THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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Depression of Synaptic Transmission from Group I Afferent During Fictive Locomotion and Scratch

BY

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

DOCTOR OF PHILOSOPHY

ABSTRACT

Presynaptic inhibition is a process controlling neurotransmitter release from the synaptic terminals of sensory afferents in the spinal cord. Information about limb muscle length and force affects ongoing movements through activation of spinal reflex circuitry and presynaptic inhibition is an important mechanism for regulating reflex magnitude in the mammalian spinal cord. Early investigations on presynaptic inhibition in anaesthetised cats focussed on the ability of limb sensory afferent activity to evoke a presynaptic reduction in synaptic transmission from the same and other afferents. Intraxonal recordings from group I muscle spindle and tendon organ afferents showed that this sensory evoked presynaptic inhibition was associated with a depolarization of the terminals of sensory afferent fibres. Sensory evoked presynaptic inhibition is now known to be produced by the action of spinal interneurons releasing neurotransmitter on the terminals of sensory afferents. This inhibition can serve to reduce the impact of additional sensory information coming to the spinal cord.

About 15 years ago recordings made during fictive locomotion found rhythmic changes in the membrane potential of group I (and other) sensory afferents during the step cycle. Because these fluctuations occurred during fictive locomotion in the absence of rhythmic sensory input, they were evidence that the central circuitry producing locomotion also exerted a presynaptic control of sensory information transmission during stepping. It was assumed that this centrally evoked, rhythmic, depolarization of sensory afferents would contribute to a phasic modulation of reflex gain.

Results obtained in part during my M.Sc. research provided evidence for a second type of presynaptic inhibition affecting synaptic transmission from group I afferents during locomotion. We found that the monosynaptic excitation of lumbar motoneurons by group Ia muscle spindle afferents was tonically depressed at the onset and continued to be depressed for some time after locomotion. This depression was likely presynaptic in origin and often occurred without any evidence for a rhythmic presynaptic component.

This thesis further characterizes the tonic and rhythmic presynaptic inhibition occurring during locomotion in the adult decerebrate cat. **The three hypotheses addressed are: 1) centrally evoked presynaptic inhibition produces a uniform depression of**

transmission from group I muscle afferents terminating in different regions of the spinal cord, 2) the rhythmic motor behaviours of unilateral hindlimb scratch and quadrupedal locomotion both involve centrally generated presynaptic inhibition of transmission from group I muscle afferents, and 3) the mechanism of centrally-evoked, like that of sensory-evoked, presynaptic depression is strictly associated with a depolarization of sensory afferent terminals.

In the first part of the thesis, 2 microelectrodes were used to simultaneously to record pairs of monosynaptic, group I evoked, field potentials in two areas of the spinal cord during fictive locomotion in decerebrate cats. The results show that although the presynaptic reduction in sensory transmission is on average the same in different areas of the spinal cord, there are often large regional differences in the amount of depression occurring in 2 locations during a bout of locomotion. This suggests that both tonic and phasic centrally-evoked presynaptic depression may result from mechanisms that can be expressed locally. The similarity of the mean field potential depression in the ventral horn and intermediate nucleus suggests that centrally-evoked presynaptic inhibition produces a generalized and tonic reduction of transmission from group I afferents contacting both spinal interneurons and motoneurons.

In the decerebrate cat, fictive locomotion can be evoked by electrical stimulation of the brainstem and fictive scratch can be evoked by topical application of curare on the cervical cord. A comparison of group I field potentials recorded during both locomotion and scratch found a similar presynaptic depression during the two behaviours. This suggests that depression seen during fictive locomotion is not a consequence of brainstem stimulation *per se* but is associated with the operation of the central circuitry that generates rhythmic movements of the limbs. Moreover, the similarity of the presynaptic depression in scratch and locomotion is consistent with the notion that their generation is based on common neuronal circuitry.

Finally, we investigated whether the locomotor related, like the sensory evoked, presynaptic inhibition is a consequence of the depolarization of primary afferents (PAD). The occurrence of PAD in group I afferents was assessed by examining changes in the

effectiveness of intraspinal microstimulation to excite group I afferents. The results show that although group I extracellular field potentials are consistently depressed during locomotion, locomotion produces inconsistent changes in afferent terminal excitability. This is in contrast to the strict association between field potential depression and afferent depolarization seen during sensory-evoked presynaptic inhibition. This suggests that PAD is unlikely to be the primary mechanism underlying centrally produced presynaptic inhibition during fictive locomotion. Consequently, measurements of PAD may not accurately reflect centrally generated presynaptic inhibition. The results of these studies further our understanding of the presynaptic inhibitory processes occurring in the spinal cord during rhythmic behaviour. Hopefully they will lead to a better understanding of the control of reflexes during locomotion.

ACKNOWLEDGEMENTS

Many thanks to all those involved in this project in one way or another over the past $3^{1/2}$ years. In particular:

My supervisor Dave McCrea, without his patience, support and guidance this work would have not been possible.

My PhD committee, Brian Schmidt, Susan Shefchyk, Brent Fedirchuk for timely suggestions and keeping me on track throughout, and my external examiner J-P Gossard for his novel suggestions on my thesis.

All those who provided support in the preparation for, and completion of these experiments, including our technician Sharon McCartney, electronics guru Matt Ellis, programming wizard Gilles Detillieux, and machinist Ken Rodie as well as my lab-mates past and present: Katinka Stecina, Myriam Lafreniere, Samit Chakrabarty, and Jorge Quevedo.

Those fellow graduate students who kept me on the right path, Jason MacLean, Zhiyu Jiang, and Kevin Carlin.

Finally, I'd like to thank my family. My parents Pam and Joe for maintained support through thick and thin. Nadia, James and Lily for providing a getaway in the Ozarks whenever I required some relaxation, and of course, to my wife Amanda, for her unwavering support, encouragement, and devotion to the cause over the past two years. Recognize.

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GENERAL INTRODUCTION

Initiation and control of locomotion.

Walking is a complex task requiring precise coordination of dozens of muscles. One of the first theories to account for the mechanisms underlying locomotion came from Charles Sherrington (1910) who emphasized that proprioceptive reflexes, and in particular, the flexion reflex were organized in a fashion that supported locomotion. However, it was shown by Graham Brown (1914), that locomotor-like activity persisted following transection of the dorsal roots, and thus the removal of proprioception. Graham Brown suggested that a central pattern generator (CPG) existed in the spinal cord that activates and coordinates the muscles involved in locomotion. The presence of a CPG in the mammalian cord has been supported by the ability of monkeys (Fedirchuk *et al.* 1998), rats (Kudo and Yamada, 1987) and cats (Grillner and Zangger, 1974) to produce stepping movements in the absence of afferent input. It is clear, however, that proprioception plays a major role in the development of muscle force and the control of the timing of the flexor and extensor phases during locomotion.

Further support for Graham Brown's locomotor central pattern generator (CPG) hypothesis came from the discovery that, in the absence of both a forebrain and afferent input, stimulation of different regions in the midbrain can cause animals such as cats and rats to walk. This was first demonstrated in the cat by Shik *et al.* (1967). One region, which when activated, can result in locomotion is the mesencephalic locomotor region (MLR). Continuous stimulation of this region in the decerebrate cat results in stable, well-coordinated locomotion on the treadmill. Following neuromuscular blockade, the rhythmic pattern of flexor and extensor efferent activity persists. This is referred to as fictive locomotion.The MLR or its analogue has been shown to produce the motor program for flying in birds (Steeves *et al.* 1987), walking in tetrapods (Shik *et al.* 1966), and swimming in fish and cyclostomes (McClellan and Grillner, 1984).

Because the MLR is defined functionally, it does not necessarily correspond to a single anatomic nucleus. Based on the effective stereotaxic coordinates, Shik *et al.* (1967) suggested that the effective site corresponded to the cuneiform nucleus. Another region which may be part of the MLR is the pedunculopontine nucleus (PPN). The PPN is

anatomically very close to the cuneiform nucleus and it is difficult to differentiate which of these two areas is being activated by electrical stimulation or drug injection (reviewed in Jordan, 1998). Lesions restricted to either the cuneiform nucleus or the PPN often do not result in a locomotor deficit (Shik *et al.* 1968; Sinnamon and Stopford, 1987). In order to eliminate locomotion, lesions within the MLR region must be large and involve parts of both the cuneiform nucleus and the PPN (see Jordan, 1986). Studies using the activity dependant labels c-fos (Brudzynski et al. 1996) and 2-deoxyglucose (Shimamura *et al.* 1987) revealed increased activity mainly in the cuneiform nucleus following MLR-evoked locomotion, suggesting that it may play a dominant role. In addition, a much more robust pattern of chemically induced locomotion is achieved when excitatory amino acids are injected into the cuneiform nucleus as opposed to the PPN (Garcia-Rill *et al.* 1985; Garcia-Rill *et al.* 1990). Thus, the one conclusion that can be safely drawn is that stimulation of the region around and including the cuneiform nucleus can initiate locomotion.

The MLR does not contain neurons that project directly to the spinal cord (Steeves and Jordan, 1984) but it indirectly influences the cord via reticulospinal cells (Orlovsky, 1970). One brainstem area believed to be involved in the pathway for the initiation of locomotion is the medullary reticular formation (MRF) (Garcia-Rill and Skinner, 1987), which is the source of the reticulospinal cells that send locomotor commands to the spinal cord. It has been demonstrated that cooling of the MRF (Shefchyk *et al.* 1984), as well as GABA (Garcia-Rill and Skinner, 1987) or procaine injections (Marlinsky and Voitenko, 1991) into the MRF blocks locomotion induced by stimulation of the MLR, while activation of the MRF neurons with cholinergic agonists and excitatory amino acids results in locomotion in mammals (Garcia-Rill and Skinner, 1987; Noga *et al.* 1988; Kinjo *et al.* 1990). The reticulospinal cells originating from the MRF supply a locomotor command to spinal locomotor systems via the reticulospinal tract descending to the spinal cord via the ventral lateral funiculus (Noga *et al.* 1991). Initially it was assumed that at least part of the ventral lateral funiculus was essential for locomotion since acute lesions prevented MLR-evoked locomotion (Noga *et al.* 1991). However, it has been recently demonstrated that after chronic section of the ventral lateral funiculus cats can walk on a treadmill (Brustein and Rossignol, 1998). Therefore, although the reticulospinal is likely the primary

pathway for MLR-evoked locomotion, the pathways in the dorsolateral white matter must also be capable of transmitting locomotor commands to the spinal cord.

Other descending pathways are likely sufficient for the initiation of locomotion, including descending pathways containing serotonin or noradrenaline. In a series of experiments performed in the 1960's Lundberg and colleagues observed that activation of the noradrenergic system by systemic L-DOPA administration can result in locomotor-like activity (Jankowska *et al.* 1967). In addition, the α-noradrenergic receptor agonist clonidine can induce hindlimb stepping on a treadmill in acutely spinalized cats (Forsberg and Grillner, 1973). The serotonergic system, presumably via the raphespinal tract, is effective for the induction of locomotor activity in the isolated neonatal rat spinal cord (Cazalets *et al.* 1992; Cowley and Schmidt. 1994, see Schmidt and Jordan, 2000). In the neonatal and spinal rabbit preparation (Viala and Buser, 1969), application of serotonin antagonists have been shown to block pharmacologically induced rhythms (MacLean *et al.*, 1998). Interestingly, both DOPA and the serotonin precursor 5-HTP injections modify the locomotor-like bursts from hindlimb flexor and extensor nerves in the rabbit. DOPA injections increase extensor activity while 5-HTP injections increase flexor activity (Viala and Buser, 1969). It has been suggested that the activation of flexors and extensors in locomotion depends upon different neurochemical mechanisms (Viala and Buser, 1969)

The role of afferent input in locomotion.

Although the basic locomotor pattern results from activation of a spinal CPG by descending systems, there is abundant evidence that the step cycle can be modified by afferent input. Shik *et al.* (1966) showed that the mesencephalic cat walking on a treadmill matches its locomotor speed to that of the treadmill. Recently, Hiebert and Pearson (1999) investigated the total contribution of afferent feedback to extensor burst generation by allowing one hindlimb to step into a hole in the treadmill belt on which the animal was walking. The absence of ground support results in a large decrease in sensory feedback to the spinal cord (i.e. activation of the Ib afferents). This caused the level of activity in knee and ankle extensor muscles to fall to ~30% of normal. Thus, they estimated that in decerebrate cats, up to 70% of the force produced during the stance phase of locomotion is due to afferent input in the group Ib pathway (Hiebert and Pearson, 1999). Although other studies

give lower estimates (35% Stein *et al.* 2000; 30% Stephens and Yang 1999), it is accepted that a substantial portion of the force produced during stance is due to afferent input.

In addition to reflexes evoked by group Ib afferents, activity in group Ia muscle spindle afferents can similarly shape the locomotor pattern. The stimulation of extensor group I afferents (both Ia and Ib fibres- Conway *et al.* 1987; Pearson *et al.* 1992; Gossard *et al.* 1994) or just group Ia afferents (e. g. Guertin *et al.* 1995) during locomotion results in the oligosynaptic excitation of extensor motoneurons. In contrast, the same stimulation at rest results in an inhibition (non-reciprocal inhibition see Jankowska 1992) of the same motoneurons. It would appear that during locomotion, as in the case for reflexes evoked in anaesthetised preparations, both Ia and Ib afferents evoke similar reflexes. This justifies using the term "group I" when describing these locomotor dependent excitatory reflexes (McCrea 2001). The reorganization of group I evoked reflexes during locomotion is accomplished by both a suppression of reflexes present at rest and the emergence of locomotor dependent reflexes not elicited at rest. Locomotor dependent group I excitation is due to the recruitment of previously unrecognized types of spinal interneurons (McCrea 2001).

As will be discussed, one of the mechanisms that may underlie reflex suppression during locomotion is a presynaptic depression of synaptic transmission from hindlimb afferents to spinal interneurons (see also Perreault *et al.* 1999). This presynaptic depression may also serve to reduce the enormous amount of afferent input to the lumbar cord during locomotion. It has recently been estimated that 800,000 action potentials per second travel to the spinal cord from muscle afferents in each hindlimb during locomotion (Prochazka and Gorassini, 1998). Perreault *et al.* (1999b) hypothesized that such intense afferent input could produce reflexes that disturb the smooth operation of the CPG. One hypothesis is that in order to avoid disruption of the motor system by intense proprioceptive input, the gain of the Ia monosynaptic, as well as other reflex pathways, needs to be reduced during locomotion. This is supported by studies which have demonstrated that the magnitude of the monosynaptic reflex is decreased during walking as opposed to standing (Capaday and Stein, 1986; 1987). Since this reflex pathway is monosynaptic, its regulation could occur either postsynaptically, at the level of the motoneuron or presynaptically by regulation of

transmitter release from the primary afferent fibres. Since it has been demonstrated that motoneuron excitability is actually increased during locomotion (Brownstone *et al.* 1992; 1994; Krawitz *et al.* 2001; Dai *et al.* 2002) it is likely that at least some of the regulation must take place presynaptically.

The next section provides a brief history of the experiments that led to the discovery of presynaptic inhibition and the theories put forth to explain its mechanism. I will then describe the evidence supporting and refuting these theories, and describe the role of presynaptic inhibition during locomotion.

Mechanisms of presynaptic control

The majority of investigations into the control mechanisms that function at the level of the primary afferent terminal to modulate the transmission of sensory impulses from the periphery has focussed on the type of presynaptic inhibition described originally by Eccles and his co-workers, which will be referred to in this thesis as sensory evoked presynaptic inhibition. Recent work suggests that presynaptic inhibition may be a more complicated process than has been previously assumed, and in fact several different control mechanisms (which will be described below) can be exerted over transmitter release from impulses entering the cord via primary afferent fibres.

The first observations leading to the discovery of presynaptic inhibition of group I afferents were made in 1925 by Ballif *et al.* who observed that long lasting inhibition of the knee jerk reflex resulted when single conditioning shocks were delivered to ipsilateral hindlimb nerves. At the time this was attributed to the release of an inhibitory substance in the spinal cord. In 1938 Barron and Matthews noted that following electrical stimulation of a neighbouring dorsal root, the amplitude of the resulting discharge from the ventral root was decreased. Barron and Matthews also noted the presence of dorsal root potentials (DRPs). DRPs were described as a wave of negative potential recorded in the dorsal root that correlated with the reduction of the ventral root recording. Initially these were attributed to current spread from the discharge of afferents and interneurons nearby (Barron and Matthews, 1938). Twenty years after the discovery of DRPs, Frank and Fourtes (1957) observed that following conditioning stimulation of a flexor nerve, monosynaptic excitatory postsynaptic potentials (EPSPs) in extensor motoneurons were decreased in amplitude

without changes in the membrane properties of the cell. Although at first Frank and Fourtes (1957) suggested that the inhibitory process occurred on the dendrites of the motoneuron and was not "seen" by the recording electrode ('remote inhibition'), it was later concluded (Frank, 1959) that a presynaptic inhibition was responsible for these observations. At first this conclusion was refuted by Eccles who suggested that the EPSP amplitude decrease was due to accommodation (Eccles et al. 1960). However, later experiments by Eccles *et al.* (1961) in which they recorded dorsal root reflexes in group I afferents led them to believe that a presynaptic mechanism may be responsible.

In the late 1950's and early 1960's Eccles and colleagues performed a series of experiments that helped them to formulate a hypothesis regarding the mechanism of sensory evoked presynaptic inhibition. First, Eccles and Krnjevic (1959) recorded intra-axonal potentials from afferent fibres during conditioning stimuli and observed that they were depolarized. Eccles *et al.* (1962) noted that the time course of depolarization of the afferent fibres was coincident with a depression of Ia EPSPs. From these observations, they suggested that presynaptic depolarization is the causal factor in the inhibition responsible for the EPSP depression. The presynaptic depolarization, termed primary afferent depolarization (PAD), was thought to depress the amplitude of the presynaptic spike potential which resulted in reduced transmitter release from the terminal (Eccles *et al.* 1963). This process was thought to be due to a transmitter acting on the presynaptic terminals, via axo-axonic synapses, causing an increased ionic permeability (Eccles *et al.* 1963). The presence of axo-axonic synapses on muscle afferents were confirmed by Gray (1962) using the electron microscope.

The ionic conductances involved in PAD were largely worked out first in the frog spinal cord. Padjen *et al.* (1973) demonstrated that when recording from afferents in the isolated frog spinal cord, there is a reduction of membrane resistance during PAD suggesting increased ionic conductance. They also noted that the PAD was reduced by fibre depolarization and increased by fibre hyperpolarization. Barker and Nichol (1973) demonstrated that the early component of the dorsal root potential (DRP - extracellular recordings of the summed depolarization of several afferent terminals), which is produced by conditioning stimulation, is reduced by exposure to low chloride (Cl^-) Ringer's solution. This

provided evidence that at least part of the PAD is due to a change in Cl^- permeability. The theory that Cl^- permeability plays a role in PAD was strengthened by reports demonstrating that GABA depolarizes primary afferent fibres, since activation of the GABA receptor opens a Cl^- channel (Krnjevic and Schwartz, 1967). In a groundbreaking set of experiments by Eccles *et al.* (1963) it was demonstrated that PAD is not glycinergic as was initially assumed, but GABAergic as evidenced by its blockade by picrotoxin, a GABA antagonist. Picrotoxin is now known to be an antagonist of the $GABA_A$ subtype of receptors.

