurones and cells of Clarke's column; Brink *et al.* 1983; Hongo *et al.* 1983). Bipolar electrodes were also positioned on ipsilateral and contralateral thoracic dorsal columns for antidromic

activation of ascending tract cells (see Fig. 1)

Assessment of MLR-evoked Facilitation of Group I Disynaptic EPSPs

As described in Angel *et al.* (1996), peripheral nerve and MLR stimuli were delivered independently. On occasion a MLR stimulus will precede a peripheral nerve stimulus by an interval that may facilitate the segmentally-evoked disynaptic pathway should this convergence exists. To assess this, intracellular recording from motoneurones from five cats of a previous study using the identical preparation to the one here (Angel *et al.* 1996) were re-analysed in the following way. Intracellular records obtained during the extension phase were aligned at the time of delivery of the nerve stimulus. The records were then sorted into three groups: group 1, those in which a stimulus from the MLR preceded the peripheral nerve stimulation by 1-10 ms; group 2, those without preceding MLR stimulation and group 3, those in which MLR stimuli occurred within a 10 ms epoch after the disynaptic EPSP. Averages of the disynaptic EPSPs taken from traces that did not have a preceding MLR stimulus (group 2) were compared to those with preceding MLR stimuli (group 1). Facilitation of the disynaptic EPSP was concluded when the peak to peak amplitude of the disynaptic EPSP with the preceding MLR stimuli was larger than the sum of the MLR EPSP and the disynaptic EPSP which was not preceded by an MLR stimulus i.e. group 1 $>$ (group 2 + group 3).

Results

Data on interneurones were collected from six cats in which group I-evoked disynaptic EPSPs in extensor motoneurones were first recorded during MLR-evoked fictive locomotion. Figure 2 is an example of disynaptic excitation recorded in a LGS motoneurone. It shows averaged intracellular recordings obtained while stimulating the homonymous nerve (1.1T) during the extension (solid) and flexion (dotted) phases of the fictive locomotor step cycle. The superimposed traces show a disynaptic EPSP occurring at the peak of the Ia monosynaptic EPSP during extension with the arithmetic difference plotted underneath. This preparation was thus deemed suitable for seeking the responsible interneurones. Because disynaptic EPSPs may not be obtainable during an experiment as the preparation deteriorates (see Discussion in Angel *et al.* 1996), it was important to show the presence of disynaptic excitation before searching for the relevant interneurones. When either locomotion began to fail, or during the latter portion of the experiment, the tungsten stimulating electrode (see Fig. 1) was replaced by an intracellular recording electrode to test again for the presence of group I disynaptic excitation. If disynaptic EPSPs in extensor motoneurones were not found, the experiment was terminated. Intracellular motoneurone recording and demonstration of disynaptic EPSPs was thus performed at least twice in each experiment. Data from interneurones obtained in the period between the demonstration of disynaptic EPSPs in extensors and the termination of the experiment were discarded and are not reported.

The initial search for interneurones was made in the L6 region of the spinal cord with the antidromic electrode placed more caudally in L7. These sites were chosen because of previous success in locating populations of interneurones with group Ia and Ib input (Jankowska *et al.* 1981) of which some are those mediating non-reciprocal inhibition (Brink *et al.* 1983; Hongo *et al.* 1983). In the first two experiments no interneurones were found which fulfilled the criteria for being candidates in the disynaptic excitatory pathway. In the subsequent four experiments the search for interneurones was carried out more caudally and closer to the site of the tungsten electrode used for antidromic stimulation.

Lumbosacral areas L7-S1 proved to be the most reliable for locating candidate interneurones.

Locomotion-induced inhibition of interneurones mediating non-reciprocal inhibition from group I afferents

Although cells with strong group I input at rest were usually rejected from further analysis, Fig. 3 illustrates two such interneurones that were studied during fictive locomotion. Panels *A-C* illustrate a lamina V-VI interneurone in L7 that is antidromically activated from the L4 DLF (3A, asterisk) and activated at monosynaptic latencies by stimulation of the MG nerve in the absence of locomotion (3B). This unit was also monosynaptically activated by Pl group I afferents and disynaptically activated by low threshold cutaneous afferents (not shown). In panel *C*, there is a marked reduction in MG group I-evoked responses during fictive locomotion. In panel *D*, another interneurone antidromically activated from the G-S motor nucleus was inhibited during MLR-evoked fictive locomotion. The arrows in Fig. 3D show the resumption of group I, FDHL-evoked action potentials within one second after terminating MLR stimulation. These two interneurones are likely to mediate non-reciprocal inhibition. The depression of non-reciprocal inhibition during fictive locomotion (McCrea *et al.* 1995; Angel *et al.* 1996) is, therefore, associated with an inhibition of laminae V-VI inhibitory interneurones.

Identification of Candidate Excitatory Interneurones

Eight interneurones in the present study displayed at least some of the criteria suggesting their identification as interneurones mediating disynaptic excitation of extensors during fictive locomotion. None of the candidate interneurones could be antidromically activated by stimulation of the DLF, ipsilateral or contralateral thoracic cord. Seven of these interneurones were weakly responsive or unresponsive to extensor group I afferent input in the absence of locomotion. During the extension phase of locomotion, however, all seven were activated by group I stimulation at monosynaptic latencies. Four of these seven cells were antidromically activated from an extensor motor nucleus and thus fulfilled

all the requirements (outlined in Introduction) for being considered as possibly mediating disynaptic excitation. The eighth interneurone was antidromically activated from the motor nucleus but was not activated by group I afferents from single nerve stimuli during locomotion or at rest. To provide the clearest illustrations of identification criteria, Figs 4, 5 & 6 illustrate data obtained from the same interneurone. Results from other candidate interneurones were qualitatively similar.

Figure 4*A* illustrates two properties of interneurones considered as candidates for mediating disynaptic excitation. Panel *A* is a series of extracellular recordings from an interneurone obtained shortly after the disynaptic EPSP was recorded in the motoneurone illustrated in Fig 2. This interneurone was antidromically activated by 22μA intraspinal stimulation of the ankle extensor motor nuclei. The threshold current for antidromic activation was 15μA and the cell body was located at the same rostrocaudal level as the tungsten stimulation electrode. The top three traces show the lack of effect of stimulation of ankle extensor nerves even at group II strength (5Tx3) in the absence of fictive locomotion. The position of the tungsten stimulating electrode was closest to the LGS motor nuclei as assessed from the size of the efferent discharge recorded from several extensor peripheral nerves (not shown). Figure 4*B* shows the estimated location of this interneurone using a camera lucida tracing of the section of spinal cord containing a prominent outline of the path of the electrode (dotted lines). A ten percent shrinkage factor from processing was taken into consideration. The interneurone was found around lamina VI. Interneurones reported were located at depths between 1.8 mm to 2.69 mm from the dorsal surface of the spinal cord in mid to caudal L7 segments; one was located in L6.

Interneurones mediating disynaptic excitation of extensors should be activated by extensor group I stimulation only during the extensor phase of fictive locomotion and at monosynaptic latencies (see Introduction). Fig. 5 shows that this interneurone is recruited by group I strength stimulation during extension but not flexion. For example, a spike occurs after each of the three 2T stimuli in the first vertically oriented extracellular recording of the interneurone (see also 5B), whereas at rest (4A) 5T stimulation was ineffective. The central latency for spike generation in this unit was 0.9 ms (5B) from the

group I afferent volley and is consistent with a monosynaptic linkage. MG and Pl group I afferents evoked similar effects in this interneurone during locomotion (not shown). The range of latencies of activation of the four interneurones that met all the criteria for possibly mediating disynaptic excitation was 0.8-1.1 ms from the incoming group I afferent volley.

Three of the seven interneurones with both weak group I activation in the absence of locomotion, and strong monosynaptic activation by group I afferents during locomotion were not antidromically activated from the motor nucleus. These three interneurones were also without an ascending axon. No attempt was made to systematically tract with the tungsten electrode to locate their axonal projections. Once the tungsten electrode was placed within the motor nuclei it was only moved in the dorsal-ventral plane. Thus interneurones projections to motoneurones at locations beyond the current spread from the tungsten electrode may not have been activated by the intraspinal stimulation. Furthermore, stimulus artefacts were sometimes large and may have prevented seeing an antidromic spike. During the search for interneurones, the microelectrode used for recording extracellular spike potentials was placed at an angle of 25 degrees from the vertical (tip pointing caudally) and the tungsten stimulating microelectrode positioned at 15 degrees from the vertical (tip pointing rostral). The recording and stimulating electrodes were often less than 1 mm from each other in the rostrocaudal plane at the surface of the cord. At a depth corresponding to the motor nucleus and intermediate nucleus, this distance separating the two is reduced substantially. Therefore these three interneurones may well have had an undetected projection to extensor motor nuclei.

The short axonal length of the interneurones as judged by the proximity of the stimulation and recording microelectrodes also precluded the use of collision to determine if the spike evoked from the motor nucleus was ortho- or antidromic. Antidromic identification was confirmed when an interneurone responded to antidromic stimulation at constant latencies and was able to follow trains of stimuli at 400Hz. Latencies of antidromic activation of five interneurones was 0.5, 0.6, 0.65, 0.8 and 1.1 ms from the tungsten stimulus artefact.

Activity of candidate excitatory interneurones during fictive locomotion

The top trace of Fig. 6 shows rhythmic activity of several (at least 3) interneurones during fictive locomotion without peripheral nerve stimulation. These records were obtained immediately following those illustrated in Fig. 5; the largest unit is the interneurone illustrated in Figs 4 & 5. The other units were not detectable by group I stimulation prior to fictive locomotion. The largest unit was isolated from the others using a window discriminator and its activity indicated by the small vertical lines above interneurone record. The large unit had a firing rate of approximately 15 Hz during extension. The tight coupling of interneurone activity to the extension phase of fictive locomotion is evident for all of the interneurones in Fig. 6.

Five other cells were found that were weakly responsive or unresponsive to group I input at rest but activated by group I afferents at monosynaptic latencies during extension. These cells were also rhythmically active during the extension phase of locomotion. Two of these five were antidromically activated from the extensor motor nucleus and together with the cell illustrated in Figs 4-6, were three of the four cells encountered that fulfilled the criteria for candidate interneurones mediating locomotor-dependent group I disynaptic excitation. An additional five interneurones in the same location in the spinal cord, but not activated by group I afferents from single nerves (for example see Fig. 6), or from ascending tracts, were active during the extension phase of fictive locomotion.

Rhythmic excitation of another interneurone during fictive locomotion is illustrated in Fig. 7. Panel *A* shows five superimposed sweeps: in the absence of locomotion, stimulation of Pl (2Tx3) only activates this cell once on the third stimulus of a 3 shock train (300 Hz). During locomotion, the same stimulus can activate this interneurone on any of the three shocks (latency 0.8 ms on the third shock). Group I stimulation did not discharge this cell during flexion. This cell did not have an ascending axonal projection and we were not able to antidromically activate it from the motor nucleus. The top trace in panel *B* is an extracellular recording of this interneurone during fictive locomotion.