The current theory as to how PAD causes a decrease in transmitter release is as follows. GABA is released from axo-axonic synapses and activates presynaptic $GABA_A$ receptors that open Cl^- channels. Due to the higher concentration of Cl^- inside the presynaptic terminal relative to outside (Alvarez-Leefmans *et al.* 1988) Cl^- rushes out of the terminal causing a relative depolarization (PAD) that increases conductance in the terminal and shunts incoming action potentials (see Willis, 1999). The diminished action potential results in a decrease in the amount of Ca^{2+} entering the terminal, and therefore a reduction in the amount of transmitter released from the terminal.

Recording techniques used to measure PAD

There are currently 5 methods commonly used to identify and measure PAD. Three of these give a measure of the PAD occurring in populations of afferents while two provide a measure of single fibre PAD. The first measure of population PAD is the dorsal root potential (DRP). The DRP is an extracellular recording, usually from small dorsal root filaments, of the summed depolarization resulting from the passive antidromic propagation of the PAD occurring in the axon terminal. The drawback to this technique is that the recording is made from an arbitrary collection of axons in the dorsal root and it is, therefore, not possible to determine the type of fibre receiving PAD. The dorsal root reflex (DRR) is similar to the DRP except that the recording electrode is placed on a peripheral nerve and records antidromic action potentials. It is therefore possible to determine which cutaneous or muscle afferents are receiving PAD. Only those afferent fibres in which the PAD is large enough to bring the axon to threshold and initiate an action potential will, however, show activity (the dorsal root reflex) recorded in the periphery. The Wall technique (Wall, 1958) is arguably the most effective method for measuring the PAD of a group of afferents. In this

method, a stimulating electrode is inserted into the spinal cord and a fixed amount of current is passed into the extracellular space. An antidromic discharge is recorded in a peripheral nerve of those fibres which were brought above action potential threshold by the intraspinal current. A PAD is indicated by an increase in the size of the peripherally recorded antidromic discharge; i.e. by an increase in the number of afferents excited by the intraspinal current.

The following two methods give a measure of PAD in single fibres. The first of these is the intrafibre recording method (Eccles and Krnjevic, 1959). This technique provides a direct measure of the membrane potentials of single afferents and thus requires the impalement of primary afferent fibres. A major drawback to this technique is that the intra-axonal impalements are made in the dorsal horn since this is the only area in which the primary afferent fibre is large enough for impalement to be successful. This results in an underestimation of PAD amplitude since the recording site is electrically distant from the primary afferent terminal. The continuous excitability test was developed by Madrid *et al.* (1979). This is a modification of the Wall technique in which single fibre PAD is measured. In this technique a window discriminator is used to isolate single afferent antidromic discharges in the peripheral nerve evoked by small intraspinal current injection. The activity of the afferent is fed back into the computer which regulates the amplitude of stimulating current being passed through the intraspinal electrode in order to maintain a constant firing probability. Typically a stimulus strength is chosen that will cause the fibre to fire 50% of the time. In addition to providing a measure of single fibre PAD, this technique allows one to determine the conduction velocity of single afferents in identified peripheral nerves.

Extracellular field potentials as a measure of presynaptic inhibition.

Another method that can be used to assess presynaptic inhibition is an examination of the ability of stimulation or behavioural states to reduce monosynaptic extracellular field potentials. Extracellular field potential recordings provide a measure of the flow of ionic current in the vicinity of the recording electrode. A schematic of a group I extracellular field potential is illustrated in Fig 1A.

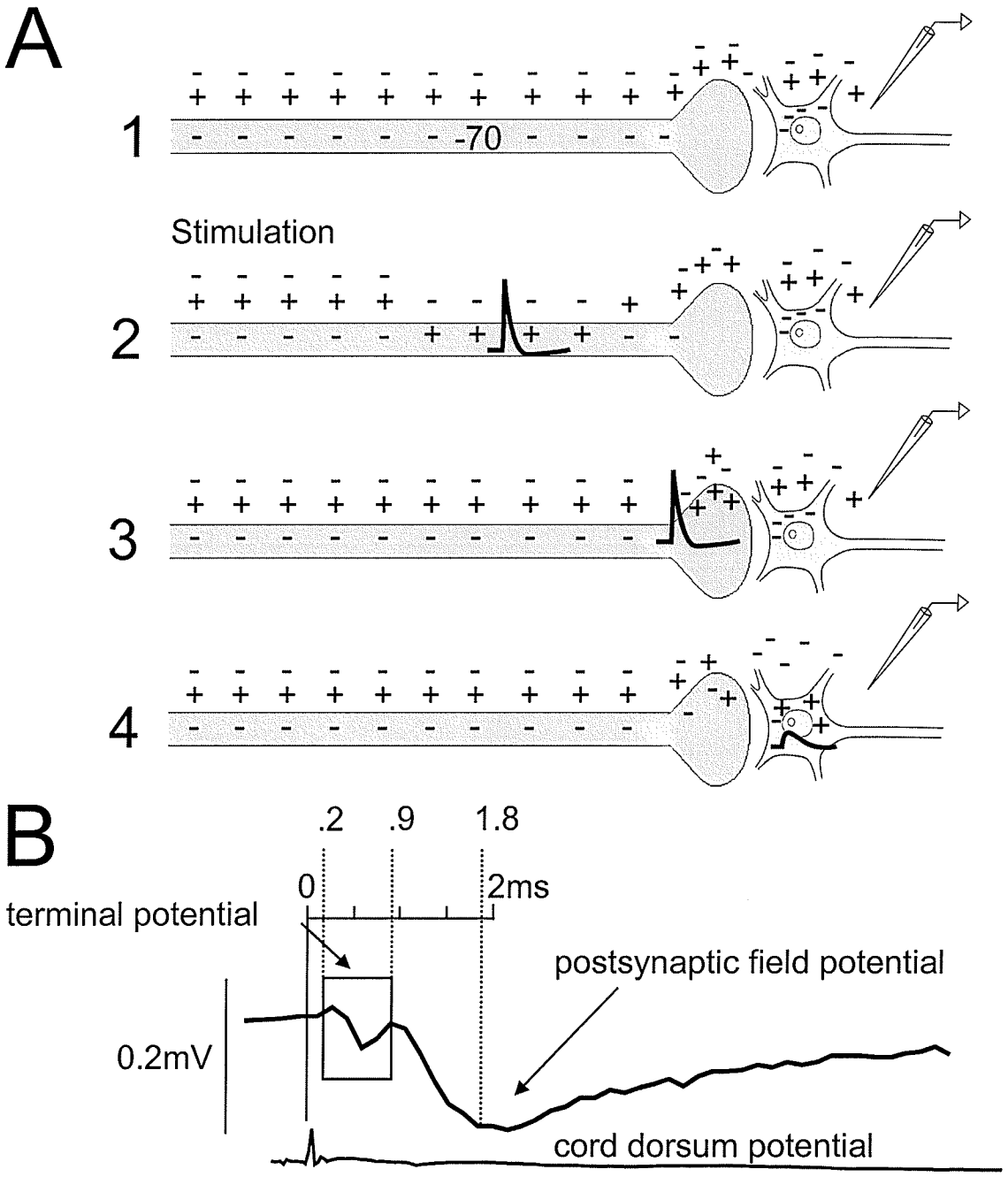

Figure 1. *Illustration of a group I extracellular field potential recording.* A. An action potential travelling towards the afferent terminal initially has no effect on the extracellular field recorded outside the motoneuron (2). As the depolarization gets closer to the terminal it causes an small initial positivity followed by a negativity as charge in the vicinity of extracellular record moves to the site of terminal depolarization and repolarization (3). This produces the terminal potential. After release of excitatory synaptic transmitter postsynaptic depolarization produces a large negative extracellular field (4) B. An extracellular field potential produced by stimulation of muscle afferents and recorded in the ventral horn of the spinal cord.

Panel A illustrates the movement of ions as an action potential approaches the afferent terminal and excites a postsynaptic neuron. Panel B shows the resulting extracellular field potential evoked by the stimulation. This is an actual record of the extracellular field potential recorded in the ventral horn of the spinal cord following stimulation of the extensor digitorum longus (EDL) nerve at 1.4T. In panel A1 the presynaptic afferent and postsynaptic neuron are illustrated at rest. The lack of change in the extracellular current flow, would produce a flat baseline in the field potential recording. In panel A2 the action potential travelling towards the afferent terminal results in a movement of negative ions away from the recording electrode. This produces change in the neutrality in the area of the electrode as reflected initially by a positive deflection recorded by the extracellular electrode. Panel B shows this initial positivity (upward deflection) with a latency of approximately 0.2ms from the arrival of the afferent volley at the cord dorsum. In panel A3 the action potential has reached the afferent terminal which results in the entry of Ca^{2+} into the afferent terminal and thus the movement of positive ions away from the extracellular electrode (negative extracellular potential). Terminal depolarization is thought to reflect the spread of depolarizing current into the presynaptic boutons in the region of the microelectrode tip (see Sypert *et al.* 1980). These initial positive and negative deflections collectively make up the presynaptic terminal potential (enclosed area in Fig. 1B).

In panel A4 release of synaptic transmitter and opening of postsynaptic channels depolarizes the motoneuron. The entry of positive charge into the postsynaptic cell leaves a relative abundance of negative charge outside and a large negative deflection in the extracellular recording. The resulting negative field potential deflection is shown by the vertical arrow in panel B with a latency of approximately 0.6ms. This is the focal synaptic potential which in this case has an onset of about 0.9 ms (i.e. is monosynaptic) and peak deflection of 0.23 mV about 1.8ms after the arrival of the afferent volley at the cord dorsum. Since the recording electrode is located outside the vicinity of several postsynaptic neurons, the negative extracellular field potential recording is a measure of the population depolarization of a group of neurons close to the recording electrode.

Figure 1 panel B illustrates a group I extracellular field potential and cord dorsum

potential evoked by EDL nerve stimulation (1.4x threshold) recorded in the experiments described in paper #1 of this thesis. The onset of the terminal potential occurs at.2ms after the arrival of the afferent volley at the cord dorsum. Measurements of monosynaptic group I field potentials similar to this will be used to asses centrally-evoked presynaptic inhibition in both papers included in this thesis.

Organization of PAD

Eccles and Krnjevic (1959) used intrafibre recordings to demonstrate that not all group I afferent fibres display the same pattern of PAD in response to segmental conditioning stimulation. It has since been demonstrated that different group I fibres display different patterns of PAD in response to conditioning stimulation from cutaneous muscle and descending fibres (see Rudomin and Schmidt, 1999). For example, group Ia afferents are depolarized by conditioning stimulation in flexor nerves but not extensor nerves, while Ib afferents are depolarized by conditioning stimuli in both flexor and extensor nerves (Eccles *et al.* 1962). Another difference is that Ia fibres are depolarized by conditioning volleys in both Ia and Ib afferents, while Ib fibres are only depolarized by volleys in Ib afferents (Eccles et al. 1961). Studies by Rudomin *et al.* (1983, 1986), using the continuous excitability method and Jimenez *et al.* (1988), using the intrafibre method, demonstrated that descending input can also result in a depolarization of group I fibres which is expressed differently in various fibre types. Stimulation of the vestibular nuclei has been shown to produce PAD in Ia afferents, while stimulation in the bulbar reticular formation, the red nucleus and the pyramidal tract did not produce PAD but rather inhibited PAD produced by group I and vestibulospinal fibres. In contrast, Ib fibres were depolarized following stimulation of rubrospinal, reticulospinal and corticospinal fibres. Cutaneous fibre stimulation has been shown to cause PAD in some Ib fibres and inhibit it in others.

Interneurons mediating PAD

Patterns of PAD suggest the existence of several groups of PAD producing interneurons differing in their segmental input and in fibres with which they form axo-axonic contacts. Although there is still much to be learned there are some indications of the location of the interneurons responsible for releasing the transmitter onto afferent terminals thought to mediate PAD. Eccles *et al.* (1962) recorded from a group of interneurons located at the base

of the dorsal horn which they referred to as D cells. The discharge patterns and input convergence of these cells were consistent with their involvement in the PAD of muscle and cutaneous afferent terminals. More recently, it has been demonstrated that microstimulation within the intermediate zone (lamina V,VI) of the spinal cord produces DRPs and a monosynaptic PAD of group I afferents (Jankowska *et al.* 1981) suggesting that this is the probable site of the interneurons responsible for the PAD of group I afferents. Another group of interneurons which are located in the dorsal horn of the sacral cord are likely responsible for the PAD of group II afferents in the lumbar cord (Jankowska and Riddell, 1995). The Rudomin lab has used spike triggered averaging of DRPs and ventral root potentials (VRPs) to reveal connections of suspected PAD interneurons with afferent fibres and motoneurons. They found two groups of interneurons that responded to stimulation of group I muscle afferents. Activity in class I interneurons is time locked to inhibitory VRPs and likely mediate non-reciprocal inhibition (Rudomin *et al.* 1987) and class II interneurons that mediate long lasting VRPs and DRPs (Solodkin *et al.* 1984). This latter group is thought to mediate the sensory-evoked PAD of group Ib and possibly group Ia afferents (Enriquez *et al.* 1996).

Local control of PAD

Eguibar *et al.* (1994; 1997) used the continuous excitability test to investigate the characteristics of the PAD recorded simultaneously in two terminals of the same group I afferent. These experiments sought to determine whether different collaterals of the same afferent are subject to different amounts of PAD depending on their input and termination. To do this, they investigated the effect of conditioning stimulation from the cerebral cortex on the PAD evoked in pairs of intraspinal collaterals (two simultaneous excitability tests) of the same afferents ending in the intermediate nucleus. They found that conditioning stimulation of the cortex results in a differential PAD between the two collaterals, the degree of which could be varied by altering either the stimulus strength or stimulation site in the motor cortex. A differential control of segmentally evoked PAD has also been demonstrated in pairs of collaterals of single muscle spindles (Lomeli *et al.* 1998). It was shown that the differential PAD could be removed during cold conduction block of the thoracic spinal cord suggesting that descending mechanisms play a role in the differential expression of PAD

(Lomeli *et al.* 1998). The observations from both of these studies provide support for the theory that there is a focal control of PAD in selected intraspinal arborizations of muscle afferents that regulates the monosynaptic activation of selected neuronal targets by segmental afferents. The likely explanation is that PAD of group I afferents is evoked by spinal interneurons located near to the group I terminals to which they project. Descending (and segmental) systems could regulate the excitability of individual PAD producing interneurons and hence their actions on afferent terminals.

Relationship between PAD and presynaptic inhibition.

Presynaptic inhibition has been defined by Eccles and colleagues as the depression of EPSPs unaccompanied by a concomitant change in postsynaptic excitability (Eccles, 1964). Due to the close correspondence between the time course of the PAD and EPSP depression and the similar pharmacological responsiveness of the two phenomenon, depolarization of the afferent terminal was implicated as the mechanism causing presynaptic inhibition (Eccles *et al.,* 1963). This has not been tested directly in mammals because the small size of the primary afferent terminal precludes intracellular recording. There is, however, strong circumstantial evidence supporting this hypothesis. Specifically, in the squid giant axon there is a steep relationship between terminal action potential size and transmitter release (Takeuchi and Takeuchi, 1962; Katz and Miledi, 1967). Based on this evidence, the assumption was made that postsynaptic EPSP amplitude is related to the presynaptic action potential amplitude, which has been shown to be decreased following depolarization of afferents (Eccles *et al.* 1962). Also, Eccles and Krnjevic (1959) demonstrated that artificial depolarization of a dorsal root resulted in a decreased primary afferent spike and EPSP amplitude while artificial hyperpolarization resulted in an increased primary afferent spike and EPSP amplitude. Recently, this hypothesis has been strengthened by the observations of Cattaert *et al.* (1992) who impaled pairs of primary afferent terminals and their corresponding motoneuron in the crayfish and demonstrated that PAD elicited by conditioning stimuli drastically reduced the amplitude of the EPSP. The size of the depolarization evoked in the afferent terminal was inversely related to the size of the evoked EPSP.

How PAD effects transmitter release has been debated for the past 30 years. Numerous modelling studies (Segev, 1990; Graham and Redman, 1994; Lamotte D'Incamps *et al.* 1998) have investigated whether it is possible that such small depolarizations in the afferent terminal can account for such large decreases in transmitter release. The consensus of these studies is that while the shunt of the terminal action potential may decrease spike height, very large synaptic conductances are required to induce a significant reduction of the action potential. Therefore, it has been suggested that while PAD may not be the sole mechanism underlying sensory-evoked presynaptic inhibition, the two processes are closely associated.

Presynaptic inhibition during locomotion

Up to this point our discussion of presynaptic inhibition, and specifically PAD, has referred exclusively to sensory evoked presynaptic inhibition evoked from the periphery. As was previously mentioned, Perreault *et al.* (1999b) raised the possibility that during movement, and especially during more complex movements like locomotion, the central nervous system is exposed to a bombardment of afferent input that may put a strain on its overall information processing ability. In support of this, it has been demonstrated in man (Capaday and Stein, 1986; Faist *et al.* 1996; Andersen and Sinkjaer, 1999) and in cat (Bennett *et al.* 1996) that there is a decrease in transmission in the group I monosynaptic pathway during locomotion. This is likely due to a centrally evoked presynaptic inhibition of afferent information by the locomotor central pattern generator. In the following section I will discuss the possible mechanisms which result in this centrally evoked presynaptic inhibition.

PAD has been shown to be closely associated with sensory evoked presynaptic inhibition and, as was previously mentioned, PAD has been shown to occur in the absence of sensory stimulation during locomotion. This was first demonstrated by Bayev (1978) in a series of experiments in which he observed fluctuating DRPs within the step cycle. PAD has also been observed during locomotion in the crayfish (Cattaert *et al.* 1992; 1994), locust (Burrows and Laurent, 1993; Burrows and Matheson, 1994), and lamprey (El Manira *et al.* 1997). In the cat, it was determined that Ia and Ib afferents of both flexor and extensor muscles were more excitable (and thus more depolarized) during the flexor phase than during

the extensor phase of the step cycle (Baev, 1980; Bayev and Kostyuk, 1982). In addition, Duenas and Rudomin (1988) observed that the amount of current required to fire single group I afferents was reduced during the flexion phase, suggesting the presence of a PAD. In addition, they showed that there is a tonic increase of afferent fibre excitability throughout locomotion. There is some debate regarding this latter finding since Bayev and Kostyuk (1982) reported a tonic hyperpolarization of group I afferents during locomotion. However neither of these findings has ever been corroborated.

Intra-axonal recordings during fictive locomotion have demonstrated that hindlimb muscle afferents are depolarized twice per step cycle, with the maximum depolarization in the flexor phase and a smaller depolarization in the extensor phase (Dubuc *et al.* 1988; Gossard *et al.* 1991; Gossard 1996). The same pattern is also seen in cutaneous fibres (Gossard *et al.* 1989). Due to the previous findings demonstrating an association between PAD and sensory evoked presynaptic inhibition, the presence of a PAD during locomotion has been interpreted to suggest that there is a presynaptic inhibition during locomotion that is larger during flexion (e.g. Rossignol, 1996).

In 1996 Gossard performed the only experiment that could prove that PAD recorded in the cat was a reliable reflection of presynaptic inhibition. He simultaneously impaled group Ia primary afferent fibres and their motoneuron targets in order to observe if the maximal PAD occurred at the same time as the minimal EPSP amplitude in the motoneuron. Due to the extreme difficulty of this experiment, he was only able to record from 6 pairs of afferents and motoneurons. In all 6 afferents the maximal depolarization occurred in the flexor phase, as had been previously found. However, in 4 of the 6 cases, the EPSP was larger in flexion (i.e. the 'wrong' phase). Gossard has since suggested that "locomotor-related PADs do not contribute importantly to monosynaptic reflex modulation during fictive locomotion" (see Menard *et al.* 1999).

Despite the dissociation between PAD and monosynaptic Ia EPSP amplitude, the fact remains that there is an inhibition of sensory information during movement and more specifically during locomotion. As was previously mentioned, numerous studies have demonstrated that the amplitude of the monosynaptic H-reflex is decreased during locomotion when compared to that measured when the subject is standing (Capaday and

Stein, 1986; Faist *et al.* 1996; Andersen and Sinkjaer, 1999. Also Bennett *et al.* (1996) observed that the gain of the stretch reflex in the cat is decreased during locomotion. This phenomenon has recently been investigated in a set of studies in the McCrea lab.

Perreault *et al.* (1999b) demonstrated that monosynaptic group I field potentials in the intermediate nucleus are tonically decreased by a mean of 20% during fictive locomotion. In addition to this tonic reduction, there were cyclic variations between the flexor and extensor phases, but these were not consistently larger in either phase. After locomotion the field potentials remained depressed for up to 2 minutes. Since field potentials reflect transmembrane currents resulting from synaptic transmission between afferents and their target neurones, field potential depression likely indicates a presynaptic inhibition of transmission from Ia afferents in these regions during locomotion. Gosgnach *et al.* (2000) demonstrated that group Ia monosynaptic EPSPs and field potentials in the ventral horn are tonically reduced, by 30% and 28% respectively, during fictive locomotion. Similar to the observations of Perreault *et al.* (1999b) EPSPs and field potentials in the ventral horn sometimes had small cyclic variations superimposed on the tonic depression. These were not consistently larger in either phase. EPSP depression did not recover to control values until minutes after the end of locomotion. In addition, the Gosgnach *et al.* (2000) study investigated whether the depression of monosynaptic EPSPs during locomotion could be due to a postsynaptic increase in motoneuron membrane resistance. Although, on average there was a decrease in motoneuron membrane resistance during locomotion, it appeared to be too small to account for the amount of EPSP depression observed (see Redman and Walmsley, 1983; McCrea *et al.* 1990). Due to the long time course of recovery of group I EPSPs and field potentials, as well as the lack of a consistent phase of locomotion in which the EPSP is larger, it was suggested (Gosgnach *et al.* 2000) that this presynaptic inhibition may be dissociated from the locomotor related PAD described above.

Mechanisms of presynaptic inhibition other than PAD

Although the majority of the work investigating presynaptic inhibition has focussed on $GABA_A$ receptor mediated PAD, this is not the only mechanism that results in presynaptic inhibition of group I fibres in the spinal cord. Five other mechanisms that may result in presynaptic inhibition are discussed below. It should be noted that virtually all of

this evidence was obtained during conditioning stimulation of segmental afferents or descending systems and not during locomotion.

1) The activation of descending monoaminergic pathways results in the release of serotonin (5-HT) and noradraneline (NA) which are thought to have presynaptic actions on group I fibres in the lumbar cord. It has been demonstrated that stimulation in the 5-HT rich raphe magnus area of the medulla evokes long lasting DRPs in group I afferents of the lumbar cord which are reduced by administration of 5-HT antagonists (Proudfit and Anderson, 1974). However iontophoretic application of 5-HT and NA into the intermediate nucleus of the cord has no effect on the amplitude of group I field potentials (Bras *et al.* 1990). In addition, Noga *et al.* (1992) demonstrated that stimulation of the locus coeruleus and subcoeruleus results in a depression of group I field potentials in the lumbar cord by a mean of 20%. More recent studies have demonstrated, however, that locally applied 5-HT and NA have little effect on synaptic actions from group I afferents (Jankowska *et al.* 2000) and appear to facilitate transmission from group II afferents in certain area. Although the effect of monoamine release in the cord is complex, it is clear that monoamines can presynaptically affect transmitter release from group I afferents. Since fictive locomotion occurs due to stimulation of the MLR and activation of descending monoaminergic systems (see Grillner *et al.* 1998), it is possible that monoamines are involved in modulating group I synaptic transmission in the lumbar cord.

2) Activation of $GABA_B$ receptors on primary afferent terminals is another mechanism that may be responsible for the centrally generated presynaptic inhibition during locomotion. From the PAD studies it is assumed that GABA released in the vicinity of the primary afferent terminal from interneurons activated by the conditioning stimulation reduces group I synaptic transmission. It has also been shown that, in addition to $GABA_A$, $GABA_B$ receptors are located on primary afferent terminals in the spinal cord (Price *et al.* 1984). The systemic administration of $GABA_B$ receptor agonist baclofen, increases the threshold for evoking the stretch reflex (Capaday, 1995) and in cat (Jimenez *et al.* 1991) reduces monosynaptic EPSP amplitude the bullfrog (Peng and Frank, 1989). Activation of $GABA_B$ receptors have since been shown to cause a presynaptic inhibition following conditioning stimuli in the absence of a PAD. A series of experiments by Curtis and colleagues demonstrated that the

presynaptic effect of the $GABA_B$ agonist baclofen in the spinal cord is associated with a reduction of the duration of presynaptic action potentials in the absence of afferent fibre depolarization (Curtis *et al.* 1981; 1997; Curtis and Lacey, 1998; Lacey and Curtis, 1994).

The proposed mechanism of $GABA_B$ receptor mediated presynaptic inhibition is as follows. GABA, released from axoaxonic synapses binds to $GABA_B$ receptors and causes a reduction in the duration of the presynaptic action potential without a depolarization. The reduction of action potential duration is thought to be due to reduced inward Ca^{2+} currents through the N and P/Q type Ca^{2+} channels (see Rudomin, 1994). When an action potential invades the afferent terminal, the reduced Ca^{2+} entry decreases neurotransmitter release and thus decreases the amplitude of the postsynaptic EPSP. Since GABA released from axo-axonic terminals binds to both $GABA_A$ and $GABA_B$ receptors, it is possible that the action of both $GABA_A$ and $GABA_B$ receptors may be the cause of the presynaptic inhibition during locomotion. Alford and Grillner (1991) found that $GABA_B$ receptor activation results in the activation of a G-protein coupled second messenger cascade which causes the presynaptic inhibition (see also Takahashi *et al.* 1998). Both the Perreault *et al.* (1999b) and Gosgnach *et al.* (2000) studies demonstrated that the presynaptic inhibition of the group I field potential or EPSP persists for a period of up to minutes after cessation of locomotion. This is consistent with a long-lasting G-protein events occurring in the terminal of Ia afferents as has been suggested for other synapses. $GABA_B$ mediated presynaptic inhibition is one mechanism by which a decreased release of neurotransmitter from afferent fibres could occur in the absence of PAD.

3) It is possible that a non-specific liberation of K^+ ions into the extracellular area surrounding the primary afferent terminal results in depolarization of the terminals by a mechanism other than $GABA_A$ mediated PAD (Barron and Matthews, 1935; Eccles and Malcom, 1946). Accumulation of extracellular potassium is caused by the repetitive activation of interneurons near primary afferent terminals (Krnjevic & Morris, 1974). K+ accumulates outside the cell due to an outward movement of K^+ during the action potential. This buildup of K^+ in a restricted area surrounding the axon could result in the depolarization of nearby axons simply by altering the K^+ outside/K^+ inside ratio. K^+ accumulation has been shown to result in a depolarization comparable in amplitude to DRPs in the neonatal rat

(Kremer and Lev-Tov, 1998). It is unlikely, however, that an increase in extracellular K^+ is the mechanism responsible for a presynaptic inhibition of group I afferents since previous studies have demonstrated a clear dissociation between extracellular K^+ accumulation and both sensory evoked (Jimenez *et al.* 1984) and centrally evoked (Gosgnach *et al.* 2000) presynaptic inhibition.

4) Branch point failure is a mechanism intrinsic to the primary afferent that can result in presynaptic inhibition of transmitter release. In the vertebrate CNS Ia afferent fibres have many axon collaterals projecting to a wide variety of locations (Hongo *et al.* 1978). A single Ia axon has many arborizations giving off synapses to many neurons making synaptic contact on nearly all of the homonymous motoneurons of a given motor pool and on many close synergists (Munson and Sypert 1979). It is frequently assumed that an action potential is propagated along an axon into all of its synaptic terminals. This is not necessarily the case. If the action potential in the stem axon does not invade all of the collaterals, certain synapses or even groups of synapses will remain silent, a form of presynaptic inhibition. Such branch point failure can occur due to geometric irregularities between the stem axon and daughter axons (if the diameter of a collateral is larger than the stem axon the action potential is less likely to travel down this collateral), or differences in the excitability of different regions of the axon (see Luscher 1998). It is unknown whether branch point failure is a mechanism exploited by the nervous system to systematically regulate transmitter release during movement.

5) Although dorsal root reflexes are a result of depolarization of the primary afferent, they can result in a presynaptic inhibition by a mechanism other than that commonly associated with PAD. As was previously mentioned, the dorsal root reflex is an action potential recorded in the peripheral nerve following a suprathreshold PAD. To be recorded in the peripheral nerve the depolarization must propagate antidromically to the peripheral nerve. While travelling to the periphery the depolarization will collide out any orothodromic action potentials and thus result in another mechanism of presynaptic filtering of sensory information. In addition to propagating antidromically, it is interesting to note that depolarization of the primary afferent terminal also travels in the orthograde direction. If the depolarization is large enough, it could theoretically result in an EPSP in the postsynaptic

neuron. If this were the case PAD would be a mechanism whereby postsynaptic excitation occurred. In other words, primary afferent terminals could act as last order "interneurons" for systems evoking PAD. Although possible, this does not seem to occur. Cattaert *et al.* (2002) impaled motoneurons and their afferent fibres during fictive locomotion in crayfish. They observed that depolarizing spikes in the afferent fibre were without coinciding EPSPs in the motoneuron.

Due to the association between PAD and presynaptic inhibition it is commonly assumed that presynaptic inhibition is due to the shunting of the incoming action potential due to increased terminal conductance. The preceding 5 mechanisms described provide other means by which a presynaptic inhibition may occur. Any of these could contribute to the centrally generated presynaptic inhibition observed during locomotion.

The need for an alternate preparation to assess centrally evoked presynaptic inhibition.

In anesthetized cats, stimulation of brainstem areas involved in the initiation of locomotion have a large presynaptic depressive effect on group II afferents and also depresses lumbar group I field potentials by an average of 20% (Noga *et al.* 1995). This presynaptic inhibition is thought to be exerted on the lumbar cord via reticulospinal tract activation. Thus, areas that evoke locomotion in decerebrate cats can depress group I field potentials in anesthetized cats in the absence of locomotion. From this work one of two conclusions can be reached. It is possible that there is an area in the region of the cuneiform nucleus which can produce a presynaptic inhibition of group I and group II fibres independent of the activation of the locomotor circuitry. The second, and more likely conclusion is that activation of the central circuitry producing locomotion by MLR stimulation results in presynaptic inhibition of group I and group II fibres as part of the locomotor program that is only partially activated in anaesthetised preparations.

If the first explanation is correct, it is possible that the 30% depression of the monosynaptic EPSP observed during brainstem evoked fictive locomotion (Gosgnach *et al.* 2000) is mainly due to stimulation of the cuneiform nucleus and a depressive action of the reticulospinal tract on group I transmission rather than a presynaptic inhibition resulting from locomotor network activity. This alternate argument, that central circuitry producing locomotor activity causes the presynaptic inhibition, would be strengthened if a similar

presynaptic depression could be obtained in a rhythmic, alternating behaviour in which components of the same circuitry are activated in the absence of MLR stimulation.

The scratch reflex is a behaviour that fits this description. It is a site-specific motor response to tactile stimulation on the body surface of mammals, reptiles and birds in which a limb will reach and rhythmically rub against the stimulated site to remove the irritating object from the skin. In the cat the receptive field from which the scratch reflex can be evoked involves only the head and neck (Deliagina *et al.* 1975). The scratch reflex can be evoked while the animal is lying, sitting or standing and is divided into 3 components: approach, cyclic and return (see Kuhta and Smith, 1990). In the approach phase the hindlimb ipsilateral to the stimulated ear is lifted to the head by the activation of hip and ankle flexors and knee extensors. During the cyclic period there is a rhythmic alternation of flexor and extensor muscles as the paw follows a circular path at a frequency of 4-8Hz. The cyclic period can be divided into: 1) the pre-contact phase which constitutes 26% of the scratch cycle, is initiated by knee extension followed by activation of ankle extension as the paw is moved towards the ear, 2) the contact phase which occupies 50% of the cycle and is marked by hip knee and ankle extension and 3) the post-contact phase which accounts for 24% of the cycle, is initiated by knee flexor then ankle and hip flexor activity (Kuhta and Smith, 1990). The return phase is simply the reverse of the approach phase as the hindlimb returns to the ground (Kuhta and Smith, 1990). Fictive scratch can be evoked in the decerebrate animal by applying curare or strychnine to the dorsal surface of the cervical spinal cord (see Perreault et al. 1999a). This suggests that propriospinal neurons are under inhibitory influence and these influences must be decreased in order to evoke scratch.

Although both scratch and locomotion involve rhythmic alternation of flexor and extensor nerves, there are some differences between the two behaviours. These include a much faster rate of rhythmic activity during scratch and a pre-scratch preparatory phase consisting of no extensor activity and tonic ipsilateral flexion (see Gelfand *et al.* 1988; Kuhta and Smith, 1990). Some minor differences in the distribution of cells expressing c-fos during fictive locomotion and scratch have been noted (Barajon *et al.* 1992). Despite these differences there are some striking similarities between scratch and locomotion. These include similar muscle groups associated with flexion and extension phases of both rhythmic

behaviours (see Gelfand *et al.* 1988), the presence of disynaptic group I EPSPs evoked in extensor motoneurons during both behaviours (Perreault *et al.* 1999a), a decrease in motoneuron membrane resistance in both behaviours (Perreault 2002), and similar activities of interneurons during both behaviours (Bayev and Kostyuk 1981).

These studies provide evidence that the central circuitry for scratch and locomotion share some elements. Since fictive scratch does not involve electrical brainstem stimulation this is an excellent model to determine whether presynaptic inhibition during rhythmic alternating activity, such as locomotion, is a component of activation of the central circuitry underlying locomotion.

Overview of the thesis

A need to control the intense bombardment of afferent input during locomotion has been postulated. There is abundant evidence for a centrally-evoked presynaptic inhibition and PAD during locomotion. Based mainly on the analogy to sensory-evoked presynaptic inhibition it has been assumed that this PAD is the mechanism accounting for the presynaptic inhibition. Recent experiments from our lab suggest that these two processes may be dissociated (Perreault *et al.* 1999b; Gosgnach *et al.* 2000). In this thesis I will use the decerebrate cat preparation to further characterize the tonic and phasic presynaptic inhibition that occurs during fictive locomotion.

The thesis is divided into two parts. In the first part, I will examine whether the presynaptic inhibition is expressed differentially in different lumbar spinal regions during MLR-evoked fictive locomotion. Simultaneous recordings of monosynaptic group I evoked field potentials will be made using 2 microelectrodes positioned in different areas of the spinal cord during rhythmic activity. These experiments address the question of whether centrally evoked presynaptic inhibition is expressed uniformly in different spinal regions and test **hypothesis #1, that centrally evoked presynaptic inhibition produces a uniform depression of transmission from muscle afferents terminating in different regions of the spinal cord**. In the same section I will compare changes in group I field potentials during MLR evoked fictive locomotion and fictive scratch evoked by topical application of curare. These results will address the question of whether the presynaptic inhibition observed during MLR evoked fictive locomotion is an epiphenomenon of MLR stimulation or a component

of centrally generated rhythmic motor behaviour and test **hypothesis #2, that the rhythmic motor behaviours of unilateral hindlimb scratch and quadrupedal locomotion both involve centrally generated presynaptic inhibition of transmission from group I muscle afferents**. In the second part of the thesis, I will attempt to determine the underlying mechanism of the presynaptic inhibition, by investigating whether the centrally-evoked presynaptic inhibition occurring during locomotion and scratch is strongly associated with a primary afferent depolarization (PAD). I will test **the third hypothesis, that the mechanism of centrally-evoked, like that of sensory-evoked, presynaptic depression is strictly associated with a depolarization of sensory afferent terminals.** While it is clear that sensory-evoked PAD is strongly associated with presynaptic inhibition in non-locomoting preparations, there is mounting evidence that this does not hold true during fictive locomotion (Gossard 1996; Perreault *et al.* 1999b; Gosgnach *et al.* 2000). The results of these studies will further our understanding of the presynaptic inhibitory processes occurring in the spinal cord during rhythmic behaviour and allow us to better understand the control of reflexes during locomotion.

PAPER # 1

Local expression of group I field potential depression during fictive locomotion in the decerebrate cat.

SUMMARY

In decerebrate cats, fictive locomotion produced by electrical stimulation of the midbrain reduces the amplitude of monosynaptic field potentials evoked in the lumbar spinal cord by stimulation of hindlimb group I muscle afferents. The effects of fictive locomotion on pairs of group I fields were examined to determine whether this centrally-evoked presynaptic inhibition is expressed uniformly within intermediate and ventral regions of the lumbar spinal cord. Fictive scratch was also evoked to assess the depression of monosynaptic field potentials and EPSPs recorded in lumbar motoneurons during a rhythmic motor behaviour without MLR stimulation.

The majority of the group I field potentials (124/149) examined were depressed during fictive locomotion. Although the mean depression of field potentials recorded in the intermediate nucleus (27%) and ventral horn (29%) was similar, in many cases the depressive effects of fictive locomotion were larger on one field than the other. Dissimilar depressions were also seen for pairs of fields recorded within the intermediate areas or within the ventral horn. Further evidence for local differences in the expression of centrally-evoked presynaptic inhibition was the differential depression of two group I fields recorded in close apposition, the differing time courses for the recovery from depression of fields recorded in different locations, and the differences in the locomotor phase in which maximum field potential depression occurred. The depressive effects of fictive scratch on monosynaptic field potentials and EPSPs were similar to those occurring during fictive locomotion. This suggests that the presynaptic depression observed during fictive locomotion is due to operation of the central rhythm generating circuitry and not MLR stimulation *per se*.