Relationship between extension and the activity of candidate interneurones

Recruitment of candidate excitatory interneurones during fictive locomotion appears to require sufficient activity in the CPG to activate at least some extensor motoneurones. The asterisk in Fig. 6 shows a fictive locomotor step where there was an incomplete but still visible extensor phase (see small activity in FDHL and the reduction in TA and Sart activity). Both the largest unit and another unit fired action potentials during this brief extension phase. In Fig. 7, MLR stimulation resulted in a period of extensor bursts before the alternating bursts in flexor and extensor nerves signalled the onset of fictive locomotion. During this period there were tonic discharges in the interneurone recorded at this location. When the flexor nerves (TA, RF) became rhythmically active, the activity of this interneurone became phasic with discharges only during extension.

The activity of candidate excitatory interneurones during extension thus resembles the gating of group I-evoked disynaptic EPSP in extensor motoneurones. Occasionally during fictive locomotion, spontaneous failures in action potential generation in some pools of motoneurones occur. Figure 8 shows intracellular records from a SmAB motoneurone at low gain (vertical trace) and high gain (horizontal records). For clarity, only the high gain intracellular records obtained with Pl nerve stimulation during extension are shown. Five depolarizing periods in this extensor motoneurone labelled a-e correspond to the extension phase, and alternate with flexor (Sart) ENG activity. The vertical dashed line is plotted 1.4 ms following the arrival of the Pl nerve volley at the cord dorsum (arrow). Note the presence of disynaptic EPSPs during each depolarizing period except that labelled *d*. The small depolarization of the SmAB motoneurone (*d*) is accompanied by an absence of ankle extensor (LGS) ENG activity. Simultaneous with this reduction in drive from the central pattern generator to extensor motoneurones is a reduction in the ability of group I afferents to evoke disynaptic EPSPs in motoneurones (intracellular records adjacent to *d* on right panel of Fig. 8).

MLR activation of candidate excitatory interneurones

Analysis was performed to determine if candidate excitatory interneurones were excited from the MLR. Figure 9*A* consists of fifty-one superimposed sweeps aligned with

the MLR stimulus (vertical dashed line) showing MLR stimulus-evoked discharges of the interneurone illustrated in Figs 4, 5 & 6, and activity in the LGS ENG. The shortest latency between shock artefact and interneuronal activity was 6.8 ms. Since the earliest descending volley from the MLR is recorded in the lumbar cord dorsum with a latency of 3.4 ms (Noga, Fortier, Kriellaars, Dai, Detillieux & Jordan, 1995), MLR activation of this candidate excitatory interneurone is not monosynaptic. In addition to the one illustrated in Figs 4-6, two other interneurones that were weakly or unresponsive to group I afferents at rest but activated by group I afferents at monosynaptic latencies during extension were activated by the MLR at similar latencies. One of the three was also antidromically activated from the motor nucleus (see Fig. 4). Two additional interneurones, located in the immediate vicinity of the candidate excitatory interneurones, with rhythmic activity during extension but not activated by the group I afferents tested were also activated by the MLR at polysynaptic latencies. Figure 9*A* also shows that MLR stimulation evokes activity in the candidate excitatory interneurone and LGS ENG at similar latencies. The low resolution of the ENG recording prevented a more accurate comparison of interneurone and ENG latencies. The records, however, are consistent with the possibility that the MLR-evoked activation of LGS motoneurones is mediated in part by candidate interneurones found in the present study.

The possibility that MLR stimulation could facilitate group I-evoked disynaptic EPSPs was tested in eight motoneurones recorded during a previous study (Angel *et al.* 1996). All records in Fig. 9*B* & *C* are averages of data obtained during the extension phase of MLR evoked fictive locomotion. Figure 9*B* illustrates MLR facilitation of the Pl-evoked disynaptic EPSP recorded in a SmAB motoneurone. The solid line in 9*B* is the disynaptic EPSP preceded by MLR stimulation (n=25, see Methods). The dashed line is an average (n=187) of the Pl group I-evoked disynaptic EPSP without preceding MLR stimuli. Stimulation of the MLR alone produced the dotted line (n=17). This average is illustrated in third trace in panel B. By aligning the slopes of the MLR EPSP in the conditioned disynaptic EPSP trace with that of the pure MLR EPSP it is possible to compare the disynaptic EPSP with and without the conditioned MLR stimulus. The larger EPSP (solid

trace) suggests that MLR stimulation resulted in a facilitation of the group I disynaptic EPSP. Panel *C* shows a similar MLR facilitation of a disynaptic EPSP in a different SmAB motoneurone evoked in this case, by selective activation of Ia afferents from triceps surae and plantaris by single brief stretches of the Achilles' tendon (see Methods in Angel et al. 1996).

The evidence for MLR-evoked spatial facilitation of disynaptic EPSPs together with MLR-evoked spikes in some interneurones is suggestive that the interneurone in the group I excitatory pathway may also relay a descending locomotor command from the MLR to extensor motoneurones.

Discussion

The primary objective of the present study was to locate candidate excitatory interneurones that mediate group I disynaptic EPSPs in extensor motoneurones during fictive locomotion. It was thus important to first show that stimulation of group I ankle extensor afferents evoked EPSPs and not IPSPs in extensor motoneurones. The finding that there is tonic suppression during fictive locomotion of interneurones mediating group I non-reciprocal inhibition, suggests that their inhibition may account for the reduction in non-reciprocal inhibition recorded in motoneurones during fictive locomotion (see McCrea *et al.* 1995; Angel *et al.* 1996). During fictive locomotion there is also release from inhibition of other interneurones that can be activated by extensor group I afferents during extension; some of these cells are also rhythmically active during extension. It is postulated that this newly encountered class of interneurones mediates the group I-evoked disynaptic excitation of extensor motoneurones. The lack of evidence to show that these interneurones are excitatory is the biggest limitation to the interpretation of present results. This study, however, presents data on the characteristics and location of candidate interneurones that will provide the foundation for future investigations on these interneurones.

Four interneurones in this study fulfilled criteria suggesting they could mediate disynaptic excitation from group I afferents. Three of the four were located in the mid to caudal L7 region of the spinal cord between 1.8 mm and 2.7 mm below the dorsal surface of the cord (i. e. in the intermediate laminae) and the fourth in L6. These interneurones were antidromically activated from an extensor motor nucleus, weakly or unresponsive to group I stimulation in the absence of locomotion, and activated by group I afferents during the extension phase of locomotion at monosynaptic latencies. The latencies for activation from the group I volley of the four most fully characterized interneurones were 0.8-1.1 ms. With a short conduction distance and an additional synaptic delay of 0.3-0.5 ms it is expected that the PSP produced in their target motoneurones by this pathway would be in the range of group I-evoked EPSPs observed in extensor motoneurones (1.1-1.9 ms, mean 1.55 ms, Angel *et al.* 1996). Based on all interneuronally mediated reflexes known to be

evoked by group I afferents at rest (Eccles, Eccles & Lundberg, 1957b; Jankowska, McCrea & Mackel 1981a,b) and during locomotion (Gossard, *et al.* 1994, Schomburg & Behrends, 1978, Shefchyk, Stein & Jordan, 1984, McCrea *et al.* 1995, Angel *et al.* 1996), the activity of the candidate interneurones reported here resemble the activity pattern of interneurones that would likely mediate disynaptic excitation of extensors recorded during locomotion.

The present study did not systematically investigate the relative strength of activation from group I afferents from all nerves. Ankle extensor group I afferents were stimulated during locomotion because they evoked the widest pattern of disynaptic EPSPs in motoneurones (Angel et al. 1996). Actions from group I afferents from each of the ankle extensor nerves were not tested during locomotion in every case nor was input from other nerves surveyed routinely during locomotion. The pattern of disynaptic EPSPs evoked in hindlimb motoneurones suggests that there are multiple populations of excitatory interneurones differing in the sources of their group I input (Angel *et al.* 1996). A further and probably intracellular analysis is needed for the assessment of the pattern of group I (and other) afferent input to these interneurones.

Is this a new population of spinal interneurones?

It is unlikely that the candidate interneurones in this study are lamina V-VI interneurones mediating group I non-reciprocal inhibition. The majority of lumbosacral lamina V-VI inhibitory interneurones have an ascending axon collateral in the DLF (Fern *et al.* 1988). All are recruited by group I strength stimulation in non-locomoting preparations and approximately 50% of them receive disynaptic cutaneous input (Jankowska & Harrison 1985a). Candidate excitatory interneurones on the other hand, neither projected to Clarke's column nor received strong group I or cutaneous input (e.g. sural or SP) in the absence of locomotion. The inhibition of the two inhibitory interneurones reported here (Fig. 3) and the suppression of non-reciprocal inhibition in motoneurones during fictive locomotion (Gossard *et al.* 1994; McCrea *et al.* 1995; Angel *et al.* 1996) argues against the possibility that the candidate interneurones are those

previously identified as mediating non-reciprocal inhibition.

Gossard *et al.* (1994) recorded from interneurones believed to be interposed in a locomotor related polysynaptic group I pathway. These interneurones were located in L6 in lamina VII and were maximally activated from group I afferents during flexion with a minimum central latency of 3 ms. This contrasts the monosynaptic latency of the interneurones reported here. Furthermore there were few discharges from interneurones during flexion in the present study. Thus, based on location, latency of group I activation and activity during fictive locomotion, the interneurones described by Gossard *et al.* (1994) are a different population than those reported here.

It is unlikely that candidate excitatory interneurones are Ia inhibitory interneurones. Ia interneurones are located in the ventral horn adjacent to lamina IX (Jankowska & Lindström, 1971) whereas the interneurones in the present study were located in the intermediate nucleus. Ia inhibitory interneurones can be readily activated by group I stimulation both in non-locomoting preparations and during fictive locomotion (e. g. McCrea, Pratt & Jordan, 1980) are thus unlike the candidate excitatory interneurones. Finally, it is unlikely that any interneurones in this study were Renshaw cells because Renshaw cells respond to activation from motor axon collaterals with a characteristic repetitive firing in the MLR fictive locomotor preparation (McCrea *et al.* 1980). Stimulation of peripheral nerves at 5T (eg. Fig. 4) would have recruited most of the motoneurone axons antidromically and thus produced discharges in Renshaw cells.

To our knowledge, there have been no previous attempts to identify the interneurones described in the present study. Because most studies in non-locomotion preparations have used stimulation of peripheral afferents as a means to locate interneurones, previous surveys of interneurones with group I input would likely have missed those that only become activated by group I afferent stimulation during fictive locomotion. Figures 4 & 6 illustrate the difficulty in finding candidate excitatory interneurones. Their lack of activity at rest made tracking for them difficult and the spontaneous activity of other neurones in the immediate vicinity during fictive locomotion complicated assessment of their fictive locomotor activity. According to the change in

response to group I input that occurs between non-locomoting and fictive locomotion conditions, however, we suggest that the interneurones in the present study are a previously undescribed population of interneurones.

Spontaneous activity during fictive locomotion

Spontaneous firing of candidate excitatory interneurones during the extension phase of fictive locomotion was an unexpected finding in the present study. If these interneurones turn out to be excitatory, then they must contribute to the rhythmic locomotor excitation of extensor motoneurones in addition to evoking disynaptic excitation from group I afferents. The question arises as to whether these interneurones are an integral part of the central pattern generator for locomotion. The main line of evidence that suggests otherwise stems from the observation that some preparations with coordinated fictive locomotion may not display group I-evoked disynaptic EPSPs in extensor motoneurones (Angel *et al.* 1996, Guertin *et al.* 1994). Thus the generation of the basic locomotor rhythm in motoneurones does not require the contribution of the interneurones subserving group I-evoked disynaptic EPSPs. Nevertheless, the tight coupling of the activity of candidate interneurones to the extensor phase (Fig. 6), and the covariance of group I disynaptic EPSP amplitude and extensor drive from the CPG (Fig. 8) suggest that the activity of candidate interneurones is closely regulated by the interneurones of the CPG.

MLR-Evoked Facilitation of Disynaptic EPSPs

By examining the presence of spatial facilitation in motoneurones, and from direct recording from interneurones, evidence has been presented that the MLR can activate interneurones of the group I disynaptic pathway. The motoneurone data presented in Fig. 9*B* & *C* is preliminary evidence suggesting that stimulation of the MLR activates pathways excitatory to interneurones producing group I disynaptic excitation of extensors.

MLR-evoked EPSPs in cat hindlimb motoneurones have been previously examined (Shefchyk *et al.* 1984). These EPSPs are found in both extensor and flexor motoneurones and are largest during the phase of locomotion in which motoneurones are depolarized and

occur with latencies of 4.2 to 8.2 ms from the MLR stimulus (Shefchyk *et al.* 1984). The lumbar interneurones relaying this excitation from the MLR to extensors have yet to be described. It is, therefore, unknown if interneurones that produce MLR-evoked EPSPs include interneurones in the locomotor-dependent group I disynaptic excitatory pathway. Their latency of activation from the MLR (Fig. 9*A*) is consistent with such a possibility.

In summary this study reports on a previously unknown population of interneurones that can be activated by stimulation of ankle extensor group I afferents during the extension phase of fictive locomotion. The hypothesis is that these are excitatory interneurones interposed in a disynaptic pathway from group I afferents to extensor motoneurones. Further work is needed to show that such interneurones are indeed excitatory as well as to examine the organization of descending and segmental input to these cells. In addition, further investigation is required to identify the mechanisms that appear to tonically inhibit these interneurones in the absence of locomotion condition and allow their phasic excitability during extension.

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Figures and Figure Legends

Figure 1. *Experimental arrangement used to locate interneurones.*

A, recording microelectrode and tungsten stimulating electrode were inserted through the L7 dorsal columns. Within the grey mater stimulating and recording microelectrode tips were often separated by $<$ 1 mm in the rostral-caudal plane. Bipolar stimulating electrodes were placed on the ipsilateral dorsolateral funiculus (DLF) at L4 and on both ipsi- (i) and contralateral (co) thoracic dorsal columns for antidromic activation of laminae V-VI inhibitory cells and ascending tract cells respectively.

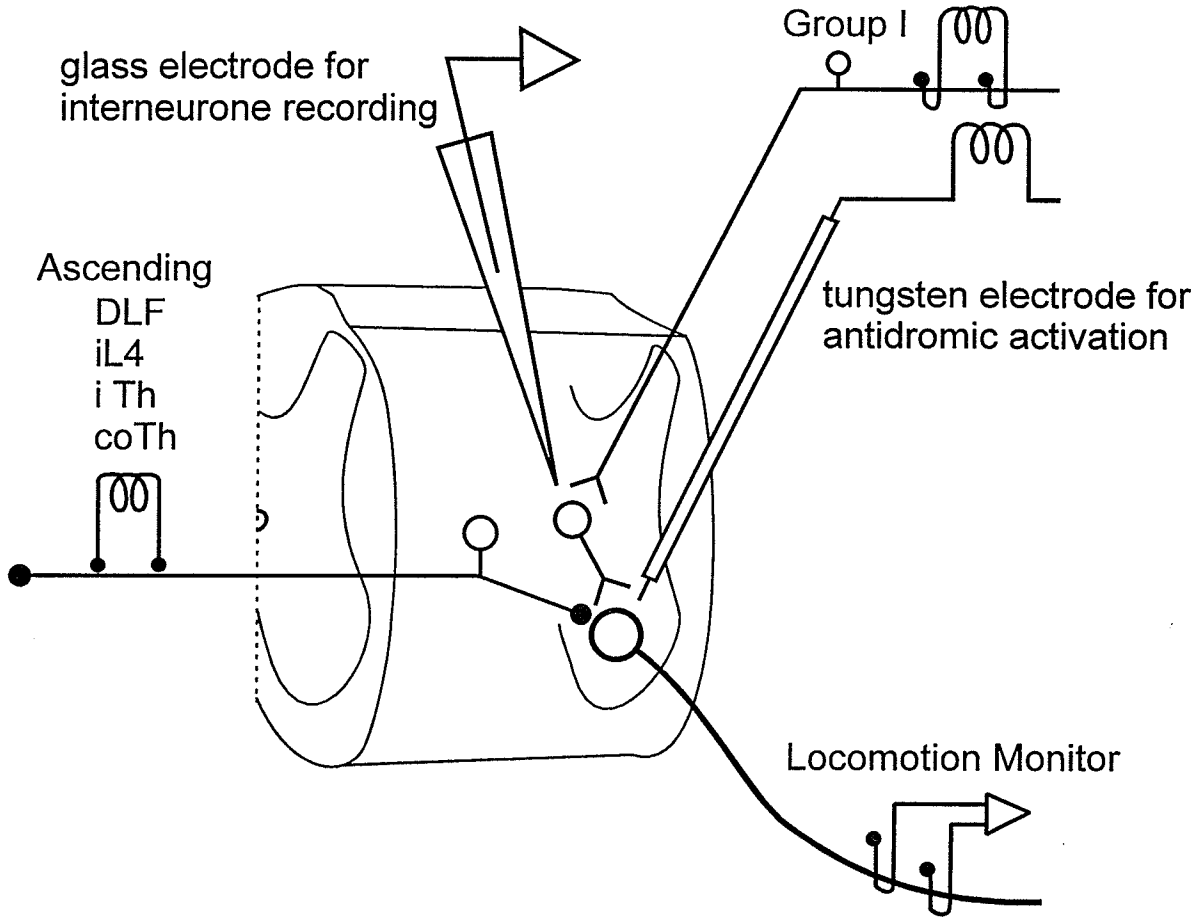

glass electrode for
interneurone recording
Group I
Ascending
DLF
iL4
i Th
coTh
tungsten electrode for
antidromic activation
Locomotion Monitor

Figure 2. *Disynaptic EPSPs in extensors indicate the suitability of the preparation for locating candidate interneurones.*

A, the LGS-evoked disynaptic group I EPSP recorded in a LGS motoneurone (filled arrow and trace marked 'difference') indicates the preparation was suitable for locating the interneurones that mediate group I disynaptic EPSP during extension.

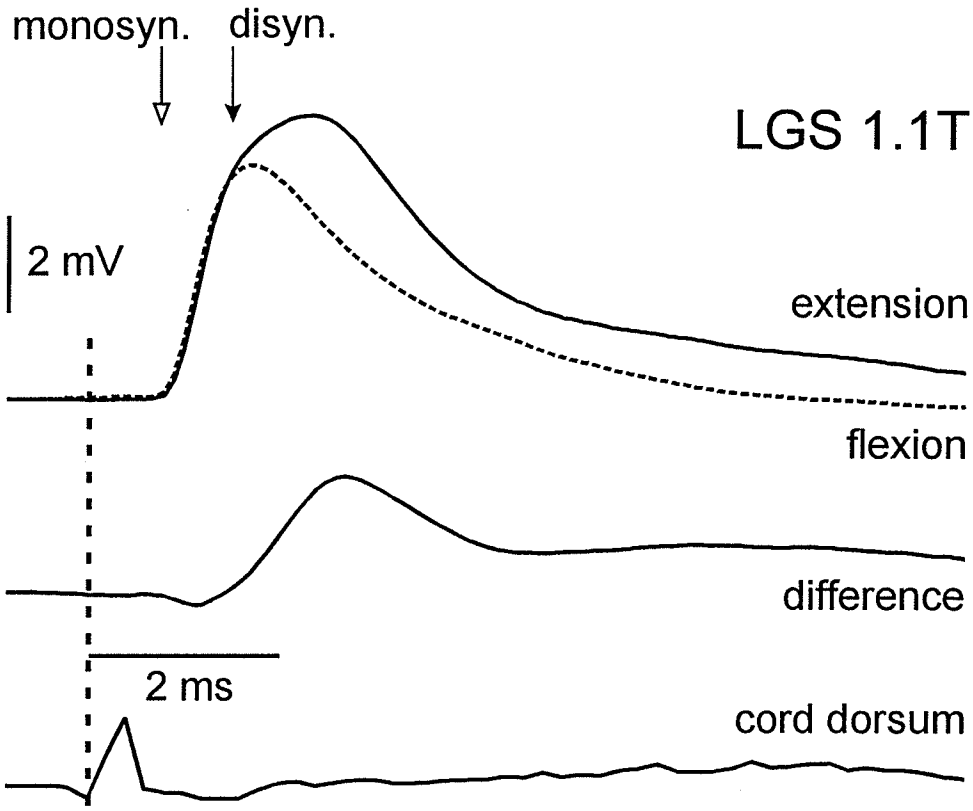

monosyn.
disyn.
LGS 1.1T
2 mV
extension
flexion
difference
2 ms
cord dorsum

Figure 3. *Locomotor inhibition of interneurones mediating group I non-reciprocal inhibition of motoneurones.*

A-C, records from an interneurone antidromically activated by stimulation of the L4 dorsolateral funiculus (*A*, antidromic activity indicated by asterisk) and activated by MG group I afferents at monosynaptic latencies in the absence of locomotion (*B*). The bottom traces in A-C are recordings of the cord dorsum potentials. In *C*, activation of this interneurone is less effective with this neurone MLR-evoked locomotion. In *D*, another interneurone (displayed vertically) was monosynaptically activated by group I afferents at rest, failed to respond to FDHL stimulation during locomotion, but became responsive to the same stimuli (arrows) within 1s after terminating MLR stimulation (horizontal bar).