The present results are consistent with the hypothesis that the centrally generated control of synaptic transmission from group I afferents during locomotion and scratch is produced through actions of interneurons acting locally within circumscribed areas of the spinal cord. In turn, this organization is consistent with that suggested to exist for presynaptic inhibition evoked by stimulation of hindlimb sensory nerves.

INTRODUCTION

In the cat, conditioning stimulation of a hindlimb muscle nerve can depress the amplitude of monosynaptic EPSPs evoked by activation of muscle spindle primary (group Ia) and golgi tendon organ (group Ib) afferents through presynaptic mechanisms (see Willis 1999). The time course of this sensory evoked presynaptic inhibition of monosynaptic group Ia and Ib EPSPs is similar to that of the associated depolarization of group I afferent terminals (Eccles *et al.* 1962). This depolarization, termed primary afferent depolarization (PAD), is thought to depress the amplitude of the presynaptic spike potential and thereby reduce transmitter release from the terminal (Eccles *et al.* 1963).

Sensory evoked primary afferent depolarization (PAD) elicited by conditioning stimulation of hindlimb nerves can be of different magnitudes in collaterals of the same cutaneous (Eguibar *et al.* 1997) or muscle spindle afferent (Lomeli *et al.* 1998) within the lumbar spinal cord. Blocking descending fibres by cooling the thoracic spinal cord reduces differences in the amount of PAD evoked in collaterals of the same afferent. This suggests that descending systems regulate the PAD of different terminals via actions on interneurons that act locally within the lumbar spinal cord (Lomeli *et al.* 1998). The observation that PAD of group I afferents can be directly evoked by stimulation of the motor cortex is strong evidence that descending systems may exert a presynaptic control of sensory transmission during movement. Although observations on PAD are not direct measures of presynaptic inhibition, the prevailing opinion is that PAD accompanies a presynaptic reduction in transmitter release and that changes in PAD reflect changes in presynaptic transmitter release (see Willis 1999, Rudomin and Schmidt, 1999). The second section of the thesis will more directly examine this association between group I PAD and presynaptic inhibition during fictive locomotion.

Further evidence for the differential regulation of sensory-evoked presynaptic inhibition in some sensory fibre terminals during movement is the observation of larger presynaptic inhibition of transmission from group Ia afferents ending on certain groups of motoneurons during voluntary contraction in man (Hultborn *et al.* 1987). As revealed by the increase in afferent fibre excitability (Duenas and Rudomin, 1988) and by direct intra-axonal recordings (Gossard *et al.* 1991) there is a PAD of group I afferents during fictive

locomotion. Since fictive locomotion occurs without sensory stimulation, this PAD has been interpreted as a reflection of presynaptic inhibition evoked by the central circuitry producing locomotion (Duenas *et al.* 1990; Gossard and Rossignol, 1990; Gossard *et al.* 1990; El Manira *et al.* 1991;1997 Cattaert *et al.* 1992). This interpretation is at least partially supported by the finding that monosynaptic group I field potentials in the intermediate nucleus (Perreault *et al.* 1999) and ventral horn (Gosgnach *et al.* 2000) are depressed during fictive locomotion. Since field potentials reflect trans-membrane currents resulting from synaptic transmission between afferents and their target neurons, this field potential depression indicates an inhibition of transmission from group I afferents to neurons in these regions during locomotion. The depression of monosynaptic Ia EPSPs recorded in motoneurons is direct evidence for a presynaptic depression of transmission from Ia afferents to motoneurons during fictive locomotion (Gosgnach *et al.* 2000) and may underlie the reduction of group I reflex gain during locomotion in the cat (Bennett *et al.* 1996). Thus it appears that the operation of the circuitry underlying locomotion includes a process by which the synaptic transmission from afferent fibres can be regulated.

Since group I reflexes have been shown to be reorganized during locomotion, one question that arises is the extent to which a centrally evoked presynaptic inhibition of group I transmission during fictive locomotion is expressed uniformly throughout the lumbar spinal cord. In other words, are there local or regional variations in the magnitude of centrally-evoked presynaptic inhibition which may preferentially suppress transmission from group I afferents in certain areas of the spinal cord? To address this question, the present study will compare the degree of field potential depression during fictive locomotion occurring simultaneously in two locations of the lumbar spinal cord. Comparisons will be made of two field potentials recorded in the intermediate laminae where both Ia and Ib afferents terminate, as well as in the ventral horn where only Ia afferents terminate.

A second goal of the present experiments is to determine whether field potential depression occurs during another rhythmic motor behaviour, fictive scratch. Fictive scratch is a behaviour with rapid alternation of flexor and extensor muscles in one hindlimb (Deliagina *et al.* 1975; Perreault *et al.* 1999) that mimics the movements occurring during real scratch in intact animals (e.g. Kuhta and Smith, 1990). It has been argued that the

rhythmic component of scratch is produced by circuitry also involved in fictive locomotion (e.g. Gelfand *et al.* 1988). There are two reasons to investigate field potential depression during scratch. First it is important to know whether the locomotor induced presynaptic inhibition represents a unique control strategy during movement or if centrally generated presynaptic inhibition is a feature of other rhythmic movements such as scratch. Second, is the issue of whether the presynaptic inhibition observed during fictive locomotion is an epiphenomenon produced by electrical stimulation of the midbrain. This later possibility is raised by the observation that stimulation of brainstem areas involved in the initiation of locomotion (i.e. the MLR) in the anaesthetised cat results in a depression of group I field potentials by an average of 20% but does not evoke locomotion (Noga *et al.* 1995). The occurrence of presynaptic depression during fictive scratch would support the argument that presynaptic inhibition during cuneiform stimulation in the anaesthetised cat results from partial activation of the locomotor circuitry. Preliminary results have been presented in abstract form (Gosgnach *et al.* 2001).

METHODS

Experiments were performed on 12 cats of either sex weighing 2.1-3.4 kg. All surgical and experimental protocols were in compliance with guidelines set out by the Canadian Council for Animal Care and the University of Manitoba. For the surgery cats were anaesthetized with halothane delivered in a mixture of 30% oxygen and 70% nitrous oxide. Two veins were cannulated to administer drugs and fluids, and blood pressure was monitored from the carotid artery. A tracheotomy was performed. Atropine (0.12mg s.c.) and dexamethasone (2mg i. v.) were given at the beginning of the surgery and a 5% glucose and 0.84% bicarbonate solution was delivered intravenously throughout the experiment at a rate of 5ml/hr. Supplemental saline or dextran infusions were given as required to maintain blood pressure. Selected left hindlimb nerves were dissected and cut in preparation for electrical stimulation and for monitoring fictive locomotion. The dissected nerves included the left semimembranosus and anterior biceps (taken together as SmAB), posterior biceps and semitendinosus (taken together as PBSt), sartorius (Sart), quadreceps (Q), medial gastocnemius (MG), lateral gastrocnemius and soleus (LGS), plantaris (Pl), tibialis anterior

(TA), and extensor digitorum longus (EDL). The Sart and Q nerves were placed in a bipolar cuff electrode for stimulation and recording. Other femoral, sciatic and obturator nerve branches, as well as tendons around the hip, were cut bilaterally. Following an L4-L7 and C1-C2 laminectomy, the cat was placed in a rigid frame. A further 2 mg of dexamethasone was administered and a mechanical precollicular-postmammillary decerebration was performed with removal of both corticies and all tissue rostral to the transection. The anaesthetic was discontinued and the cat was paralysed with pancuronium bromide (Pavulon, 0.1 mg $kg^{-1}h^{-1}$) and artificially ventilated. Mineral oil pools over the spinal cord and both hindlimbs were warmed by radiant heat. Dissected nerves were placed on bipolar electrodes for stimulation and recording. A lethal injection of barbiturate anaesthetic was administered at the end of the experiment.

Glass microelectrodes filled with 2M sodium citrate (tip diameter 1.8-2.5μm; resistance 2-4 MΩ) were used for extracellular field potential recording in the ventral horn and intermediate nucleus. Fictive locomotion was evoked by unilateral or bilateral stimulation of the MLR (50-500μA, 1ms pulses at 12-30Hz; see Guertin *et al.* 1995). Fictive scratch was evoked by placing a piece of cotton soaked in a 0.01-0.03% solution of d-tubocurarae on the ipsilateral (left) C1 or C2 spinal root and touching the pinna. Intracellular recordings from antidromically identified lumbar motoneurons during fictive scratch were made with similar electrodes (Gosgnach *et al.* 2000).

Selected peripheral nerves were electrically stimulated using strengths expressed in multiples of threshold current. Threshold current (T) was defined as the smallest current producing a detectable extracellular compound action potential volley at the cord dorsum recording electrode. The peripheral nerve stimulation strength (100μs constant current pulses delivered at 3-5Hz) was adjusted when recording in the ventral horn to reduce antidromic activation as much as possible and the extracellular recording electrode positioned to avoid obvious antidromic spikes (≤2T, see Gosgnach *et al.* 2000). To make recordings from two areas of the spinal cord, two stereotaxic arcs were used and the microelectrodes moved independently to locate monosynaptic group I field potentials. In all cases when recording from the intermediate nucleus and in some cases when recording from the ventral horn, the electrode was inserted into the spinal cord medial to the dorsal roots. For analysis purposes,

group I extracellular field potentials were considered to be located in the intermediate nucleus if they were recorded at a depth between 1.3 and 2.4mm from the dorsal surface of the spinal cord. In the remaining cases, when recording from the ventral horn the dorsal roots were reflected dorsally and the electrode was inserted lateral to their entry zone and recordings were made at a depth of 1.2- 1.9mm from the surface of the cord. Field potentials were considered to be monosynaptic if they had a central latency of 0.7-1.0ms.

To examine whether presynaptic inhibition acts focally at circumscribed areas of the lower lumbar cord we used the "two location protocol" in which 2 electrodes were placed in the lumbar cord, either both in the intermediate nucleus (see Fig. 2), both in the ventral horn (see Fig. 3), or one electrode in each area (see Fig. 1). Peripheral nerves were stimulated to evoke monosynaptic extracellular field potentials at each electrode location. In some regions field potentials were evoked at the same location by stimulation of 2 or 3 nerves (e.g. Fig. 3). Following a control period of data collection, fictive locomotion was then evoked for approximately 1 minute. Following termination of brainstem stimulation, the field potential was recorded from for 4-5 minutes to permit recovery to control amplitude. The recovery timed given (e.g. 10s) indicates the beginning of a 10 second period in which the average field potential recovered to within 5% of its control amplitude.

To compare synaptic transmission depression from group I afferents during fictive scratch and fictive locomotion we used the "two behaviour protocol" (see Figure 5) in which a monosynaptic field potential was recorded during a short (~10s) period of no rhythmic activity (control), and then during approximately 30s of either fictive locomotion or scratch. Following the cessation of this rhythmic behaviour, the animal was given a 2-5 minute recovery (shorter if it was determined that the field potential had recovered to control amplitude) and the alternate rhythmic behaviour was then elicited. This recording period was then followed for up to 2-5 minutes after fictive behaviour to examine the recovery time. The amount and time course of depression for each activity was analysed and compared.

In order to obtain consistent results and avoid contamination by extracellular action potentials, field potential amplitude was measured just before the peak of the negative deflection on field potentials at latencies of 0.6-0.9ms from arrival of the volley at the cord dorsum. All measurements were made from averages of monosynaptic field potentials.

Activity in a rectified, integrated ENG was used to divide the step cycle into flexion and extension phases. Field potentials evoked during flexion and extension were sorted and separate averages calculated. Averages in the control, locomotor/scratch, and recovery period typically consisted of 40-80 field potentials. When phasic analysis was performed the averages typically consisted of 20-40 stimuli in each of the flexion and extension phases.

Field potentials were considered to be depressed if the amplitude at the point measured was reduced by more than 5% of control amplitude. Pairs of field potentials were said to be depressed by a different amount (i.e. a differential depression occurred) if the percent depression of the two fields differed by more than 5% during locomotion. Recovery was said to have occurred if a field potential returned to within 5% of its original amplitude. The 5% level was chosen conservatively, and may have resulted in an underestimation of the number of field potentials significantly depressed because of the low standard deviation of the averaged field potential (see Fig. 2).

Data capture and analysis was performed using software developed within the Winnipeg Spinal Cord Research Centre (a Pentium PC running QNX or Linux for data capture and analysis).

RESULTS

Recordings were made from 12 adult male or female cats. Eighty-four monosynaptic field potentials were recorded from 50 locations in the intermediate nucleus and 65 fields from 31 locations in the ventral horn during fictive locomotion. Almost all of the field potentials recorded in the intermediate nucleus and ventral horn were one member of a pair of field potentials obtained in two spinal locations. The effects of fictive locomotion on 43 pairs of field potentials with one electrode in the intermediate nucleus and the other in the ventral horn were examined. Thirty five pairs of group I field potentials were recorded during fictive locomotion with both electrodes in the ventral horn and 54 pairs examined with both electrodes in the intermediate nucleus. A comparison was also made of the effects fictive locomotion and fictive scratch on 26 group I extracellular field potentials.

Of the 84 field potentials recorded during fictive locomotion in the intermediate nucleus 69 were depressed by >5% (mean depression 27% ±11%) and 3 increased by ≥5%.

Sixty of these were followed for recovery after locomotion and 54 recovered to within 5% of control amplitude within 4 minutes after the end of locomotion. Of the 65 field potentials recorded in the ventral horn during fictive locomotion, 55 were depressed by a mean of 29% (±13%) and 6 increased in amplitude (mean increase 20%±12%). Forty of the 48 ventral fields had recovered within 4 minutes. The mean depression of both flexor and extensor evoked field potentials was similar ($p=0.4$) in the intermediate nucleus (28%±11% flexors, 25%±15% extensors) and ventral horn (29%±14% flexors, 29%±10% extensors).

Group I field potential depression is not uniform between intermediate and ventral spinal cord regions.

The amount of field potential depression during locomotion was compared for 43 pairs of simultaneous recordings with one electrode in the intermediate region and the other in the ventral horn (6 experiments). An example is illustrated in Fig. 1. in which 1.4T stimulation of the EDL nerve evoked a monosynaptic field potential in the intermediate nucleus (rostral electrode) that was unaffected during locomotion. The field potential produced by other fibres of the same nerve in the ventral horn (electrode positioned 2.2mm caudally) was depressed during fictive locomotion by 37% and recovered within 20s of the cessation of fictive locomotion. In this case there was a clear differential depression of monosynaptic field potentials in different regions of the spinal cord. Although the mean depression recorded in intermediate and ventral areas was similar ($p=.26$, 22%±13% depression in intermediate nucleus, 26%±12% in ventral horn, $n=43$) there were often regional variations between individual recordings.

INSERT FIGURE 1 HERE

Of the 43 pairs examined, the field potential depression during locomotion between the two recordings differed by more than 5% in 36 cases. In 14 pairs, the percent change in amplitude differed by 5 to 10% in the two areas during fictive locomotion, in 18 the amplitude change differed by 15% to 25% and in 4 it differed by more than 25%. In only 7 pairs was the amount of depression of field potentials recorded in the intermediate nucleus and ventral horn within 5% of each other during locomotion.

Of the 43 comparisons of locomotor-induced depression in intermediate and ventral lumbar spinal regions, 20 were of fields evoked from the same nerve (e.g. Fig. 1 with EDL stimulation) and 23 from heteronymous nerves (both flexor nerves or extensor nerves). The amount of depression for homonymous comparisons (intermediate regions 25%±18%, ventral 26%±16%, n=20) was similar to that for heteronymous comparisons (intermediate regions 26%±19%, ventral 28%±19%, n=23). For comparisons involving homonymous nerves there was more depression at the ventral site in 8 cases, more at the intermediate site in 6 cases and similar depressions at both sites in 8 cases.

Group I field potential depression is often not uniform in two ventral horn areas.

Perhaps it is not surprising that a comparison of the depression of intermediate and ventral fields shows so much variability when one considers that the field potentials evoked near motoneurons in the ventral horn are produced by Ia muscle spindle afferents and those in the intermediate areas by both Ia and Ib afferents with a likely predominance of Ib tendon organ afferents (see Discussion). For this reason comparisons were made of the degree of depression during fictive locomotion of 35 pairs of field potentials recorded in the ventral horn and thus evoked from Ia afferents. An example of differential depression in two ventral horn areas is illustrated in Figure 2

INSERT FIGURE 2 HERE

Figure 2 A illustrates the simultaneous recording of two ventral horn field potentials separated by 4.5mm and evoked by LGS stimulation (2T). In panel B averaged extracellular field potentials evoked by LGS nerve stimulation (2T, 4Hz) evoked before (control), during (locomotion), 10s (recovery1) and 30s (recovery2) after MLR evoked fictive locomotion are shown. The standard deviations of the averages are indicated by the dotted lines above and below the mean average in panel B. During locomotion the rostral LGS field potential was reduced by 42% while the caudal LGS field was only reduced by 25%. Given the small size of the standard deviation (during locomotion the average consisted of 42 stimulus presentations) there is a clear difference in the amount of depression of these two ventral fields. In this case both field potentials took about 30s to recover to control levels.

Of the 35 ventral pairs compared, 28 differed by more than 5% during locomotion. The percent depression of 14 pairs differed by 5 to 15%, 10 differed by 15 to 25% and 4 differed by more than 25% during fictive locomotion. The remaining 7 differed by less than 5%. From this data it is clear that pairs of field potentials evoked by Ia afferents are subject to a regional variation in centrally evoked presynaptic inhibition. Although not systematically investigated, there was no obvious tendency ($p=.13$) for rostrally (mean, 29%±11%) or caudally (mean, 24%±9%) located ventral field potentials to be preferentially affected during fictive locomotion .

Group I field potential depression is often not uniform in two areas of the intermediate nucleus.

Analysis of the depression of pairs of group I fields recorded in intermediate regions of the cord revealed variations similar to those recorded in ventral regions. An example of differential field potential amplitude modulation in two intermediate areas is illustrated in Figure 3.

INSERT FIGURE 3 HERE

Each microelectrode (separated by 4mm) recorded two field potentials, one evoked by Pl (2T) simulation, and the other evoked about 25ms later by Q (2T) nerve stimulation. This allowed for 4 comparisons. Rostral and caudal Pl evoked field potentials are illustrated in panel B, rostral and caudal Q evoked field potentials are illustrated in panel C. The rostral Pl field (dashed traces, located in mid-L6) was depressed by 17% during locomotion and recovered within 10s after cessation of rhythmic activity. The caudal Pl field (solid traces, located mid-L7) was depressed by 25% but its recovery was delayed for 30s after the cessation of locomotion. This difference in the time for recovery from similar levels of depression suggests some independence in the processes by which presynaptic inhibition of group I transmission is evoked in these two regions during fictive locomotion. Concerning the two Q fields (Fig. 2C) recorded in concert with the Pl evoked fields (Fig. 2B), the rostral Q field remained unchanged while the caudal one was depressed by 38% during locomotion.

Thus at the rostral location, the Pl field was depressed and the Q field unaffected during locomotion while at the caudal location both the Pl and Q fields were depressed.