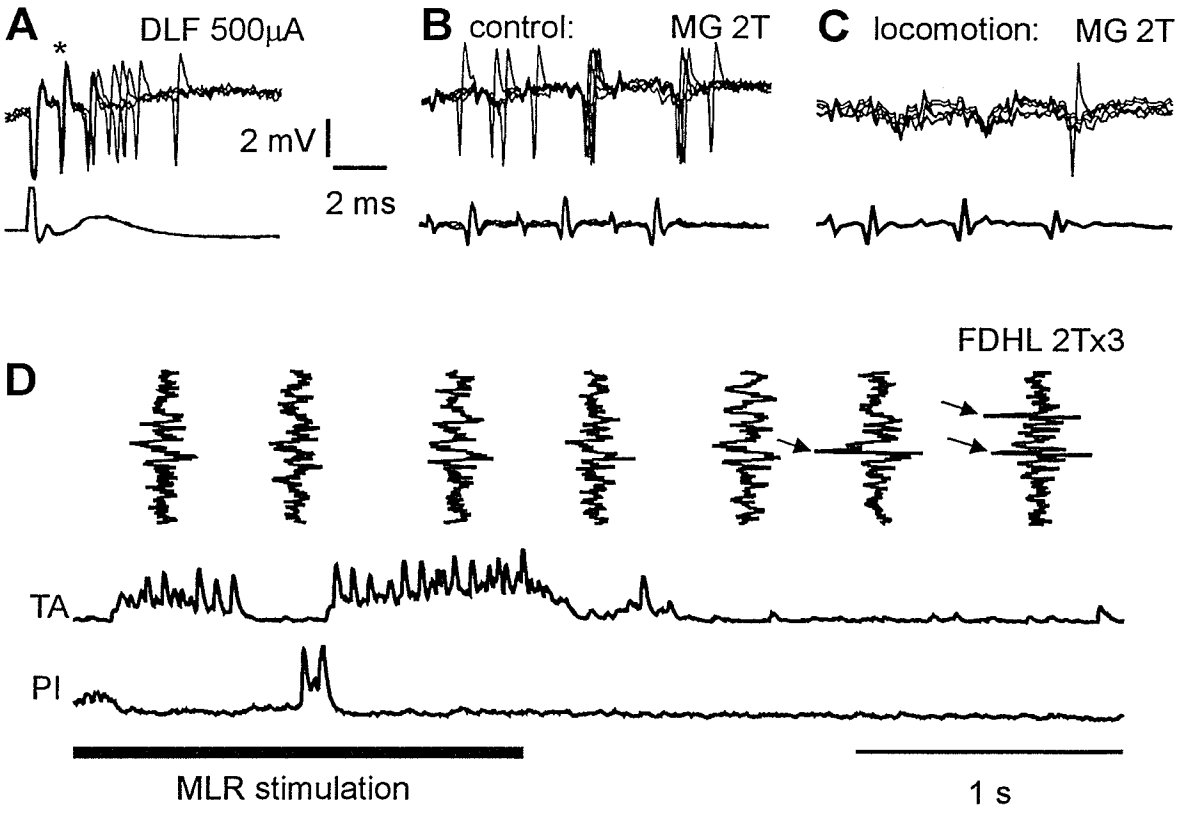
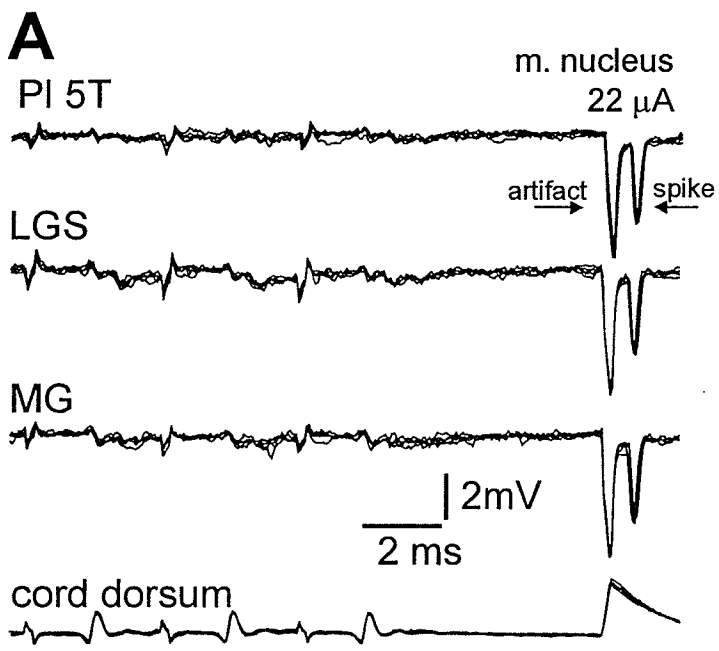

A
DLF 500μA
2 mV
2 ms
B control:
MG 2T
C locomotion:
MG 2T
D
FDHL 2Tx3
TA
PI
MLR stimulation
1 s

Figure 4. *Absence of group I activation of a candidate excitatory interneurone in the absence of locomotion.*

A, overlayed of five extracellular recordings of an interneurone antidromically activated by intraspinal stimulation of the ankle extensor motor nuclei but unresponsive to supramaximal group I stimulation (5T x3, @ 300Hz). Bottom trace is the cord dorsum recording of the afferent volley. *B*, camera lucida reconstruction of the recording microelectrode track (dotted outline) points to the estimated location of the interneurone (filled circle) illustrated in Fig. 4*A*, 5, 6, 8*A*. The scale bar takes into account a 10% shrinkage due to mounting. This candidate interneurones was located in the intermediate nucleus.


A
m. nucleus
22 μA
Pl 5T
artifact
spike
LGS
MG
2mV
2 ms
cord dorsum

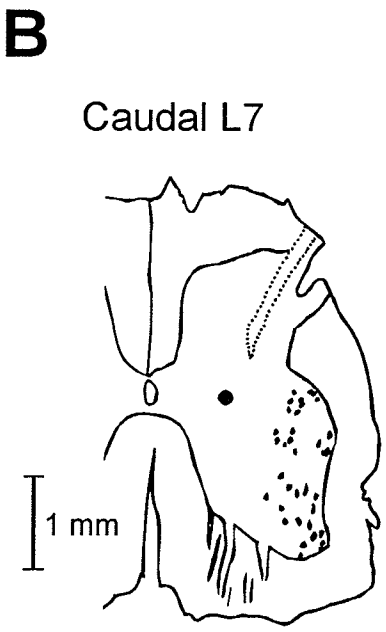
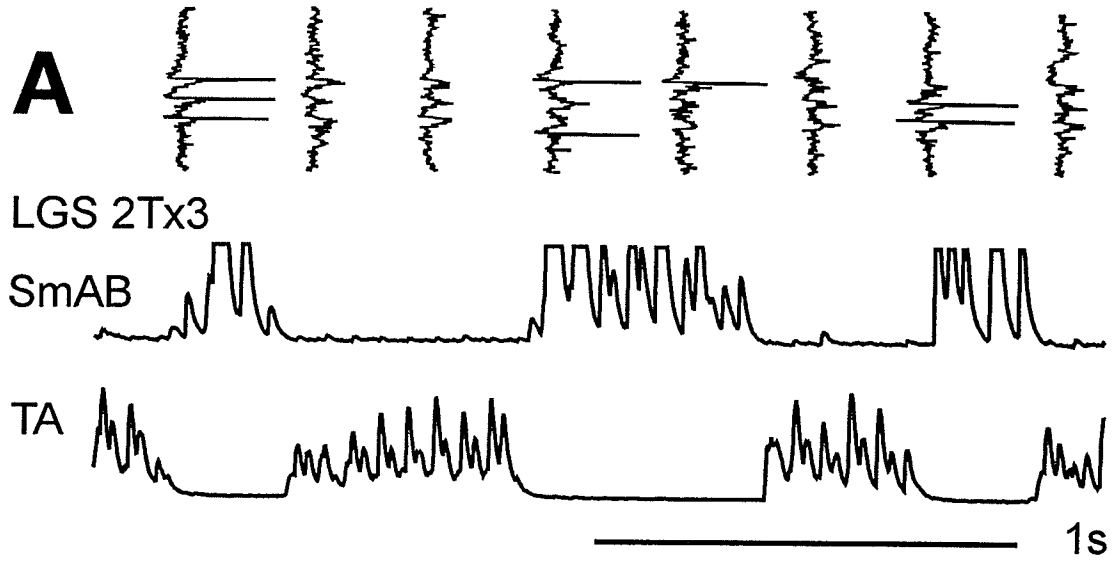

B
Caudal L7
1 mm

Figure 5. *Group I activation of a candidate excitatory interneurone during fictive locomotion.*

Extracellular recording of an interneurone during locomotion (same interneurone as in Fig. 4). *A*, top traces are vertically oriented extracellular recording of the interneurone showing its response LGS stimulation during locomotion. The extension phase of locomotion is indicated by SmAB activity. *B,* records obtained during fictive locomotion show the latency of orthodromic activation to be 0.9 ms.


A
LGS 2Tx3
SmAB
TA
1s

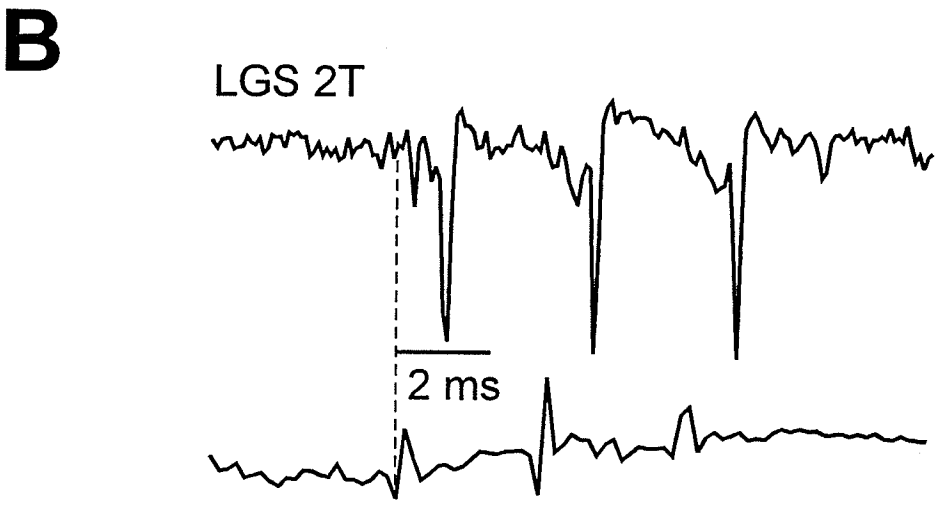

B
LGS 2T
2 ms

Figure 6. *Spontaneous activity of candidate interneurone during extension.*

Extracellular recording of a candidate interneurone during MLR-evoked fictive locomotion in the absence of peripheral nerve stimulation (largest spikes; represented by small vertical ticks). At least two other interneurones visible in the upper records are rhythmically activated. The asterisk shows that with only a minimal amount of motoneurone activity (see FDHL) the three interneurones were recruited and are thus tightly coupled to the rhythm of locomotion (see text)..