Of the 54 pairs of intermediate fields compared, 45 differed by more than 5% during locomotion. In 22 cases the percent amplitude change between the pairs differed by 5 to 15% during fictive locomotion. In 17 cases they differed by 15 to 25% and in 6 cases by more than 25%. In only 9 cases was the difference between the two recordings less than 5%. Based on this data it is apparent that there are variations in the centrally evoked presynaptic inhibition between intermediate regions as well as on different group I fibres ending in the same region.

Differential phasic depression in two regions during fictive locomotion

An analysis of the phasic depression of the amplitudes of pairs of field potentials was performed to determine the consistency of the phase of the fictive locomotor step cycle in which maximum depression occurs. An example of this analysis is illustrated in Figure 4 in which two electrodes located 2mm apart in the L5 segment recorded group I field potentials evoked by stimulating the EDL nerve (2T, 5Hz).

INSERT FIGURE 4 HERE

The rostral electrode was located in the intermediate nucleus and the caudal electrode in the ventral horn. Averages of the evoked field potentials were made before the onset of MLR stimulation and locomotion (control), during (locomotion), and after cessation of MLR evoked fictive locomotion (recovery). Panel B illustrates that the rostral EDL field (upper traces) was depressed considerably less (22%) than the caudal EDL field (lower traces, 48%). In this case, the rostral field potential was reduced more in the extension (dashed line, depressed 32%) than the flexion phase (solid line, depressed 8%), while the caudal locomotor field was depressed more in flexion (solid line, depressed 56%) than extension (dashed line, depressed 35%). The dissimilarity of the phasic modulation of these two EDL field potentials provides further evidence for a local expression of the processes responsible for the reduction of monosynaptic group I field potentials during fictive locomotion.

For comparisons involving an intermediate and ventral field potential, maximal field

depression occurred in the same phase of locomotor cycle in less than half the cases (18/43). For comparisons involving two ventral group I fields, maximal field depression occurred in the same phase of locomotor cycle in only about half the cases (19/35) and for two intermediate fields in only 15/54 cases.

Group I field potential depression in fictive locomotion and scratch is similar

A comparison was made of the effects of MLR-evoked fictive locomotion and fictive scratch evoked without electrical stimulation of the MLR on single lumbar group I field potentials. Fictive locomotion and scratch were evoked within a short period of each other and comparisons were made on field potentials under both conditions. Continuous monitoring and averaging of field potential amplitude was employed to ensure that the field potential amplitude had fully recovered before the other fictive behaviour was evoked.

INSERT FIGURE 5 HERE

An example of a field potential recorded during both fictive locomotion and fictive scratch is illustrated in Fig. 5 with averages of field potentials calculated from the periods labelled in panel A shown directly below in panel B. During fictive scratch this SmAB evoked field potential (depth 1.8mm, mid L6) was reduced by 21% and recovered within 40s of the end of the bout of scratch (recovery1). During the first locomotor period (loco1) rhythmic locomotor activity was poor and field potential amplitude was reduced to 15% of control. The break in the abscissa (hatched lines) represents a 10-15s period without data collection but with MLR stimulation before the period of more fully developed rhythmic activity (see also panel C2). During this period (loco2) the field potential was depressed to 22% of control, i.e. similar to that occurring during fictive scratch. This field potential recovered to control levels about 1 min after the cessation of fictive locomotion.

In total 26 field potentials were recorded from five cats during both fictive locomotion and fictive scratch. The mean depression during the two fictive behaviours was quite similar ($p=0.7$), 29%±14% during fictive locomotion and 28%±11% during fictive scratch. However, in only 12 of the 26 recordings was the amount of depression during locomotion and scratch within 5% of each other. This data is presented in Table 1 which also

indicates the time (within 10s) taken for the field potentials to recover and the phase in which maximum depression occurred.

INSERT TABLE 1 HERE

A small sample of the effects of fictive scratch on monosynaptic Ia EPSPs was made using intracellular recordings from 5 flexor and 1 extensor motoneuron. This data compliments the report of Perreault et al (1999) on Ia EPSP amplitude during fictive scratch in extensor motoneurons. The mean depression of the 6 monosynaptic EPSPs recorded during fictive scratch was 34%, similar to the 30% depression reported previously from this laboratory during fictive locomotion (Gosgnach et al. 2000). In two of the three cases in which a monosynaptic EPSP was recorded during both fictive locomotion and scratch, the depression was within 5% in both behaviours. In the third, the differential depression between the two was 10%. This is illustrated in Figure 6.

INSERT FIGURE 6 HERE

Panel B illustrates averaged monosynaptic EPSPs recorded in an EDL motoneuron during the time period indicated above in panel A. Monosynaptic EPSP amplitude was decreased by 45% during locomotion and 55% during scratch. In panel C the EPSPs evoked during locomotion and scratch have been separated into those evoked during flexion (solid traces) and those evoked during extension (dashed traces). Monosynaptic EPSP amplitude is depressed in both phases in locomotion (33% in flexion, 51% in extension) and scratch (27% in flexion and 58% in extension). Note the large disynaptic EPSP (open arrows in panel B and C) evoked during locomotion in this EDL motoneuron (see Quevedo et al 1999). All monosynaptic EPSP measurements were made at a fixed latency just before the peak so as to avoid contamination by the disynaptic EPSP (see Quevedo et al. 1999).

DISCUSSION

The present experiments further characterize the depression of transmission from hindlimb muscle group I afferents in the decerebrate cat which occurs at the onset of rhythmic alternating activity and may persist for a period of time after the cessation of rhythmic activity (Perreault et al. 1999, Gosgnach et al. 2000). This study used simultaneous recordings of monosynaptic group I field potentials from two sites to address the question of whether the amount of presynaptic depression is uniformly expressed in the lumbar spinal cord during fictive locomotion and scratch. The principal finding is that during both fictive locomotion and scratch, there can be considerable variability in the amount of depression of group I extracellular field potentials within different areas of the lumbar spinal cord. The depression of group I field potentials recorded in the ventral horn (28%, Gosgnach *et al.* 2000) and intermediate nucleus (20% Perreault *et al.* 1999; 28% present results) is quite similar when averaged between several preparations. In individual experiments and runs, however, large variations in field potential depression could be found for fields evoked by either flexor or extensor afferents and both in the ventral horn and intermediate laminae of the spinal cord. The paired recordings revealed that the locomotor phase in which group I field potentials were maximally depressed differed in many cases. Finally, in some cases there were differences in the amount of depression on different group I terminals ending in the same area. As discussed below, this evidence is consistent with a mechanism of centrally generated presynaptic depression that is produced through interneurons acting locally within circumscribed areas of the spinal cord.

This study also demonstrates that group I extracellular field potentials are depressed during fictive scratch as well as fictive locomotion and on average, to a similar extent. This observation may have been partially anticipated from the finding that monosynaptic Ia EPSPs recorded in extensor motoneurons are decreased during both fictive locomotion and scratch (Perreault *et al.* 1999a). It was necessary, however, to examine the presynaptic depression in both fictive behaviours as assessed from the field potentials measurements used in this and previous studies (Perreault *et al.* 1999b; Gosgnach *et al.* 2000) and to see if fields in intermediate regions are affected during scratch. The present finding was that both ventral and intermediate fields as well as EPSPs in flexor motoneurons are depressed during

scratch to an extent similar to that seen during fictive locomotion. Together this evidence strengthens the argument that the neuronal circuitries underlying scratch and locomotor behaviours may be organized on similar principles (see Gelfand *et al.* 1988; Perreault *et al.*1999a).

As mentioned in the Introduction, in anaesthetised cats, electrical stimulation of brainstem areas near to, or coincident with areas that evoke locomotion in decerebrate preparations, evokes a depression of group I field potentials (Noga *et al.* 1995). The present study shows that during scratch, evoked without electrical stimulation, there is a group I field potential depression similar to that occurring during locomotion. Interestingly, during fictive weight support (tonic motor activity induced by contralateral scratching), group I EPSPs are depressed compared to control, but are further depressed during rhythmic scratch (Perreault *et al.* 1999a). These observations add support to the argument that the presynaptic depression seen during MLR evoked fictive locomotion results from actions of the central circuitry involved in motor activity rather than MLR stimulation *per se* (see Perreault *et al.* 1999b; Gosgnach *et al.* 2000). Another argument in favour of an activity related presynaptic depression is the finding that during the delay from the start of brainstem stimulation to the initiation of rhythmic locomotor activity, group Ia EPSP amplitude is usually not reduced until rhythmic flexion and extensor activity is evident (Gosgnach *et al.* 2000; see also Perreault *et al.* 1999b).

The recognition of a centrally generated presynaptic inhibition during walking in man (e.g. Capaday and Stein, 1986; 1987) and reduced preparations (e.g. Gossard 1996; Perreault *et al.* 1999a; Gosgnach *et al.* 2000) raised the question of whether it, like the presynaptic inhibition evoked by conditioning stimulation of hindlimb nerves, is associated with PAD. The accompanying section of the thesis directly addresses this issue. Results presented there lead to the conclusion that the occurrence of PAD is not well correlated to presynaptic depression of synaptic transmission during fictive locomotion. In other words, the centrally evoked presynaptic depression during fictive locomotion results from another, non-PAD related mechanism. Nonetheless, there are some features of the expression and organization of PAD that may have relevance to the centrally evoked presynaptic depression occurring during locomotion and scratch. Microstimulation of the dorsal horn evokes short-latency and

monosynaptic dorsal root potentials in afferents terminating in the same regions of the cord where the stimulus was applied (Jankowska et al. 1981; Riddell and Jankowska, 1995). This suggests that interneurons in the vicinity of afferent terminals can be activated to produce a PAD in these afferents. These observations have led to the suggestion that PAD in the lumbar cord results from activation of interneurons that make axo-axonal contacts with nearby primary afferent fibres. These interneurons are subject to descending control and receive input from a variety of segmental afferents resulting in the patterns of PAD characterised in non-locomoting preparations (see Rudomin and Schmidt, 1999).

Local variations in the amount of PAD in two axon collaterals of a single afferent have been well documented during conditioning stimulation of hindlimb nerves (Eguibar *et al.* 1994; 1997; Quevedo *et al.* 1997). Cold (conduction) block of the thoracic cord eliminates this differential PAD suggesting that descending systems can affect select portions of the lumbar circuitry responsible for PAD (Lomeli *et al.* 1998). Moreover, stimulation of the cortex can evoke PAD (i. e. a central PAD) as well as control the ability of other hindlimb afferents to produce PAD in group Ia and Ib afferents (i. e. control of sensory evoked PAD) (Eguibar *et al.* 1994). Strong evidence supports the existence of several populations of lumbar interneurons responsible for PAD (see Rudomin and Schmidt, 1999). The next section of the thesis presents a hypothesis that reconciles some of the differences in the observations of PAD and presynaptic inhibition during locomotion. Arguments presented there suggest that the same interneurons that produce PAD may also activate other mechanisms (e.g. $GABA_B$ receptor mediated) that dominate the prolonged presynaptic inhibition seen during locomotion. We suggest that the CPG for locomotion and scratch may excite some of the PAD producing interneurons and that this results in a presynaptic inhibition of group I afferent transmission. A study of the activity of PAD producing interneurons (Solodkin *et al.* 1984) during fictive locomotion would provide the direct evidence needed to refute or support this suggestion.

Another possible mechanism underlying the local variations in group I field potentials during locomotion is a paracrinic release of neurotransmitter in the lumbar cord acting on afferent fibres variably depending on the number of presynaptic receptors on particular afferents. This seems to be the case in the serotonergic system of the lamprey

spinal cord (Christenson *et al.* 1989) and could explain the present results.

Regardless of the responsible mechanisms or interneurons, what are the functional consequences of the on average 28% tonic presynaptic depression of group I transmission in intermediate and ventral spinal areas seen during fictive locomotion and scratch? Despite the indirect nature of field potential recordings, their depression during fictive locomotion and scratch is well correlated with a depression of monosynaptic EPSPs in motoneurons (Perreault *et al.* 1999a; Gosgnach *et al.* 2000; Perreault 2002; this thesis). Consequently field potential depression likely indicates a reduction in monosynaptic activation of spinal interneurons during locomotion and scratch. A survey of field potential depression found a reduction in transmission from cutaneous, group I, and group II muscle afferents during fictive locomotion (Perreault *et al.* 1999b) but a dramatic reduction in group II transmission in the intermediate nucleus. During fictive locomotion many intermediate group II field potentials became undetectable and those that remained were reduced to about half their control size (Perreault *et al.* 1999b). In contrast, monosynaptic group II fields in the dorsal horn were affected much less and on average to about the same extent as group I and cutaneous fields recorded in that study and the group I fields recorded in the present study. The suggestion was made that this selective group II depression in the intermediate nucleus could be responsible in part for a selection (and suppression) of particular group II reflexes during locomotion (Perreault *et al.* 1999b).

Neither the present nor previous results support a similar role in reflex selection for the depression of group I transmission during locomotion. Both intermediate and ventral fields are similarly depressed by about 28% regardless of their origin from flexor or extensor afferents and in both fictive locomotion and scratch. It is now generally accepted that there is a reorganization of group I reflexes during locomotion. For example, oligosynaptic group I evoked non-reciprocal inhibition is suppressed at the onset of locomotion and replaced by a disynaptic excitation as well as other reflexes (see McCrea, 2001). Preliminary results suggest that this disynaptic excitation is evoked through interneurons located in the intermediate nucleus, an area also containing the inhibitory interneurons activated in the absence of locomotion (i.e. responsible for non-reciprocal inhibition- Angel *et al.* 2003). Thus the monosynaptic activation of interneurons in the intermediate nucleus during

locomotion is likely an important component of group I reflexes operating during locomotion. Available evidence suggests that presynaptic depression of transmission in the group I pathway in the intermediate nucleus may reduce reflex gain during locomotion but is unlikely responsible for selecting between parallel reflex pathways. The results presented in Fig. 3, however, showing the depression of fields evoked from one source of group I afferents but not another, leave pathway selection as a potentially viable hypothesis. Future experiments comparing the depression of group I transmission to interneurons in the intermediate nucleus and to ascending tract cells in Clarke's column would also be of interest in this regard.

During locomotion the activation of muscle afferents during limb movement will produce a massive sensory input to the spinal cord. Estimates of up to 800,000 sensory action potentials per second from each hindlimb travel to the cord during one locomotor step have been made (Prochazka and Gorassini 1998). It has been suggested that such sensory input may exceed the overall information-processing capability of the central nervous system and that sensory-evoked presynaptic inhibition would be an effective mechanism to reduce this input (Rudomin and Schmidt, 1999). Centrally evoked presynaptic depression could play a similar role at the onset and during locomotion in anticipation of the proprioceptive sensory input during stepping (Perreault et al. 1999b). Proprioceptive afferent input from the hindlimb plays a large role in the production of locomotion. Studies have estimated that anywhere from 30 % (Stein *et al.* 2000) to 70% (Hiebert and Pearson, 1999) of the ankle extensor output during locomotion is of reflex origin. Thus, it is crucial that mechanisms are available to regulate the effectiveness of afferent input with regard to the particular task at hand and to prevent disrupting the step cycle with inappropriate reflexes. It is possible that the principle role of centrally evoked presynaptic inhibition of group I afferents during locomotion and scratch is to provide reflex gain reduction.

The interpretation that the present results reflect a general gain reduction leads to the possibility that the particular variations in presynaptic depression seen in this study are not of great significance. Other than the striking degree of variability, there was no obvious pattern or way to predict which fields would be affected most during locomotion and scratch. Unless there is a finer level of organization not disclosed by the present approaches, group I

transmission is affected in a seemingly random manner to an overall level of about 28% in the field potentials recorded in the ventral horn and intermediate nucleus and in the Ia EPSPs recorded in motoneurons (Perreault *et al.* 1999a, 1999b; Gosgnach *et al.* 2000; present results). The extensive divergence of individual group I afferents (Hongo *et al.* 1978) to interneurons in several spinal areas, the convergence of many afferents onto individual interneurons, and the convergence of many interneurons to motoneurons could negate any specific regional variations in synaptic transmission resulting in a generalized and tonic gain reduction during fictive locomotion and scratch. According to this view, the locomotor circuitry would need only to activate the appropriate number of presynaptic inhibitory interneurons to evoke the degree of presynaptic depression needed for the ongoing locomotor task.

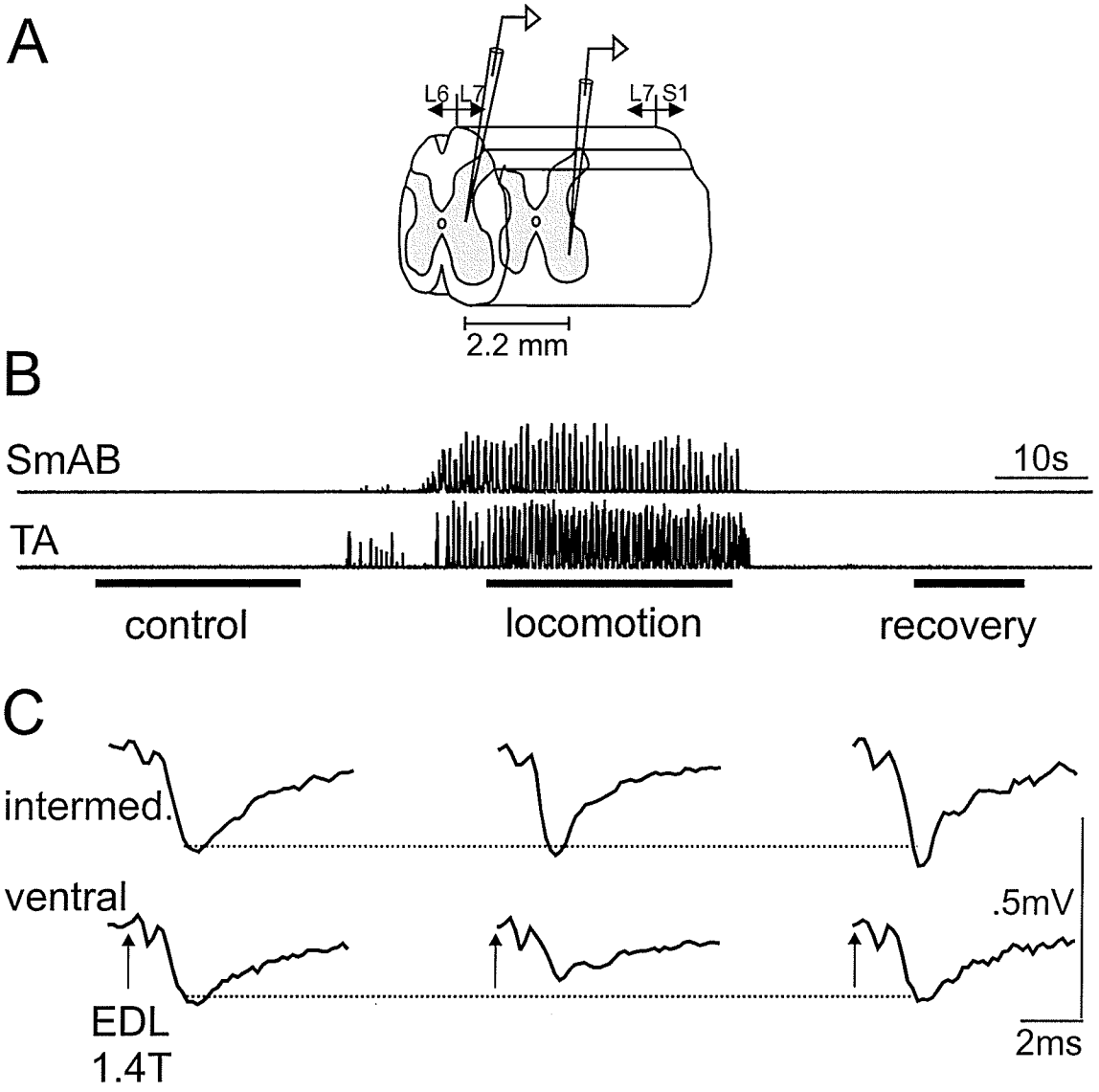

Figure 1. *Differential depression between two group I field potentials recorded in the intermediate nucleus and ventral horn*. **A.** Rostral electrode is located in the intermediate nucleus at the L6/L7 border (depth 1.8mm). Caudal electrode is located in the ventral horn of the L7 segment 2.2mm caudal to the other electrode (depth 1.7mm). **B.** Rectified, integrated ipsilateral ENGs from extensor (SmAB) and flexor (TA) peripheral nerves before, during, and after MLR-induced fictive locomotion **C.** Averaged group I field potential evoked by EDL nerve stimulation (1.4T,4Hz) recorded by the rostral and caudal electrode during the time period indicated directly above in panel B. The intermediate nucleus field potential was unaffected during locomotion, the ventral horn field potential was depressed by 37%.

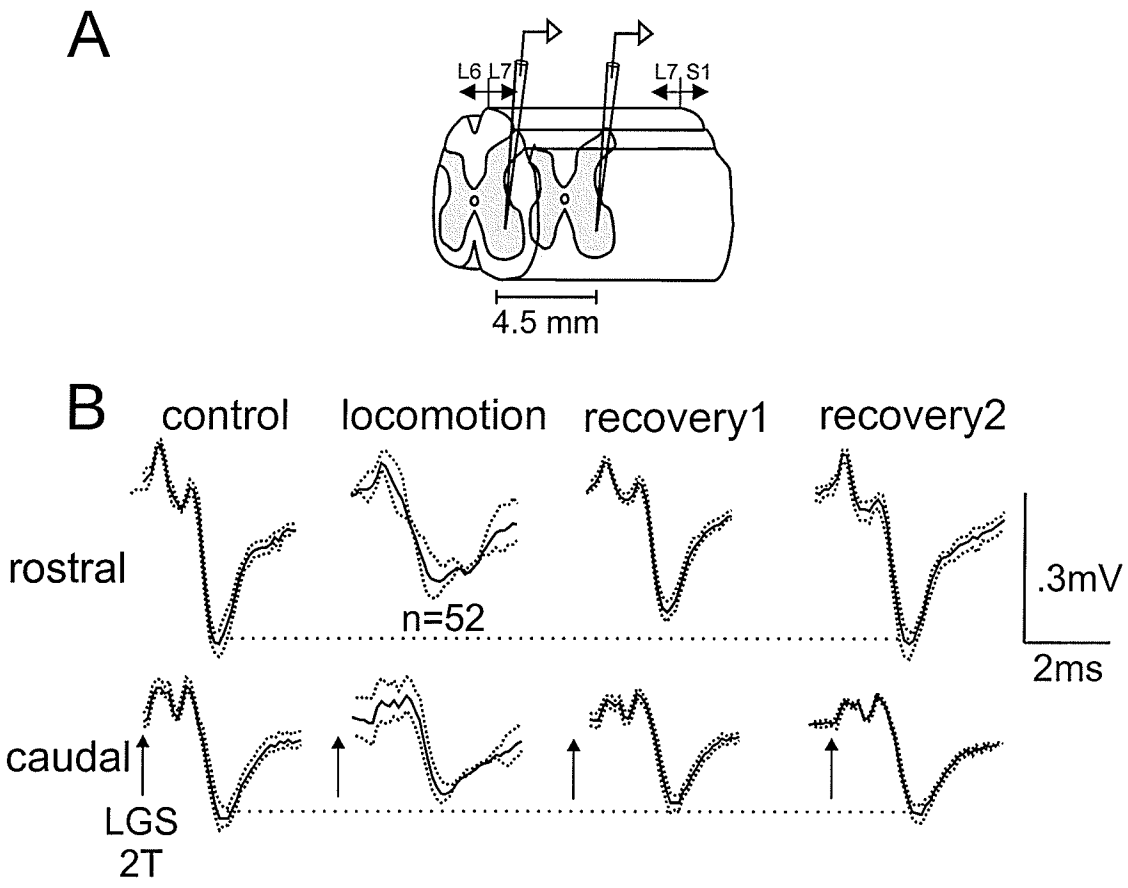

Figure 2. *Differential depression between two group I field potentials recorded in the ventral horn.* **A.** Rostral electrode is located at the L6/L7 border in the ventral horn. Caudal electrode is located in mid L7 segment 4.5mm caudal to the first electrode, also in the ventral horn. **B.** Averaged group I field potentials evoked by LGS nerve stimulation (2T, 4Hz) recorded by the rostral and caudal electrode before (control), during (locomotion), 10s after (recovery1) and 30s after (recovery2) cessation of MLR induced fictive locomotion. Dashed lines indicate standard deviations. During locomotion the rostral field potential was reduced by 42% and the caudal field potential was reduced by 25%. Both fields fully recovered within 30s.

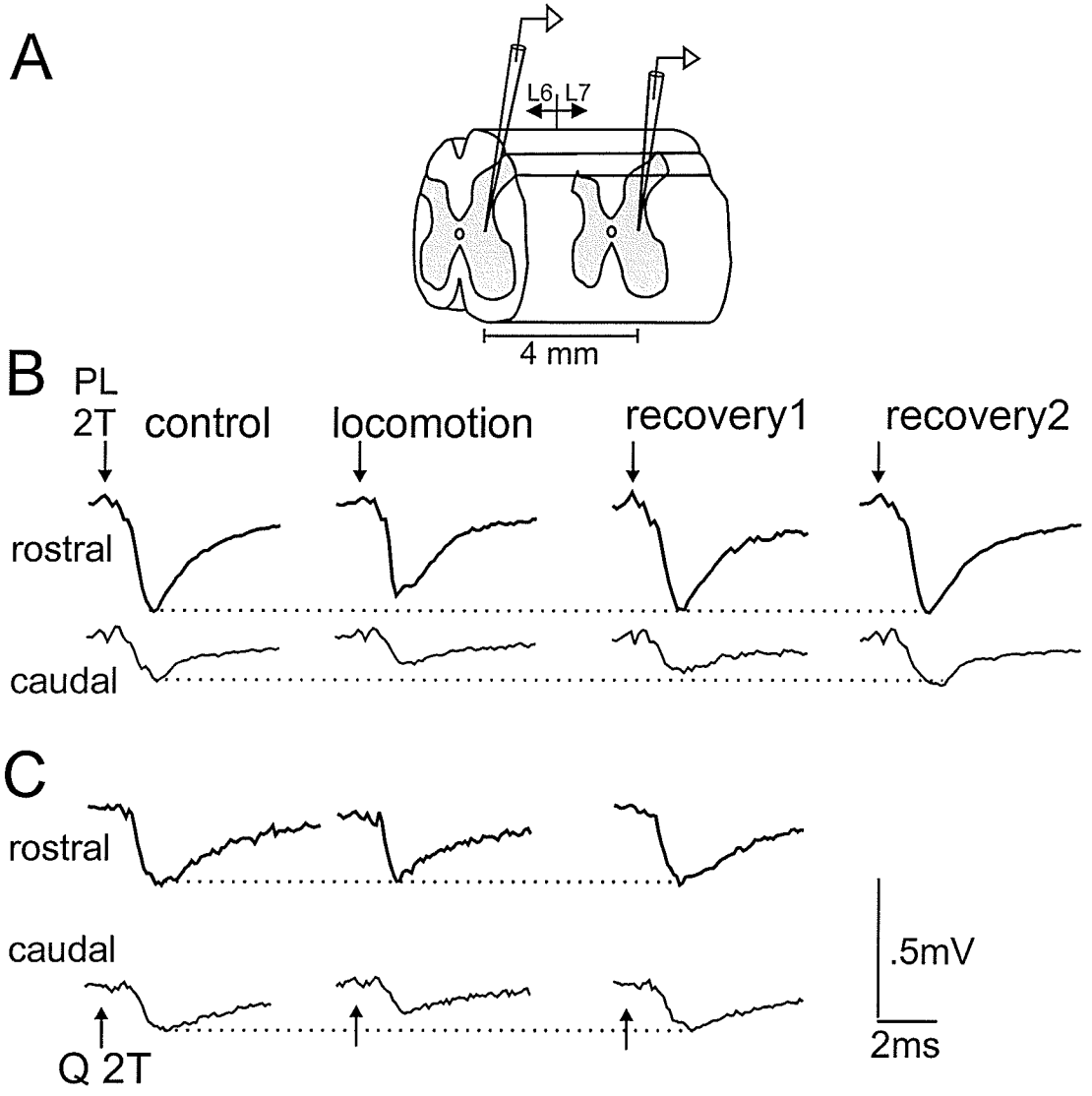

Figure 3. *Differential depressionof two group I field potentials recorded in the intermediate nucleus.* **A.** Rostral electrode is located in the intermediate nucleus in the mid L6 segment. Caudal electrode is located in the intermediate nucleus in the mid L7 segment, 4mm caudal to the first electrode **B**. Averaged group I field potentials evoked by PL nerve stimulation (2T, 4Hz) recorded by the rostral and caudal electrode before (control), during (locomotion), 10s after (recovery1) and 30s after (recovery2) MLR-induced fictive locomotion. The rostral PL field is depressed by 17% during locomotion and recovers within 10s of its cessation. The caudal field is depressed by 25% during locomotion and does not recover until 30s after its cessation. **C.** Group I field potentials evoked by Q nerve stimulation (2T, 4Hz) at the same time and in the same location as those in panel B. Rostral Q field is unaffected during locomotion, caudal field is depressed by 38% and recovers within 10s of the end of locomotion.

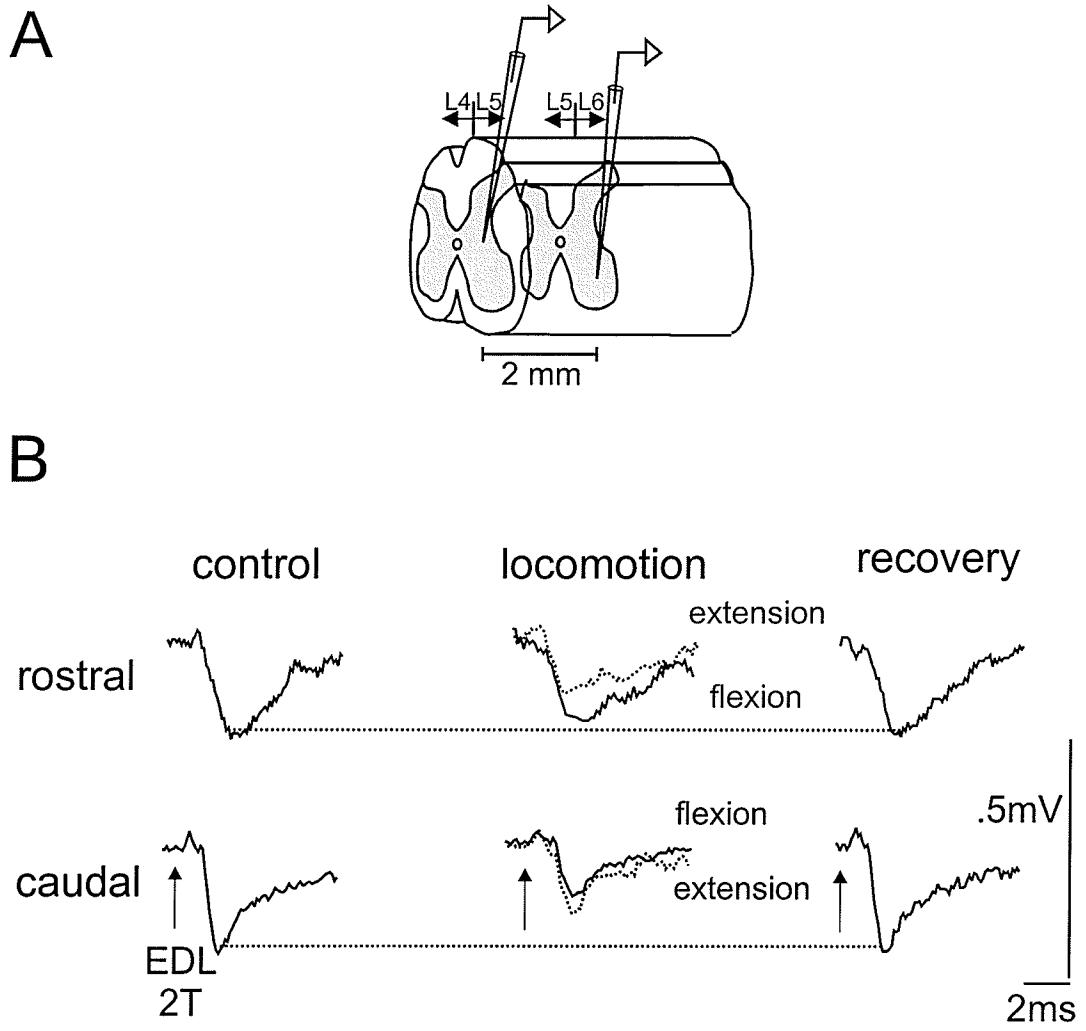

Figure 4. *Differential control of the phase of maximal group I field potential depression* **A.** Rostral electrode is located in the intermediate nucleus at the L4/L5 border. Caudal electrode is located in the ventral horn at the L5/L6 border 2mm caudal to the first electrode. **B.** Averaged group I field potentials evoked by EDL nerve stimulation (2T, 5Hz) recorded on the rostral (upper traces) and caudal (lower traces) electrodes. Fields evoked during MLR induced fictive locomotion have been divided into those evoked in flexion (solid line) and extension (dashed line). During locomotion the rostral EDL field is depressed to a greater extent during extension (32%), while the caudal EDL field was depressed to a greater extent during flexion (56%).

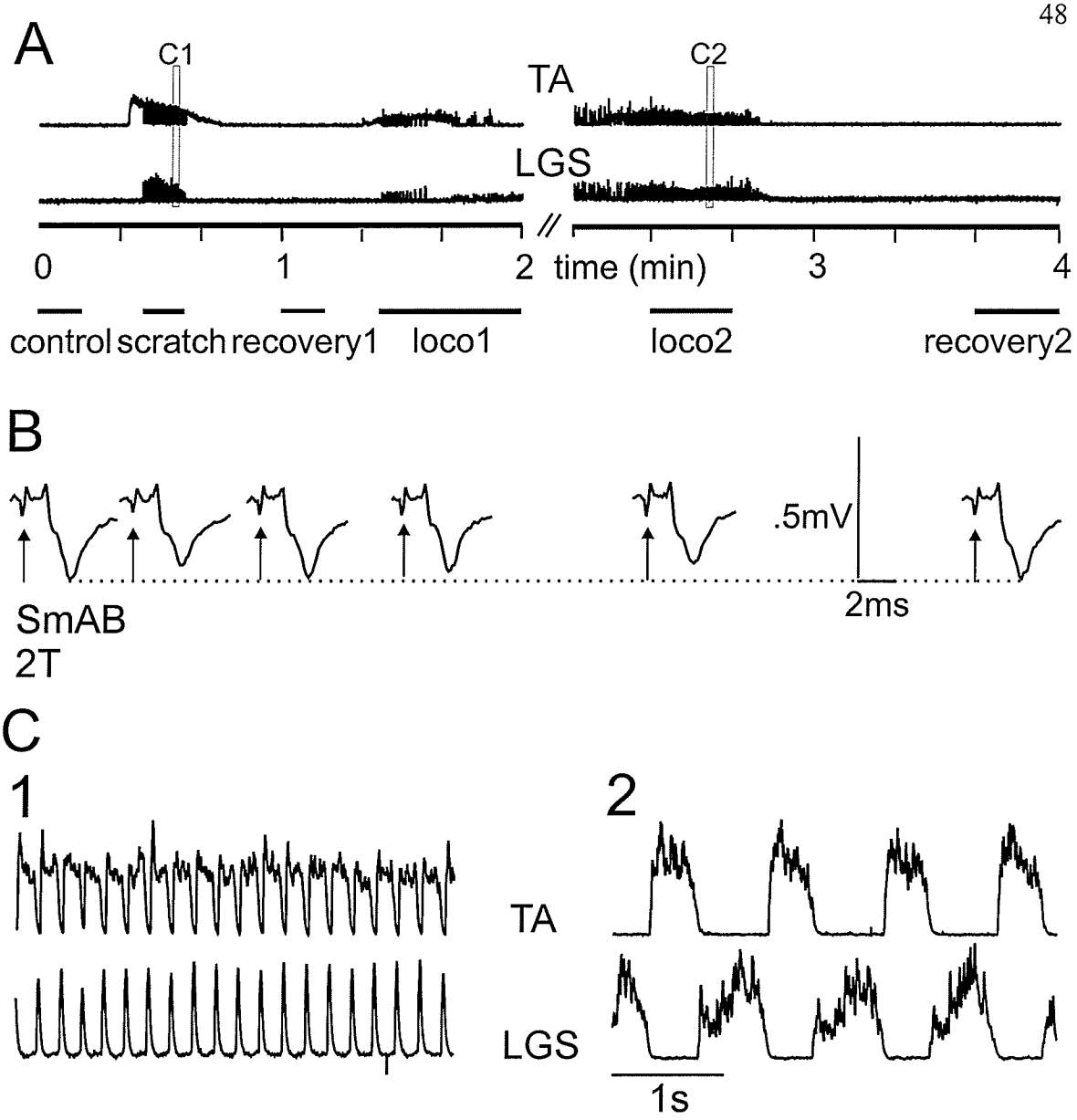

Figure 5. *Group I field potentials are depressed during both fictive locomotion and fictive scratch*. **A.** Rectified, integrated ipsilateral ENGs from flexor (TA) and extensor (LGS) peripheral nerves before (control), during (scratch), and after (recovery1) fictive scratch as well as during (loco1, loco2) and after (recovery2) MLR induced fictive locomotion evoked immediately after scratch. **B**. Averaged group I field potentials evoked by SmAB nerve stimulation (2T, 5Hz) during the time period indicated directly above (in panel A). During fictive scratch the field potential is depressed by 21% and recovers within 40s. During the loco1 time period the field potential is depressed by 15%. When the locomotor rhythm is fully developed in the loco2 time period the field potential is further depressed (22%) and recovers approximately 1 min after cessation of locomotion. **C.** Expanded portion of panel A illustrating rhythmic alternation between TA and LGS nerves during a 4s period of fictive scratch (C1) and fictive locomotion (C2).