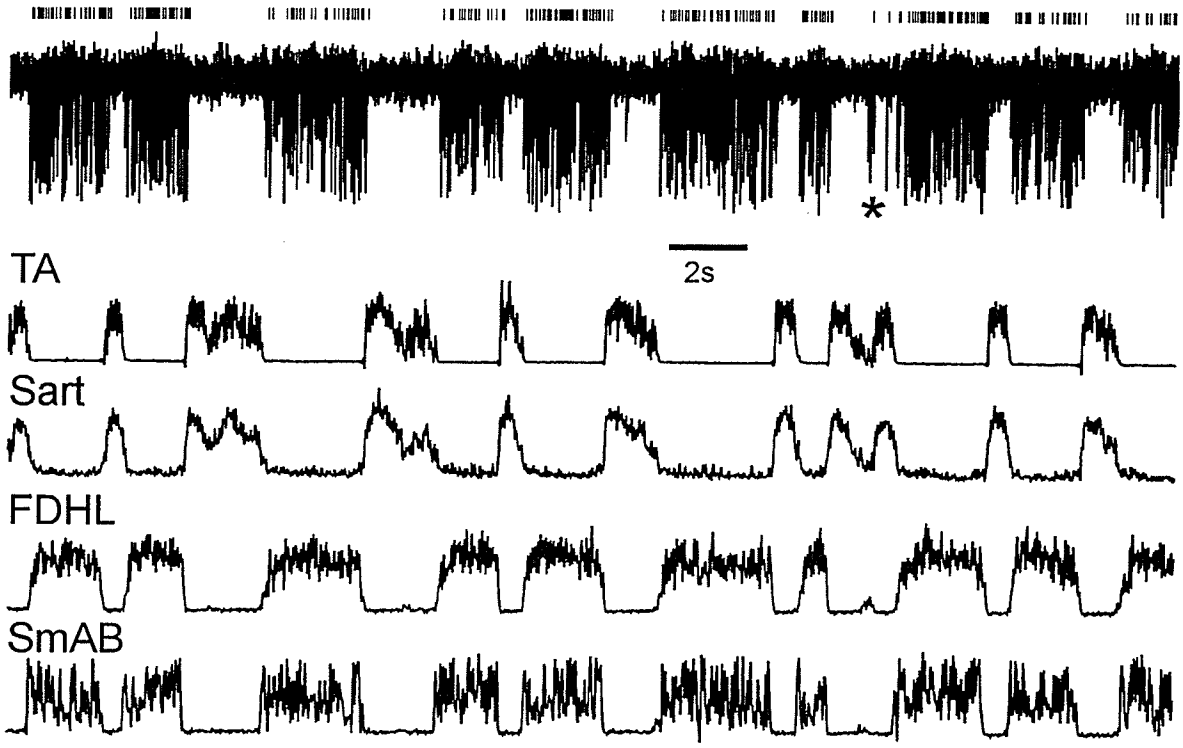

*
2s
TA
Sart
FDHL
SmAB

Figure 7.*Tonic activity of candidate interneurones in the absence of flexor nerve activity.*

The records at the top of panel *A* shows the weak activation of an interneurone by group I afferents in the absence of locomotion, and strong group I activation of the interneurone during fictive locomotion at monosynaptic latencies (0.8 ms on third stimulus) below. The top trace in panel B shows that in addition to the interneurone in *A* at least one other interneurone was activated tonically when MLR stimulation produced rhythmic activation of only extensors (MG). The interneurones discharged rhythmically only after the onset of fictive locomotion.

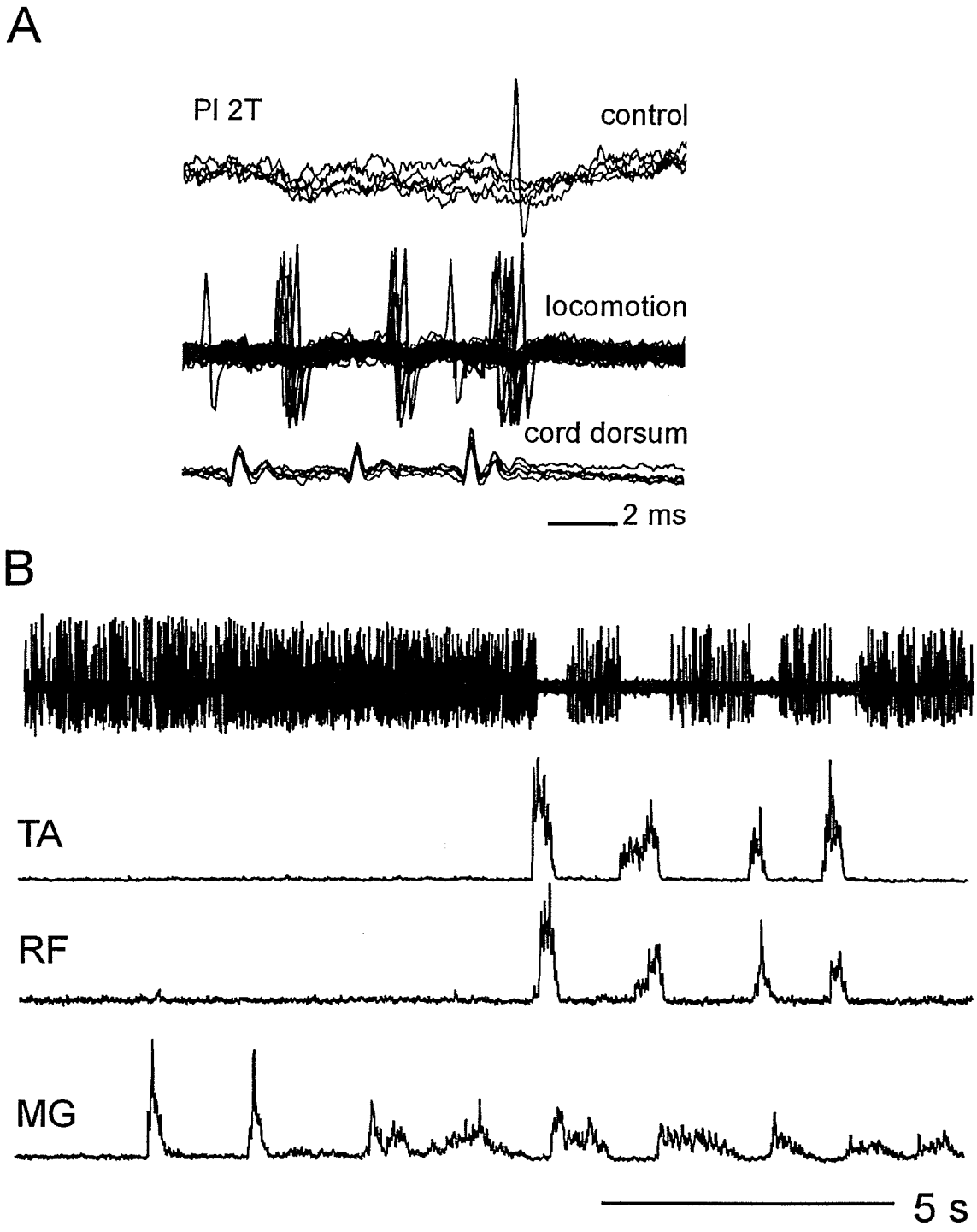

A
PI 2T
control
locomotion
cord dorsum
2 ms
B
TA
RF
MG
5 s

Figure 8.*Relationship between disynaptic EPSPs and activity of the extensor rhythm generator.*

Vertically oriented ENGs from a contralateral extensor (CoSmAB) and ipsilateral flexor (Sart) and extensor (LGS) nerve are plotted alongside a low gain intracellular record from a SmAB motoneurone during MLR-evoked fictive locomotion. Horizontally plotted high gain intracellular recordings from this motoneurone showing Pl-evoked disynaptic EPSPs during extension are positioned to the right. The afferent volley and EPSP onset are indicated by the vertical arrow and dotted vertical line respectively. Notice that the disynaptic EPSPs are largest when Pl stimulation occurs during large locomotor drive potentials (a, b, c, e). In contrast, when there is no activity in the extensor nerve (*) and the locomotor drive potential is small, the Pl-evoked disynaptic EPSPs are absent (d).

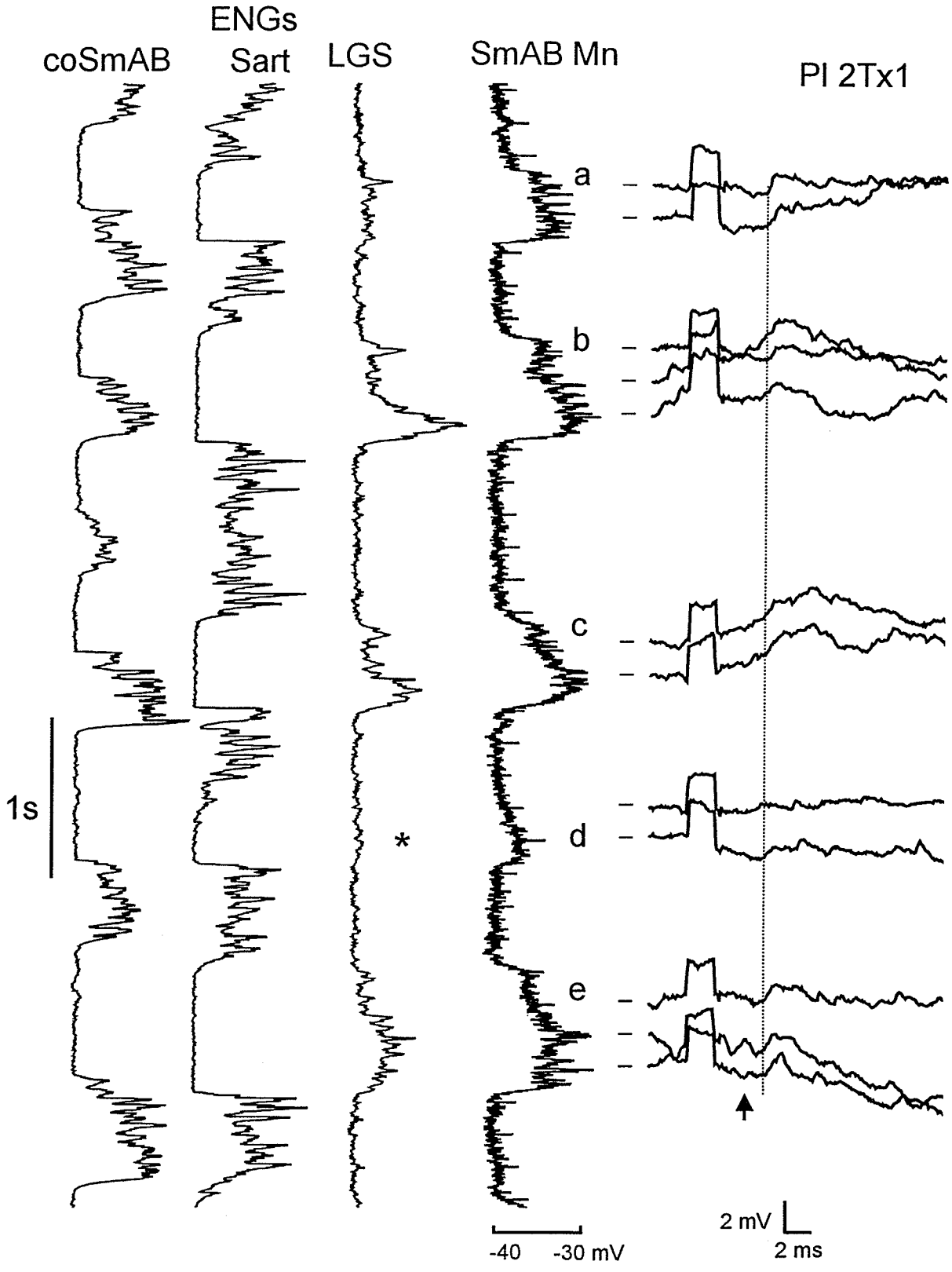
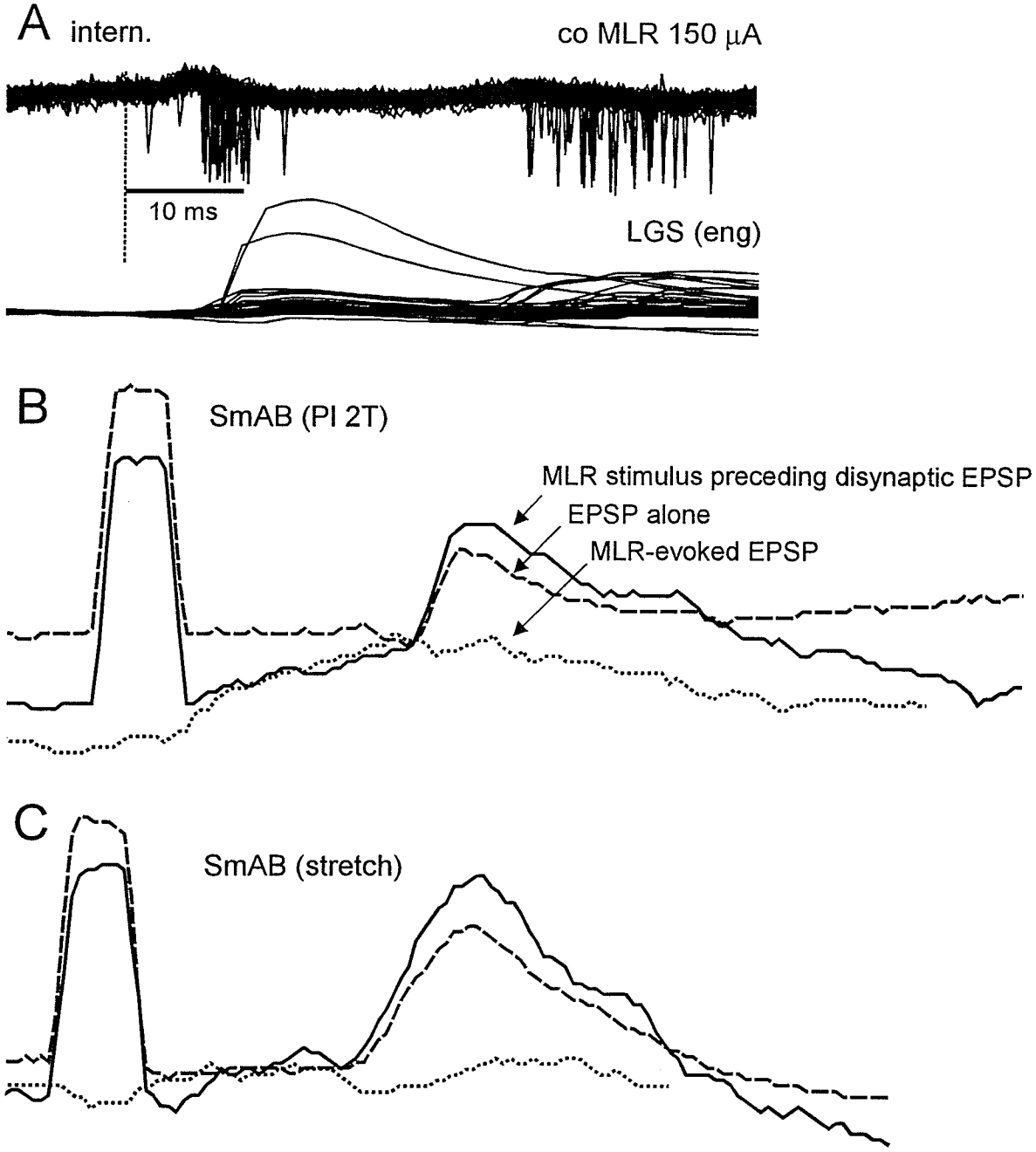

coSmAB
ENGs
Sart
LGS
SmAB Mn
PI 2Tx1
a
b
c
d
e
1s
*
-40
-30 mV
2 mV
2 ms

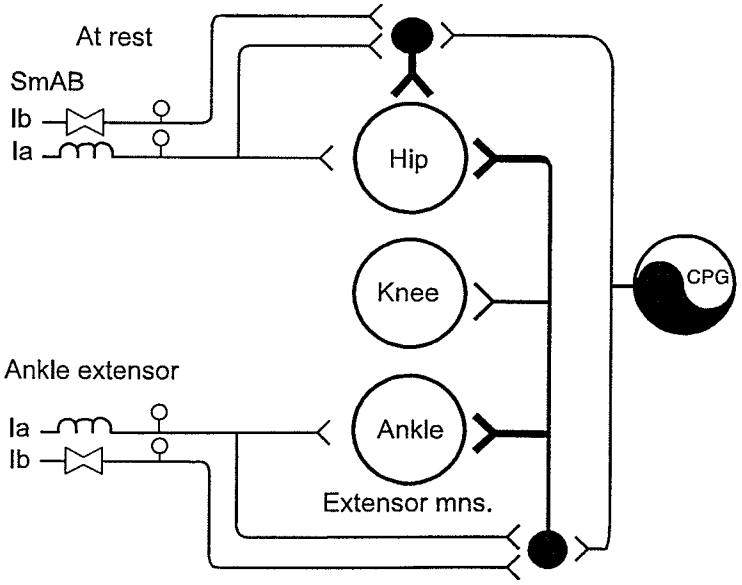
Figure 9.*Evidence for convergence from the MLR onto interneurones in the group I locomotor dependent disynaptic pathway*

A, the top raw records are MLR stimulus-locked spikes (n=51) of the interneurones already illustrated in Fig. 6 indicate a polysynaptic connection from the MLR to both the identified interneurone and the others that are recruited during fictive locomotion (150 μA @ 15Hz). The bottom overlayed traces are MLR stimulus-locked discharges recorded in the LGS peripheral nerve. *B*, top trace (solid line) is an average (n=25) of disynaptic EPSP recorded in a SmAB motoneurone evoked by Pl group I afferents with MLR stimuli preceding peripheral nerve stimulation by 1-10 ms (see text). The second trace (dashed line) is an average (n=187) of a similar peripherally-evoked EPSP without a preceding MLR stimulation. The lowest traces is an average (n=17) of MLR EPSP which randomly occurred over a 10 ms epoch. This was used to estimate the MLR EPSP component contaminating the solid traces. *C*, as in *B* but with selective activation of triceps surae and Pl Ia afferents using muscle stretch. The top two traces are averaged group I disynaptic EPSP with (n=9) and without (n=79) preceding MLR stimulation respectively, and the averaged (n=18) MLR EPSP is situated below.


A
intern.
co MLR 150 μA
10 ms
LGS (eng)
B
SmAB (PI 2T)
MLR stimulus preceding disynaptic EPSP
EPSP alone
MLR-evoked EPSP
C
SmAB (stretch)

General Discussion

The first section of this thesis described the distribution of a newly described disynaptic excitatory pathway that is activated during the extensor phase of fictive locomotion. The outstanding feature of this locomotor-related group I pathway is the widespread pattern of EPSPs evoked by ankle extensor group I afferents in extensor motoneurones of the ankle, knee and hip joints. Evidence has been presented showing that Ia afferents alone from triceps surae and plantaris muscles can elicit disynaptic EPSPs across the three main joints during locomotion. Because the disynaptic EPSP amplitude evoked by electrical stimulation of a single muscle nerve can be larger than that produced by stretching the four ankle extensor muscles in the same motoneurone, it has been argued that Ib afferents also contribute to disynaptic excitation (McCrea *et al.* 1995). The absence of any such pattern of synaptic connectivity in non-locomoting preparations leads to our conclusion that this reflex pathway is mediated by a previously undescribed population of interneurones that is disinhibited during locomotion. Further discussion of the functional implication of this pathway during locomotion can be found in the Discussion section of Angel *et al.* 1996. To the right is a simplified circuit diagram illustrating how the excitation from group I afferents changes going from rest i.e. monosynaptic excitation from Ia afferents, to locomotion i.e. monosynaptic and disynaptic excitation from both Ia and Ib afferents. The explanation for the two sets of interneurones is discussed in the Discussion section of Angel *et al.* 1996 and the contribution of the CPG is detailed in the Discussion section of Section II. The line thickness of the interneurones represents



the sampling bias of our experiments and not the relative strength of synaptic effects. Thus ankle and hip motoneurones were sampled most.

As proposed in Angel *et al.* (1996) the expression of locomotor-related group I disynaptic EPSPs may be the result of cyclic disinhibition of interneurones. This mechanism is favoured over a voltage-dependent gating mechanism at the level of the motoneurone because PBSt motoneurones, which were often more depolarized during flexion, only displayed disynaptic EPSPs during the less depolarized extension phase (Angel *et al.* 1996). Removal of tonic PAD of all group I afferents which reaches a maximum during extension is also an unlikely gating mechanism for disynaptic EPSP since Ia monosynaptic EPSPs in extensor motoneurones could be maximal during either the extension or flexion phase, or could remain unchanged across both phases of the step cycle. The unpredictable phase dependent modulation of Ia monosynaptic EPSPs is in contrast to the consistent expression of group I disynaptic EPSPs during extension (Angel *et al.* 1996). Selective PAD of only those afferent terminals which synapse on the interneurones mediating group I disynaptic excitation of extensor motoneurones might be an alternate gating mechanism. Investigation in other pathways from group I afferents have revealed that collaterals of the same afferent can be under differential presynaptic control (Eguibar, Quevedo, Jimenez & Rudomin, 1994; Zytnicki, Lafleur, Kouchtir & Perrier, 1995). If this kind of presynaptic mechanism gated group I disynaptic EPSP during locomotion, substantial selective tonic PAD of group I afferent collaterals in the intermediate nucleus would be required in the absence of locomotion. The PAD must be sufficient to prevent activation of any interneurones responsible for group I disynaptic excitation by single shock stimuli. At the onset of locomotion PAD must be reduced, then cyclically modulated such that a further reduction in PAD occurs during extension and increases during flexion. The level of PAD during flexion must be less than the tonic PAD in the absence of locomotion as disynaptic EPSPs have been shown to be evoked with short stimulus trains during flexion, but never at rest. The possibility that both PAD and postsynaptic mechanisms contribute to the gating of the excitatory interneurones in group I disynaptic pathway cannot be ruled out.