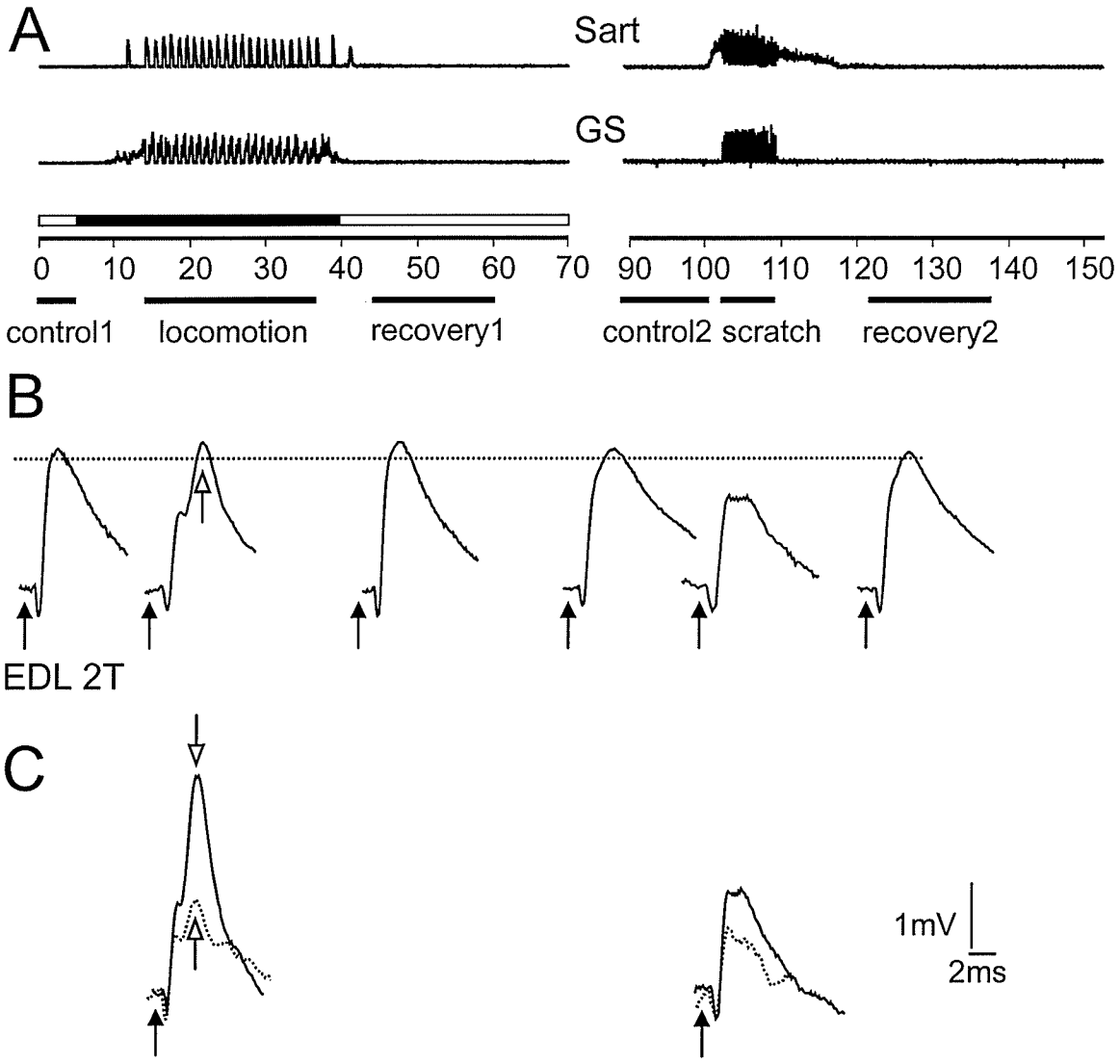

Figure 6. *Group I monosynaptic EPSPs are depressed during both fictive locomotion and fictive scratch.* **A.** Rectified, integrated ipsilateral ENGs from flexor (Sart) and extensor (GS) peripheral nerves before (control1), during (locomotion), and after (recovery1) MLR induced fictive locomotion as well as before (control2) during (scratch) and after (recovery2) fictive scratch evoked immediately after locomotion. **B.** Averaged group I EPSPs evoked by EDL nerve stimulation (2T, 5Hz) during the time period indicated directly above (in panel A). During fictive locomotion the monosynaptic EPSP is depressed by 45% and recovers within 10s (disynaptic EPSP indicated by open arrow). During fictive scratch the EPSP is depressed by 55% and recovers after approximately 10s. **C.** EPSPs evoked during locomotion and scratch have been separated into those evoked during flexion (solid traces) and extension (dashed traces). EPSP amplitude is depressed in both phases in locomotion (33% in flexion, 51% in extension) and scratch (27% in flexion and 58% in extension).

	Locomotion			Scratch		
Field	Depression (%)	Maximal depression in	Recovery time(s)	Depression (%)	Maximal depression in	Recovery time(s)
EDL	58	F	20	38	E	30
	13	-	80	36	-	80
	25	F	20	32	F	10
	42	F	80	24	-	50
	23	F	10	25	E	10
PbSt	67	-	100	14	-	40
	29	E	60	60	E	60
PI	30	E	10	23	E	20
Q	33	-	30	32	E	20
	37	F	10	25	-	20
	36	F	30	32	F	30
	36	F	20	27	F	30
Sart	34	-	20	37	-	20
	2	E	10	8	F	20
	increase	-	-	19	F	50
	36	-	80	20	E	50
	30	-	50	29	-	50
	19	E	10	23	E	10
SmAB	22	E	70	21	E	40
St	44	F	60	49	-	30
TA	27	-	30	27	-	30
	40	F	20	42	F	30
	20	F	20	20	F	20
	15	E	10	20	F	10
	8	F	10	34	-	10
	27	F	30	19	F	30

Table 1. *Group I field potential depression during fictive locomotion and scratch.* Nerves used to evoke group I field potentials are listed in the rows. Columns indicate percent depression during respective rhythmic behaviour, the phase of either fictive scratch or locomotion of maximal field potential depression (E=extension, F=flexion, - = similar in both phases) and the time taken for the field potential to recover to control amplitude after the cessation of rhythmic activity.

PAPER #2

Dissociation between PAD and presynaptic inhibition of group I field potentials in the spinal cord during fictive locomotion.

SUMMARY

In decerebrate cats, fictive locomotion produced by electrical stimulation of the midbrain reduces the amplitude of monosynaptic field potentials evoked in the lumbar spinal cord by stimulation of hindlimb group I muscle afferents. The mechanism underlying this process is unknown. The effects of fictive locomotion on nerve excitability (measured by the Wall technique) were used to determine the relationship between the centrally generated presynaptic inhibition of transmission from group I afferents and the depolarization of primary afferents (PAD) during fictive locomotion.

During fictive locomotion 25/28 group I extracellular field potentials were depressed (mean 25%) suggesting that there was a centrally generated presynaptic inhibition, however this was not consistently associated with an increase in terminal excitability. Afferent excitability could be increased, decreased or unaffected during fictive locomotion. In less than half the cases was there a phasic modulation of peripheral nerve excitability. In those cases in which there was a phasic modulation, excitability could be largest in either flexion or extension.

The results of this study demonstrate that the centrally generated presynatic inhibition which occurs during locomotion is not consistently associated with PAD and thus the depolarization of single afferent fibres is likely not the primary mechanism underlying the presynaptic inhibition of group I afferents during locomotion. We propose that measurement of afferent fibre excitability is not an appropriate technique for the study of presynaptic inhibition during locomotion in the cat.

INTRODUCTION

Hindlimb muscle afferents conducting in the group I conduction velocity range include muscle spindle primary endings relaying information about muscle length (Ia afferents) and Golgi tendon organ receptors carrying information regarding muscle tension (Ib afferents). These two proprioceptive systems make monosynaptic connections with interneurons in intermediate regions (laminae IV-VI) of the lumbar spinal cord. The common reflex actions of Ia and Ib afferents evoked through these interneurons are termed group I reflex actions and are subject to a great deal of control and reorganization under different behavioural conditions. For example, under quiescent conditions, extensor group I afferents evoke di- and trisynaptic inhibition of ipsilateral hindlimb extensors (Jami 1992). With the transition from the resting state to locomotion, these inhibitory reflexes are suppressed and extensor Ia and Ib afferents evoke a disynaptic excitation of homonymous and other extensor motoneurons (McCrea *et al.* 1995; Angel *et al.* 1996). Flexor group I afferents also evoke a disynaptic excitation of flexor motoneurons during locomotion (Degtyrenko *et al.* 1998; Quevedo *et al.* 2000). Furthermore, activity in group I afferents can control the duration and amount of motoneuron activity during locomotion. For example in some cat preparations, it is estimated that reflexes evoked from extensor group I afferents contribute as much as 30-70% of motoneuron activity during stance (Hiebert and Pearson, 1999; Stein *et al.* 2000). It is now widely believed that some of the reflex actions of group I afferents during locomotion are evoked through interneurons comprising portions of the central pattern generator for locomotion (e.g. Conway *et al.* 1987; Guertin *et al.* 1995). Given the powerful actions of the group I reflex system during locomotion, it is important to understand how group I reflex pathways are controlled in the locomotor state.

The presynaptic regulation of transmission from group I afferents to spinal neurons is a powerful process by which reflex gain can be adjusted. In the anaesthetized cat, presynaptic inhibition of hindlimb group Ia and Ib afferents can be readily evoked by conditioning stimulation of the same or other group I afferents (see Will 1999; Schmidt and Rudomin, 1999). In the spinal cord this sensory-evoked presynaptic reduction of transmitter release is strongly associated with a depolarization of the afferents (i. e. primary afferent depolarization or PAD). This PAD has been detected directly during intraxonal recordings of

the depolarization of primary afferents (e.g. Eccles and Krnjevic, 1959) and indirectly by assessing increases in the excitability of afferent terminals either with multi-fibre (e.g. Wall 1958) or single fibre (e.g. Duenas and Rudomin 1988) techniques.

During locomotion, in the absence of conditioning stimulation of peripheral nerves, there is a presynaptic inhibition of synaptic transmission from group I afferents. This centrally evoked presynaptic inhibition has been seen as a reduction in the monosynaptic excitation of motoneurons by Ia afferents in the human H reflex (Capaday and Stein, 1987; 1988 Faist *et al.* 1996; Andersen and Sinkjaer, 1999) and the cat stretch reflex (Bennett *et al.* 1996). It has also been recorded directly as a reduction in the amplitude of monosynaptic Ia EPSPs in motoneurons during fictive locomotion (Gosgnach *et al.* 2000) and scratch (Perreault *et al.* 1999a; Paper 1, this thesis) and a reduction in monosynaptic group I field potentials in both the intermediate nucleus (Perreault *et al.* 1999b; Paper 1, this thesis) and the ventral horn (Gosgnach *et al.* 2000) of the cat lumbar spinal cord.

The question arises as to whether the centrally evoked presynaptic inhibition occurring during locomotion is strongly associated with PAD of group I afferents as is the case for sensory-evoked presynaptic inhibition. Evidence for such an association includes the finding that lumbar Ia afferents are rhythmically depolarized (Dubuc *et al.* 1988; Gossard *et al.* 1991; Gossard 1996) and that the terminals of single group I afferents show increases in excitability during fictive locomotion (Duenas & Rudomin, 1988). On the other hand, at least two observations argue against the strong association between PAD and depression of transmission from group I afferents during fictive locomotion. First, a tonic primary afferent hyperpolarization (PAH) during fictive locomotion was reported by Bayev and Kostyuk (1982) when assessing the excitability of multiple group I fibres using the Wall technique (Wall 1958). The presence of a PAH would suggest that there is an increase in synaptic transmission from group I afferents during locomotion (see Eccles and Krnjevic, 1959), a suggestion contrary to all experimental observations. Second, simultaneous intracellular recordings from Ia afferents and motoneurons found a lack of correlation between the rhythmic PAD in the afferents and fluctuations in EPSP amplitude during fictive locomotion (Gossard 1996). Presumably larger PADs should be coupled to smaller EPSPs in the motoneuron if the centrally evoked presynaptic inhibitory process operating during

locomotion is like that operating during sensory evoked presynaptic inhibition.

The principle aim of the present study was, therefore, to more closely examine whether the presynaptic inhibition observed during fictive locomotion is associated with a tonic depolarization of group I primary afferents. The approach taken was to compare changes in the excitability of group I afferent terminals in intermediate regions of the lumbar spinal cord to changes in the amplitude of monosynaptic group I field potentials recorded in the same regions. Some results have been presented in abstract form (Gosgnach *et al.* 2001).

METHODS

For a more detailed description of the methods refer to Paper #1. Briefly, experiments were performed on 8 cats of either sex weighing between 2.0-3.4kg. The animals were anaesthetised and select peripheral nerves were dissected in preparation for stimulation and recording. Following this, the animals were moved to a rigid frame and a mechanical postmammilary-precollicular decerebration was performed with removal of the corticies and rostral tissue. Glass microelectrodes filled with 2M sodium citrate were used for recording extracellular field potentials evoked by peripheral nerve stimulation. Fictive locomotion was evoked by unilateral or bilateral stimulation of the MLR (80-400 μA, 1ms pulses at 15-25Hz)

For analysis purposes, group I extracellular field potentials were considered to be located in the intermediate nucleus if they were recorded at a depth between 1.3 and 2.4mm from the dorsal surface in the spinal cord. They were considered to be monosynaptic if they had a central latency of 0.7-1.0ms. Field potential measurements were made at a fixed latency just before the peak of the downward deflection. The protocol for collection and analysis of data was as follows. The glass recording electrode was mounted on a microdrive and the tungsten stimulating electrode (Microprobe Inc, Potomoc USA, 76mm length, 300kΩ tip resistance) was mounted on a second microdrive which was attached to the first microdrive at a fixed angle but could also be moved up and down independently. The electrode tips were aligned above the spinal cord using a microscope and a small x-y positioning device on the recording electrode assembly. The stimulating electrode was then

raised approximately 5mm away from the recording electrode. The recording electrode was positioned medial to the dorsal root entry zone and lowered into the intermediate nucleus of the spinal cord until a group I field potential (resulting from peripheral nerve stimulation) at least 200 μV in amplitude could be recorded. The stimulating electrode on the second microdrive was then lowered to within approximately 200μm of the tip of the recording electrode. The following protocol was employed; 1) Intraspinal stimulus intensity was adjusted (0.2ms square, negative pulse, range 4-25 μA) to antidromically activate ipsilateral muscle afferents. Care was taken to use a stimulus intensity well below (typically <1/2) that which evoked a maximal antidromic discharge in the peripheral nerve. 2) We then examined the effects of conditioning stimulation of the ipsilateral PBSt muscle nerve (3-5 shocks, 300Hz, 2-5T). An increase in antidromic discharge (>5%) was interpreted as evidence for an increased excitability of afferent terminals around the intraspinal stimulating electrode and hence PAD. 3) We determined whether the same conditioning stimulation caused a decrease in the amplitude of the monosynaptic field potential evoked by stimulation of the same or a synergist peripheral nerve and recorded through the glass microelectrode at the same intraspinal location. A reduction in monosynaptic field potential amplitude (>5%) was interpreted as evidence for a reduction in transmission from the group I afferent terminals (i.e. a presynaptic inhibition). 4) We also assessed the effect of locomotion on the antidromic response produced by intraspinal stimulation in the absence of afferent fibre stimulation (see lower panel Fig 2) 5) We assessed the effect of fictive locomotion on monosynaptic field potentials evoked by peripheral nerve stimulation (see upper panel Fig 2). In some cases steps 4 and 5 were combined so that the intraspinal stimulation was given a few milliseconds before the peripheral nerve stimulation to permit direct comparison of the effects of locomotion on afferent fibre excitability and field potential amplitude during the same period of locomotion. In those cases where a clear terminal potential (see Figure 1 General Introduction for detailed description) was observed, the peak to peak amplitude was measured in an attempt to measure the spread of depolarizing current into the presynaptic boutons (see Sypert *et al.*). No attempt was made in this study to examine changes in sensory evoked PAD during fictive locomotion (e.g. Menard *et al.* 2002).

RESULTS

In total 34 afferent terminal excitability measurements were made during control and fictive locomotion in 8 cats. In 28 cases extracellular group I field potentials were also recorded in close proximity (≤200μm) to the intraspinal stimulation site. In 26 cases, in the absence of locomotion a short stimulus train to a hindlimb flexor nerve was used to produce a sensory evoked presynaptic inhibition and its effect on the excitability of group I terminals and monosynaptic field potentials.

Correspondence between field potential depression and PAD during sensory evoked PAD.

An example of the effect of conditioning stimulation on the antidromic volley and the extracellular field potential is illustrated in Figure 1.

INSERT FIGURE 1 HERE

The upper panel illustrates the stimulation/recording setup used. A group I field potential (central latency 0.9ms) was evoked by FDHL nerve stimulation (2T, single shocks delivered at 4Hz see panel B solid upper trace, field potential indicated by open arrow) and recorded in the rostral L6 spinal segment in the intermediate nucleus (depth 2.0mm). Immediately after collecting this data (<60s), an antidromic response was evoked by intraspinal stimulation (17μA) through the tungsten electrode positioned approximately 200μm above the glass recording electrode and recorded in the LGS nerve (see panel C, solid upper trace, antidromic response indicated by filled arrow). The antidromic response had a latency of 2.1ms. Stimulation of the PbSt nerve (5Tx4) 24 ms before intraspinal stimulation was then used to evoke presynaptic inhibition. Panel B illustrates the sensory evoked reduction in the FDHL field potential of 16% (dashed line) compared to the control field potential (solid line). In panel C the effect of the same PbSt stimulation on the antidromic response in the LGS nerve is illustrated. The conditioned response (dashed line) is 40% larger than the control response (solid line).

In all 26 cases conditioning stimulation of a flexor nerve (PbSt) resulted in both an increase in nerve terminal excitability (mean 34%) and a decrease in the amplitude of the

group I field potential measured in the same spinal location (mean 21%). In those cases (n=11) in which the antidromic response and field potential were evoked at the same interval following conditioning stimulation, field potentials were depressed by an average of 21% and antidromic responses were increased by 32%. In the remaining 15 cases the antidromic response and the field potential were evoked at slightly different latencies (±7ms) from the conditioning stimulation. The significant observation is that in all 26 cases, sensory stimulation resulted in both a decreased field potential amplitude and an increase in afferent terminal excitability.

Lack of correspondence between field potential depression and PAD during locomotion.

The primary aim of this section of the thesis was to compare changes in field potential amplitude and afferent nerve excitability induced by fictive locomotion without conditioning peripheral nerve stimulation. We observed 3 different patterns of effects on terminal excitability and field potentials during fictive locomotion. The first was a decrease in amplitude of the group I field potential and an increase in amplitude of the antidromic response. An example is illustrated in Figure 2.