Effects on Motoneurone Recruitment

As suggested in Angel *et al.* (1996), the presence of fast rising disynaptic EPSPs superimposed on a motoneurone's locomotor drive potential influences motoneurone recruitment and would likely increase force output. Whether such effects are distributed uniformly to slow (S), fast resistant (FR) and fast fatiguing (FF) motoneurones is unknown; therefore, the effect of group I disynaptic excitation on recruitment order cannot be predicted. The relation between motoneurone type and incidence or amplitude of group I disynaptic EPSPs during locomotion is thus an important basis for future experiments. A preferential distribution of group I disynaptic excitation to the F-type, i.e. those recruited last under non-locomoting conditions might suggest a state dependent compression of recruitment thresholds. Such a proposal has been made based on experiments investigating the effective synaptic current (I_N) in motoneurones evoked by stimulation of rubrospinal (Powers, Robinson, Konodi & Binder 1993), and vestibulospinal neurones (Westcott, Powers, Robinson & Binder 1995) wherein trains of supramaximal stimuli applied to these descending systems in the absence of locomotion produce larger depolarizing currents in type F than in type S. Whether activation of the locomotor dependent group I pathway evokes different I_N in type F and type S motoneurones remains to be shown. In addition whether accurate measurements of I_N can even be made in the presence of the intense synaptic noise and expression of intrinsic membrane properties (Brownstone *et al.* 1994) that occur during fictive locomotion remains to be determined. Further complicating matters is the requirement that only single peripheral nerve stimuli be used to activate the disynaptic pathway during measurements of I_N since trains of stimuli applied during locomotion activate polysynaptic "resetting pathways" (Gossard *et al.* 1994; Guertin, Angel, Jimenez & McCrea 1994). Parallel activation of the longer latency group I excitatory pathway may contaminate the I_N produced solely by the disynaptic excitatory pathway.

Assessment of disynaptic EPSPs in motoneurones during locomotion requires that action potentials be blocked by QX-314. The time between motoneurone impalement and blocking of a sufficient

number of fast sodium channels such that a cell's rheobase is affected is unknown. Electrical typing of all motoneurone must therefore be performed immediately after intracellular impalement and rheobase measurements should be taken before measurements of input resistance.

Functional Implications

Based on recent experiments in the locomoting cat (Schomburg & Behrends, 1978; Gossard *et al.* 1994; Guertin *et al.* 1994; McCrea *et al.* 1995; Angel *et al.* 1996) the original scheme of reflex contribution to the control of hindlimb extensor motoneurones during locomotion must be readdressed. Firstly, in light of the group Ia contribution to the locomotor-dependent disynaptic pathway, it can be concluded that Lundberg (1969) underestimated the contribution of group Ia afferents to motoneurone activation. Not only do extensor motoneurones receive homonymous group I disynaptic excitation (Schomburg & Behrends, 1978; Angel *et al.* 1996), but each ankle extensor can evoke disynaptic EPSPs in extensor motoneurones at each of the three main hindlimb joints during extension (McCrea *et al.* 1995; Angel *et al.* 1996). This would amplify the depolarization of motoneurones evoked by activation of Ia afferents as well as increase the Ia receptiveness of different motoneurones. Secondly, inhibition from Golgi tendon organ afferents is replaced by disynaptic excitation which may summate with the Ia effects. Finally group I (Ia & Ib) afferents can regulate the cycle period by impinging on interneurones of the CPG and influence the onset and duration of flexion and extension (Duysens & Pearson, 1980; Conway *et al.* 1987; Pearson *et al.* 1992; Guertin *et al.* 1995 a, b, see also PhD. Thesis of P. Guertin). Group I afferents therefore appear to mediate their effects on extensor motoneurones largely, if not predominantly, through excitatory interneuronal pathways activated during locomotion and in doing so, activate motoneurones that lie outside their restricted "Ia monosynaptic pattern".

In summary, Lundberg's proposition that the functional organization of Ia reflex excitation of extensor motoneurones is to promote weight support and forward propulsion via the monosynaptic reflex should now be revised to include interneuronally mediated Ia and Ib excitation with a far more reaching pattern that affects extensor motoneurones

throughout the hindlimb. The distribution of disynaptic EPSPs in the cat appears to make great sense for maximizing proprioceptive-evoked facilitation of extension during locomotion. The deep lengthening contraction of the ankle extensors (Engberg & Lundberg, 1969; Goslow *et al.* 1973) which is made possible by the cat's digitigrade posture, puts ankle extensor proprioceptors in the appropriate position for transducing ground forces into sensory signals. Accordingly ankle extensor group I afferents evoke the widest distribution of both disynaptic and longer latency EPSPs (Angel *et al.* 1996; Guertin *et al.* 1994). It is of interest that following chronic deafferentation of one hindlimb, cats display a lack of full angular excursion of all of the main hindlimb joints during the extension phase of walking (Wetzel, Attwater, Wait & Stuart, 1976). Although this deficiency is likely due to the absence of group I, group II, joint and cutaneous afferents, the lack of full extension corresponds well with a loss of "group I effects."

The deafferentation experiments raise the issue of the relationship between group I afferents and the spinal central pattern generator. From the standpoint of normal locomotor kinematics, it seems that group I afferents may be considered as an integral component of the 'functional central pattern generator.' That is to say, although the rudimentary rhythm has already been proven to be centrally generated (Graham-Brown, 1914), proprioceptors are probably necessary for the fully developed behaviour of locomotion i.e. normal angular excursions of the joints and hindlimb trajectories on a step-to-step basis (for example see Wetzel *et al.* 1976). The notion that the disynaptic and longer latency group I excitatory pathways are required only during perturbation of locomotion such as uphill walking or during an unexpected loading of the limb is probably inaccurate. Furthermore, it has been suggested that the group I extension enhancement pathways are less excitable in the treadmill preparation than in the fictive locomotor preparation (Pearson, personal communications). This claim stems from observations that the group I stimulus-evoked increase in extensor EMG during treadmill locomotion appears to be smaller than when a similar stimulation is applied during the extensor phase of fictive locomotion. If the interneuronal pathways mediating extension enhancement are being activated on a step to step basis (and thus providing rhythmic depolarization during

"control extensor activity") in the treadmill cat, the effects of electrical stimulation of group I afferents might be occluded causing a smaller stimulus-locked increase in extensor activity. This would make the interneurones producing extension enhancement appear less excitable. In contrast, the absence of rhythmic proprioceptive feedback during fictive locomotion might enable group I afferent stimulation to recruit a larger portion of interneurones mediating group I extension enhancement resulting in a larger stimulus-evoked increase in extensor motoneurones. Thus in fictive locomotion, the extension enhancement pathways might appear more excitable.

These differences in group I stimulus-evoked activity of motoneurones may indeed be different in the two locomotor conditions. However, the differences might not be due to a reduction of activity of the excitatory interneurones in the pathways. Instead the perceived smaller effects in the treadmill cat might result from occlusion of the electrically-evoked activity of interneurones due to the normal recruitment of these interneurones as part of the functional central pattern generator during real locomotion.

Is There a Disynaptic Group I Pathway in Humans?

In humans, Dietz *et al.* (1979) showed that the peak medial gastrocnemius EMG amplitude reaches levels 2-3 times larger during the lengthening contraction of extension than during maximum voluntary isometric contraction. In addition they showed a reduction of EMG in gastrocnemius following ischemic silencing of group I afferents, thus providing evidence that reflexes from Ia afferents provide a considerable amount of excitation to motoneurones during running. The latency of reflex enhancement of the medial gastrocnemius ENG from ground contact was 35-45 ms, and it was concluded that Ia monosynaptic excitation mediated these effects. More recently Yang, Stein & James (1991) have concluded that 30-60% of the force generated in soleus during walking in humans is produced by sensory feedback from Ia afferents. They too attributed these effects to the monosynaptic linkages of Ia afferents. Therefore, as in the cat, it appears that reflexes from group I afferents in humans are also suited to reinforce the extension phase of walking. From experiments using fictive locomotion and treadmill locomotion it has been

established that both group Ia and Ib afferents mediate their effects on motoneurones through excitatory interneuronal system that are activated only during locomotion, in addition to the Ia monosynaptic excitation. Whether locomotor-dependent excitatory interneuronally-mediated reflexes are present during the stance phase of walking or running in human remains to be shown. A Ia homonymous non-monosynaptic excitation of quadriceps motoneurones has been demonstrated in humans which is under cortical control (Fournier, Meunier, Pierrot-Deseilligny & Shindo 1986). This pathway however is believed to be mediated by propriospinal neurones and thus may be a different interneuronal system than the interneurones mediating the locomotor dependent group I disynaptic EPSP in the cat. A study using peristimulus time histograms (PSTH) of single motor units following stimulation of group I afferents from lower limb extensors during walking or running in humans is probably suitable for determining the presence of a human locomotor-dependent group I disynaptic pathway.

Identification of such a pathway in humans might shed some light on possible mechanisms for the pathological extensor posture that can occur following injury to the central nervous system. This disorder of movement can be evoked by eliciting a stretch reflex at a single joint of the lower limb, and can result in prolonged activation of extensor muscles of the ankle, knee and hip joints (Kuhn, 1950). Such a pattern of muscle activation resembles that of locomotor-dependent group I disynaptic excitation in cats (Angel *et al.* 1996). If indeed loss of control of group I disynaptic excitation in humans contributes to exaggerated extensor tone, then identification of the transmitters involved in this disynaptic pathway may have implications on the pharmacological treatment of extensor posture or spasticity.

Candidate Interneurones in Group I Disynaptic Excitatory Pathway

Based on the characterization of disynaptic (McCrea *et al.* 1995; Angel *et al.* 1996) a set of four criteria was developed to predict the activity pattern of interneurones that would be candidates for those mediating group I disynaptic EPSPs. According to McCrea *et al.* (1995) and Angel *et al.* (1996) candidates should be weakly or unresponsive to group

I input at rest. This first proposition assumes that the gating of this new reflex pathway does not solely occur at the interneurones' presynaptic terminals (i.e. presynaptic inhibition of the interneurone). Secondly, candidate interneurones should be antidromically activated from an extensor motor nucleus. Thirdly, candidate interneurones should be more easily activated during the extensor phase of fictive locomotion, and lastly, they should be activated at monosynaptic latencies from the group I incoming volley.

The second section of this thesis describes experiments which used the previously mentioned criteria as predictors for identifying a new class of interneurones. Four of these interneurones were located in the intermediate nucleus (1.8-2.69 mm from the dorsal surface of the cord) in segments L6-S1 (only 1 was found in L6 and the remaining cell in L7-S1) close to their target motoneurones. The paucity of candidate excitatory interneurones in more rostral regions (L6) might indicate a possible caudal displacement of interneurones mediate group I locomotor disynaptic EPSPs relative to the inhibitory laminae V-VI interneurones. Also in contrast to the laminae V-VI inhibitory interneurones, no candidates were antidromically activated from the DLF. A larger sample pool of these interneurones is required before any firm comment can be made concerning their rostrocaudal distribution or projections to ascending tract cells. Further discussion of the control of candidate interneurones by the CPG can be found in the Discussion of Section II. The remaining portion of this discussion will focus on future studies that would disclose the control and operation of interneurones mediating group I disynaptic excitation of extensor motoneurones during locomotion.

Future Studies

The organization of synaptic inputs onto interneurones in the locomotor-dependent group I disynaptic excitatory pathway remains poorly understood. From intracellular records of extensor motoneurones it seems that ankle extensor group I afferents have the strongest input. Four of the interneurones reported in Section II had convergent input from group I afferents of several ankle extensors, however not all nerves were tested for each interneurone during locomotion. No candidate interneurones displayed cutaneous-evoked

discharges. It must be made explicit that the lack of either disynaptic EPSPs in motoneurones or action potentials in candidate interneurones evoked by cutaneous nerve stimulation alone during fictive locomotion does not preclude them from having subthreshold monosynatpic or oligosynaptic effects. As with other pathways, descending rubrospinal (Hongo, Jankowska & Lundberg 1969), or corticospinal (Lundberg & Voorhove 1962) facilitation may very well reveal input patterns that under some experimental conditions remain undisclosed. Preliminary evidence of spatial facilitation of group I disynaptic EPSPs with MLR stimulation, and MLR stimulus-locked discharges of a candidate interneurone suggests that this pathway is subject to facilitation. Thus intracellular recording from candidate interneurones is needed to demonstrate the full pattern of convergence from segmental (cutaneous, joint, group II & III) and supralumbar inputs. Intracellular labelling of excitatory interneurones with HRP will also be essential to examine the projections of individual interneurones and determine if, as with laminae V-VI inhibitory interneurones, subpopulation of cells exist with different axonal projections.

The numerous criteria that were used to make the initial identification of candidate interneurones in the locomotor-related group I disynaptic excitatory pathway highlight the technical difficulties involved in studying these interneurones using the *in vivo* cat preparation. The requirement of eliciting fictive locomotion makes this pathway not condusive to experiments aimed at surveying the convergence patterns from segmental and descending sources, and the germane question arises as to whether the interneurones can be somehow activated in the absence of locomotion. From preliminary studies of candidate interneurones, it appears that cells located in L7 in and around the intermediate nucleus that i) do not project to the DSCT, ii) receive no, or, weak extensor group I input at rest and iii) are antidromically activated from the triceps motor nucleus, have a predictable activity pattern during locomotion, i.e. they are facilitated by extensor group I afferents at monosynaptic latencies. A critical future experiment will be to attempt to activate these candidates with iontophoretic application of a substance (e.g. glutamate) and use the sucrose gap technique and spike triggered averaging to confirm that in fact interneurones

with these characteristics produce excitatory ventral root potentials. If glutamate (or another excitatory substance) is shown to activate these interneurones then controlling the level of excitability of the interneurone with graded amounts of iontophoresis will probably be the best approach to make surveys of convergence from both segmental and descending systems. Another advantage of being able to regulate candidate interneurones' excitability at rest is that it affords the ability to survey different supralumbar sources which may provide inhibitory input. These experiment may involve electrical stimulation of descending tracts or selective lesioning.

What Keeps the Candidate Excitatory Interneurones Tonically Inhibited in the Absence of Locomotion?

One of the remarkable characteristics of extensor group I disynaptic excitation of extensor motoneurones is the extent to which the pathway is inhibited in the absence of locomotion. The pattern of disynaptic EPSPs reported in McCrea *et al.* (1995) and Angel *et al.* (1996) has never been reported or even suggested in the literature. Thus an important route to be taken for future investigation of this pathway is the examination of sources of inhibition of these interneurones in the absence of locomotion.

In accordance with the classic lesion studies (Eccles & Lundberg 1959; Holmqvist & Lundberg 1959) it might be surprising to discover that a tonic descending inhibition is solely responsible for maintaining the interneurones in the group I disynaptic excitatory pathway quiescent since disynaptic EPSPs have never been reported in high or low spinalized non-locomoting cats. In fact, it is the group I non-reciprocal inhibition that is enhanced in the spinal state (Eccles & Lundberg 1959). At present, there is no reason to favour one supralumbar source over another since nothing is known about inhibitory projections to these interneurones, and as such it may be logical to systematically survey different areas of the CNS. The use of strychnine-induced rhythms in the cat may provide insight into possible glycinergic control of the group I disynaptic excitatory pathway. It has been hypothesized that the synchronous neuronal bursting that occurs after application of the glycinergic blocker strychnine might represent the activity of some of the interneurones

of the locomotor rhythm generator, without the reciprocal inhibition between flexion and extension, and without left and right limb alternation (Cowley & Schmidt, 1995). If the group I disynaptic excitatory pathway is activated during intravenous or intrathecally applied, strychnine-induced rhythms two question follow: 1) is the expression of group I disynaptic excitation due to partial activation of the locomotor circuitry? or 2) is the expression of group I disynaptic excitation due to the removal of their tonic glycinergic inhibition? If the relevant interneurones are released from inhibition by strychnine one might expect to regularly observe disynaptic EPSP during the quiescent phase between strychnine-induced bursts. Group I disynaptic EPSPs may however, be larger during extension if the interneurones subserving the disynaptic excitation are rhythmically activated by the CPG (Section II). Nevertheless, if disynaptic EPSPs are routinely evoked by single shocks between the synchronous strychnine bursts, then it may be concluded that the responsible interneurones are under at least some glycinergic control. It may also be possible to titrate strychnine doses that are subthreshold for evoking rhythmicity, and examine the presence disynaptic excitation.

An alternative to a tonic descending (or propriospinal) inhibition as the mechanism inhibiting interneurones in the group I disynaptic excitatory pathway might be the existence of persistent hyperpolarizing conductances in the interneurones, at rest. Several novel persistent potassium conductances with unique pharmacological sensitivities have recently be described. In neonatal rat lumbar motoneurones, a persistent hyperpolarizing outward potassium conductance at resting membrane potentials has been shown to be inhibited by intravenous application of the neuropeptide thyrotropin-releasing hormone (TRH)(Nistri, Fisher & Gernell 1990) and Substance P (Fischer & Nistri, 1993). In adult rat hippocampal pyramidal neurones, a persistent outward potassium conductance has been shown to be inhibited by both the glutamate metabotropic receptor agonist 1*S*-3*R*-ACPD, and the muscarinic receptor agonist methylcholine (Guerineau, Gahwiler & Gerber, 1994). Whether TRH or Substance P, or activation of glutamate metabotropic or muscarinic receptors have any role in gating the activity of interneurones that mediate locomotor-dependent extensor group I disynaptic EPSPs is unknown but is a reasonable, testable

hypothesis. Interestingly, intravenous application of TRH analogue CG3703 in the three-day-old rat pup has been shown to promote a postural shift to the more distal aspects of the dorsum of the paw (Clarke 1992) and initiate locomotion (Clarke & Kirby, 1994) indicating an increase in force output in extensor muscles.

The observation that group I disynaptic EPSPs can be evoked in the high spinal DOPA locomotor preparation (Schomburg & Behrends, 1978) but not in low spinal clonidine locomotor preparation (McCrea *et al.* 1995) suggests that either supralumbar systems disinhibit the responsible interneurones, or alternatively, clonidine prevents their disinhibition. Unpublished observations in the MLR fictive locomotor preparations show that group I disynaptic EPSPs can be evoked in the presence of clonidine, therefore supralumbar, and perhaps cervical propriospinal, systems may play a role in the disinhibition of the group I disynaptic pathway. It is of interest that Cowley & Schmidt (1996) have evidence that critical components for the generation of lumbar locomotor activity are located in supralumbar portions of the spinal cord in the neonatal rat.

Possibilities of the In Vitro Neonatal Rat Preparation

A relatively new, powerful technique for investigating the pharmacological control of locomotion is the *in vitro* isolated neonatal rat brainstem-spinal cord preparation (Smith & Feldman, 1987). In the presence of 5HT, with or without an NMDA receptor agonist, this preparation displays alternating locomotor-like activity in flexor and extensor nerves (Cowley & Schmidt, 1994). The advantage of this preparation over the *in vivo* cat locomotor experiments is that a variety of neuroactive substances can be repeatedly added to the recording chamber in a single experiment and the experimenter has more control of the amount of neurochemicals to which the spinal cord is exposed. Not only does this preparation display locomotor-like activity in its hindlimb nerves similar to the cat, but the effects of trains of stimuli applied to extensor group I nerves also evoke extension enhancement and resetting (Kiehn, Iizuka & Kudo, 1992) that are somewhat similar to that reported in the cat. The reflexes underlying enhanced extension in the neonatal rat are still unknown. If the neonatal rat displays group I disynaptic EPSPs during extension, then the

in vitro preparation may be the technique of choice to examine the pharmacological control of interneurones mediating locomotor-dependent extensor group I excitation. An initial assessment of group I PSPs recorded in motoneurones however, is needed to determine the resting organization of segmental interneuronal pathways from proprioceptors.

Non-reciprocal Inhibition Revisited

It has been established that reflexes from extensor group I afferents to extensor motoneurones are completely reorganized in several fictive (Conway *et al.* 1987, Guertin *et al.* 1995; McCrea *et al.* 1995; Angel *et al.* 1996) and treadmill (see Pearson, 1995) locomotor preparations. Such a massive reorganization demands the conclusion that interneurones mediating reflexes in the absence of locomotion cannot *a priori* be given a role in locomotion. In addition, the initial identification of interneurones involved in locomotion should not be attempted in preparations not displaying locomotion. It seems we are left with the following puzzling question: during what behaviours does the central nervous system utilize the classical group I non-reciprocal pathways? This system which is characterized by substantial segmental and descending control, and mutual inhibition, so far, appears to have no role in reducing level of activity of contracting muscles during locomotion in the cat.

It is unlikely that group I non-reciprocal inhibition represents a vestige of evolution since it exists in humans, and is under multisensory (Pierrot-Deseilligny, Katz & Morin, 1979) and descending (Iles & Pisini 1991; Fournier, Katz & Pierrot-Deseilligny, 1983) control. Preliminary evidence suggests that shows that, as in the cat, group I non-reciprocal inhibition in humans is also reduced during locomotion (Stephens & Yang, 1994). The group I non-reciprocal inhibitory system may be important for movements that demand a significant amount of descending voluntary control such as operating one joint independently of others during posture or exploratory movements. Accordingly, Fournier *et al* (1981) showed that non-reciprocal inhibition in human soleus is reduced during a voluntary soleus contraction, whereas during that same task, non-reciprocal inhibition is increased in quadriceps motoneurones. It may follow that interneurones subserving non-

reciprocal inhibition serve to inhibit muscles that are not relevant to the contraction instead of limiting the excitability of motoneurones that are recruited for the given task, as has been the theory since 1950. This kind of organization exemplifies the flexibility of central mechanisms to select populations of interneurones in a task specific manner such that reflex excitation and inhibition may be directed to the appropriate motoneurones to best execute a movement.

Final Remarks on the Group I System

Group I disynaptic excitation is but one of the locomotor related events that can influence motoneurone depolarization and recruitment. Together with the Ia monosynaptic EPSPs, longer latency locomotor-related group I excitation (Gossard *et al.* 1994, Guertin *et al.* 1994) and expression of motoneuronal intrinsic membrane properties (Brownstone *et al.* 1994; Guertin *et al.* 1995) the central nervous system seems to have several built-in safety factors to ensure sufficient extensor recruitment and weight support during walking. The extent to which interneurones in the disynaptic pathway receive convergent excitatory input from higher centres may indicate their relative importance as last order cells in pathways involved in the initiation of voluntary movements in addition to their segmental role in reflex activation of extensor motoneurones during the stance phase of locomotion. Understanding the pharmacological control of these cells may also have clinical implications if aberrant expression of the group I disynaptic pathway contributes to the exaggerated stretch reflex i.e. spasticity, or the reflex-evoked extensor posture in the lower limb that often occurs after injury to the central nervous system.

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