INSERT FIGURE 2 HERE

A stimulating electrode and recording electrode were placed within about 200μm of each other in the caudal L6 segment of the spinal cord (depth 2.3mm). Throughout the control and locomotor periods 15μA pulses were passed through the intraspinal stimulating electrode to produce an antidromic response in the LGS nerve. Stimulation of the MLR evoked a period of fictive locomotion represented by alternating activity between the PLong and SmAB nerves (Fig. 2A). Panel B illustrates the averages of the group I field potentials evoked by LGS nerve stimulation (2T,5Hz) before (control), and during the period of MLR-evoked fictive locomotion indicated by the horizontal bar. During locomotion the field potential is depressed by 28% (32% during flexion, 27% during extension). In panel C the antidromic responses to intraspinal stimulation recorded in the LGS nerve corresponding to each of the fields in panel B are illustrated. During locomotion the response increased by

24% (23% during flexion, 27% during extension) suggesting that the LGS afferents were more depolarized. In this example locomotion resulted in both an increase in terminal excitability and a decrease in field potential amplitude.

The second pattern observed during locomotion was one in which there was a decrease in extracellular field potential amplitude but no change in the terminal excitability.

INSERT FIGURE 3 HERE

Figure 3A illustrates the decrease in amplitude of the extracellular field potential (evoked by Pl nerve stimulation) during locomotion. The field potential during locomotion (dashed line) is reduced by 22% compared to control (solid line). The lower trace in panel A illustrates the antidromic responses recorded in the MG nerve (latency 1.8ms) evoked by intraspinal stimulation (20μA) during control (solid line) and locomotion (dashed line). The two short latency responses are superimposable. In this trace there is also a delayed response to the intraspinal stimulation during locomotion (marked by *, latency 2.6ms). This is likely an orthodromic synaptically evoked response of MG motoneurons to the intraspinal stimulation. In panel B the nerve records were averaged and separated into those evoked in the flexion (solid) and extension (dashed) phases and then averaged. The late response (*) is large in extension, when the MG motoneurons are depolarized and absent during flexion when MG motoneurons are hyperpolarized. A similar orthodromic response can be seen in Fig 2C (*).

The third pattern observed during locomotion was a decrease in extracellular field potential amplitude and a decrease in afferent fibre excitability.

INSERT FIGURE 4 HERE

Figure 4A illustrates the extracellular field potentials evoked by Q nerve stimulation (2T, 5Hz) recorded 200μm away from the intraspinal stimulating electrode. The locomotor field potential is 35% smaller than the control field with little difference in the fields evoked during flexion or extension. In Panel B antidromic responses evoked in the Q nerve by intraspinal stimulation (20 μA) are illustrated. In this case the response during locomotion is

reduced by 15% compared to control and is similarly reduced in flexion (19%) and extension (12%). This is an example of primary afferent hyperpolarization during locomotion. In this case a process other than PAD must be acting on the terminals to produce the field potential reduction.

In total 34 measurements (21 from extensor nerves, 13 from flexor nerves) of afferent excitability were made during both control and locomotor conditions. In 28 of these cases a group I field potential was also measured. This data is summarized in Figure 5.

INSERT FIGURE 5 HERE

Antidromic responses to intraspinal stimulation during locomotion are plotted, as percentages of control amplitude, in ascending order with circles indicating the mean percent modulation of antidromic volley amplitude throughout the locomotor run. The vertical lines represent phasic modulation between the phases of locomotion with the open squares indicating amplitude recorded in the flexor phase. The most compelling data in this figure is that obtained with stimulation and recording from the same peripheral nerve (i.e. starred bars in Fig. 5). In all 10 of these cases the monosynaptic field potential was depressed during locomotion. In 3 cases there was a concomitant increased in excitability during locomotion (i.e. PAD), in 2 cases there was no change in afferent excitability, and in 5 cases there was an increase in afferent excitability (i.e. PAH). In total, the excitability of the afferents increased in 14 cases, decreased in 13 cases and did not change (i.e. $\leq$5% change) in 7 cases. The mean change in nerve excitability during fictive locomotion for all 34 cases was an increase by 1% during locomotion (overall a 2%$\pm$25% decrease in extensor nerves n=21, 3%$\pm$27% increase in excitability of flexor nerves n=13). In 14/34 (9 extensor nerves, 5 flexor nerves) measurements there was a phasic modulation (defined as a greater than 10% difference between the phases) of peripheral nerve excitability. Of the nine excitability measurements made in extensor nerves, 5 were larger in extension and 4 were larger in flexion. Of the 5 flexor afferents which displayed a phasic modulation, 4 were more excitable during flexion. The extracellular field potential amplitudes (plotted as percent of control) measured in the same location as the intraspinal stimulation are represented by the open bars. Those bars that

have an asterisk at the base indicate recordings in which the same nerve was used to evoke field potentials and record the antidromic responses.

Twenty five of the 28 group I evoked extracellular field potentials were depressed, by a mean of 25% (26% depression of extensor fields, 23% depression of flexor fields). Of the field potentials that were depressed, 10 were associated with a depolarization of primary afferents during locomotion, 5 were accompanied by no change (±<5% change) in the polarization of primary afferents during locomotion, and 10 were associated with a hyperpolarization of primary afferents during locomotion. Of the 3 field potentials that increased in amplitude during locomotion, 2 were associated with a primary afferent hyperpolarization and the other with no change in afferent fibre polarization. From this figure it is evident that field potential amplitude can be depressed in the absence of PAD, and in some cases in the presence of primary afferent hyperpolarization.

DISCUSSION

There is overwhelming evidence from both human and animal experimentation that at the initiation and during movements, presynaptic mechanisms regulate transmission from primary afferents to their spinal targets (see Introduction - Capaday and Stein 1986; 1987; Hultborn *et al.* 1987; Gossard *et al.* 1991. Gossard 1996; Bayev and Kostyuk 1981; Bayev and Kostyuk 1982; Duenas and Rudomin, 1988). Our laboratory has used two techniques in decerebrate cat preparations to show that there is a general and tonic depression of synaptic depression from group I (Ia+Ib), Ia, group II and cutaneous afferents during MLR evoked fictive locomotion and during fictive scratch. Thus both extracellular records of monosynaptic field potentials in the intermediate (Perreault *et al.* 1999b; Gosgnach this thesis) and ventral horn (Gosgnach *et al.* 2000 and this thesis) and intracellular recordings from motoneurons (Gosgnach *et al.* 2000 and this thesis) show, with few exceptions, a depression of monosynaptic excitation from segmental afferents during fictive locomotion and scratch.

The present study is the first to address the question of whether increases in the excitability (i.e. PAD) of group I afferents are correlated with decreases in the monosynaptic activation of interneurons in the intermediate nucleus of the lumbar spinal cord as assessed by the population response recorded in the field potential. In the case of classical presynaptic

inhibition in which conditioning stimulation of a flexor nerve precedes the test monosynaptic excitation of neurons (e.g. Fig 1) there is a consistent correlation between the inhibition of the monosynaptic excitation (i. e. field potential depression) and the depolarization of group I afferents (i. e. increase in the intraspinally-evoked antidromic discharge recorded in muscle nerves). During fictive locomotion, however, this consistent relation broke down such that in only about 1/3 of the cases were both field potentials depressed and antidromic discharges increased. In the other cases there was either no change or a reduction in the antidromic discharge as field potentials were inhibited during fictive locomotion. This was evident for both homonymous pairs of fields and nerve recordings (Fig. 5 starred bars) obtained during different bouts of locomotion but within a few seconds of each other and for heteronymous pairs (e.g. evoking a field potential from one extensor nerve while recording from another extensor nerve) obtained during the same bout of locomotion.

The terminal potential is an early component of the extracellular field potential (see Figure 1, General Introduction for details) which is thought to reflect the spread of depolarizing current into presynaptic boutons in the region of the microelectrode tip (see Sypert *et al.* 1980). In those cases in which a terminal potential was observed, the effects of conditioning stimulation (n=13) and during locomotion (n=14) on its amplitude were measured. In both cases there was a only a very weak relationship between extracellular field potential depression and terminal potential depression (r^2=.06 conditioning, r^2=.43 locomotion).

Work in other laboratories has used changes in the membrane potential of primary afferents to make inferences about presynaptic control during locomotion or scratch. Results from those studies have provided somewhat conflicting assessments of this presynaptic control during motor tasks. For example, excitability measurements of single group I afferent terminals in the intermediate nucleus show a tonic depolarization upon which phasic depolarizations can be superimposed (Duenas & Rudomin 1988). On the other hand, Bayev and Kostyuk (1982) using the multifibre Wall technique reported a consistent hyperpolarization of group I and cutaneous afferents during fictive locomotion whereas the same laboratory reported a tonic depolarization of group I fibres during fictive scratch

(Bayev and Kostyuk 1981). The difference between afferent excitability during scratch and locomotion is surprising considering that group I monosynaptic EPSPs and field potentials are depressed by similar amounts during both fictive locomotion and scratch (see paper#1 this thesis).

Gossard *et al.* (1991) and Gossard (1996) reported rhythmic depolarizations of Ia and cutaneous afferents that often had 2 components with the larger depolarization occurring during the flexion phase. Unfortunately those studies never examined the possibility of a tonic depolarization of primary afferent terminals. In addition Gossard (1996) raised the possibility that an examination of the membrane potential of afferents may not reflect changes in synaptic transmission from Ia afferents to motoneurons. In those heroic experiments, simultaneous recordings from primary afferents and their target motoneurons showed poor correlation between the membrane potential of the afferents and the magnitude of the EPSP recorded in motoneurons. The present data is in agreement with this poor correlation between the locomotor phase in which maximal single fibre PAD and minimal EPSP amplitude occurred (Gossard 1996).

When one combines the observations that: 1) PAD of group I fibres is not well correlated with postsynaptic EPSP amplitude (Gossard 1996), 2) locomotion can be associated with a either tonic PAD (Duenas and Rudomin 1988) or PAH (Bayev and Kostyuk, 1982) 3) group I field potentials can be depressed in the presence of a PAD, PAH, or no change in afferent polarization and 4) that terminal potential changes were poorly correlated with field potential depression (present study) one is lead to the suggestion that the depolarization of single afferent fibres is not the primary mechanism underlying the presynaptic inhibition of group I afferents during locomotion. Therefore we propose that measurement of afferent fibre excitability is not an appropriate technique for the study of presynaptic depression of synaptic transmission during locomotion in the cat. This is in contrast to the case of the invertebrate in which PAD appears to be an accurate measure of presynaptic inhibition since it has been demonstrated that during locomotion the PAD amplitude is inversely proportional to the orthodromic spike amplitude which is in turn proportional to the response in the target motoneuron (Cattaert *et al.* 1992; Burrows and Matheson, 1994). The presence of PAD and PAH has also been used to suggest the existence

of presynaptic changes in transmitter release from sensory afferents during micturition (Angel *et al.* 1994; Buss & Shefchyk 1999). On the basis of the present studies showing a clear dissociation between PAD and presynaptic inhibition, this assumption may require revisiting.

One limitation of this study is that intraspinal stimulation was delivered in the intermediate nucleus where both group Ia and group Ib afferents have synaptic terminals and often end on the same interneurons (see Jankowska 1992). We are thus unable to determine whether there are differences between the occurrence of locomotor dependent PAD of Ia and Ib afferents. It remains a theoretical possibility that one subset of the group I fibres was depolarized during locomotion while the other was hyperpolarized. If that were the case, preferential activation of one fibre type would lead to the recording of a PAD in some cases, a PAH in others or a mixture of a PAH and PAD that resulted in little change in the antidromic volley recorded in the periphery. Had we stimulated within the ventral horn, only Ia terminals would have been activated. However ventral horn stimulation would also activate motoneurons antidromically and complicate discerning changes in PAD. Of course this could have been avoided by cutting ventral roots and thereby leaving only the antidromic discharge of Ia afferents in the peripheral nerve. This was not done in the present experiments since it would have greatly compromised our ability to monitor fictive locomotion.

Another limitation of the present study was the use, in some cases, of different nerves for recording antidromic excitability and for stimulating to evoke monosynaptic field potentials (bars without stars in Fig. 5). This assumes that the locomotor related presynaptic inhibition is exerted equally on synergist as well as homonymous nerves (see Gosgnach *et al.* 2000), which, as the first paper in this thesis showed, is sometimes not the case. However Figure 5 shows that almost all field potentials (25/28) are depressed during locomotion. The most compelling data is that obtained with stimulation and recording from the same peripheral nerve (i.e. starred bars in Fig. 5). In those 10 cases 3 examples of PAD were seen during locomotion, 2 of no effect on the primary afferents and in 5 cases there was a PAH. In all 10 cases the monosynaptic field potential was depressed during locomotion; a clear dissociation between PAD and presynaptic inhibition.

This paper is the fourth (Perreault *et al.* 1999b; Gosgnach *et al.* 2000; paper #1 this thesis) from this laboratory describing the characteristics of a centrally evoked presynaptic inhibition of group I afferents generated from the central pattern generator. From these studies it is evident that this presynaptic inhibition occurs at the onset of rhythmic activity, the tonic inhibition is usually greater than phasic variations, it often lasts beyond the end of locomotion (Gosgnach *et al.* 2000), and can occur in the absence of a PAD or even in the presence of a PAH. Although many studies have demonstrated that PAD induced by sensory fibre stimulation evokes a presynaptic inhibition at rest (see Willis 1999; Schmidt and Rudomin, 1999), our conclusion is that the absence of PAD during locomotion is insufficient evidence for the lack of a locomotor related presynaptic inhibition.

The fact remains that there is a presynaptic inhibition of sensory information from cutaneous, group I and group II muscle afferents during movements such as locomotion. In the case of group II afferents, terminals ending in the intermediate regions are subject to a more powerful presynaptic inhibition during fictive locomotion than terminals of the same fibres ending less than a millimetre away in the dorsal horn (Perreault *et al.* 1999b). This suggests that the locomotor dependent presynaptic depression is evoked through either a variety of mechanisms (e. g. different transmitter or receptor systems) or by differential expression on particular afferents (e. g. the number of presynaptic inhibitory contacts on individual afferents). The present results show that for group I afferents, a mechanism other than PAD is at least partially responsible for reduced transmitter release in the intermediate nucleus. The nature of this other mechanism has not been addressed. A simple explanation for our findings that is consistent with the known role of GABA in sensory-evoked presynaptic inhibition, could involve different effects mediated through $GABA_A$ and $GABA_B$ receptors. This suggestion assumes that interneurons located near the group I terminals in the intermediate spinal nucleus are activated for prolonged periods during locomotion by the CPG network. Accordingly, release of GABA from these interneurons would activate the ionotropic $GABA_A$ receptors resulting in a PAD that in some cases desensitises and is abolished after a relatively short time period. It seems reasonable to speculate that at the same time, but with a longer time course of onset, metabotropic GABA actions on $GABA_B$ receptors are activated, persist and produce a presynaptic reduction in transmitter release that

continues during and beyond the locomotor period. This potential mechanism is discussed further in the General Discussion section of this thesis with special reference to the work of Curtis and Lacey (1998) and with consideration to involvement of other presynaptic transmitter systems.

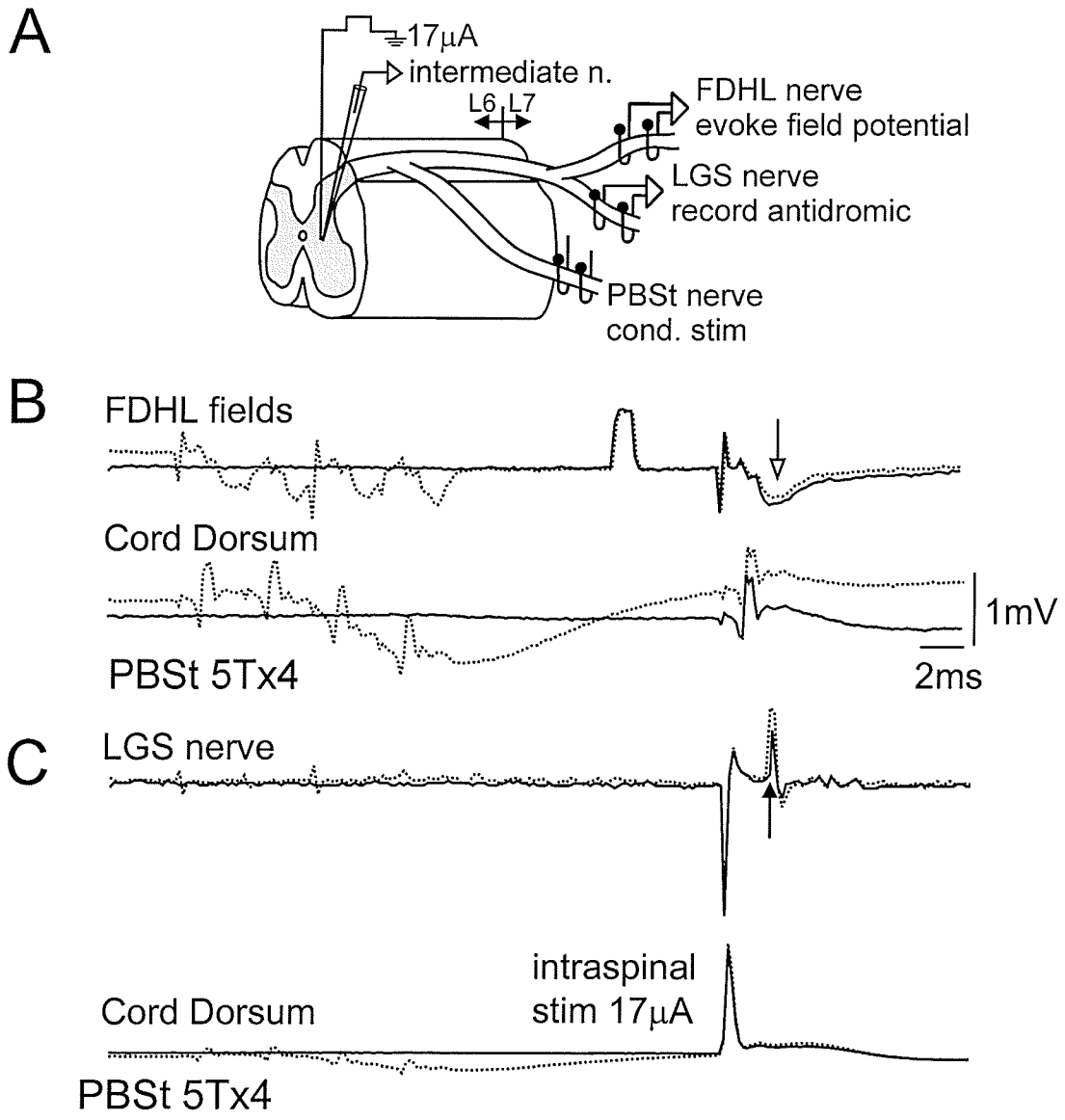

Figure 1. *Conditioning stimulation results in PAD and depression of group I field potentials.* **A** Intraspinal stimulation (17μA) in the intermediate region (depth 1.7mm) of the rostral L6 segment evokes an antidromic response in the LGS nerve. PBSt nerve stimulation (5Tx4) is used as conditioning stimulation to induce sensory-evoked PAD . A group I field potential evoked by FDHL nerve stimulation (2T, 4Hz) is recorded 200μm from the stimulating electrode. **B** The amplitude of the conditioned field potential (dashed trace) is reduced by 16% compared to the control field potential (solid line). **C** When the LGS nerve is conditioned with PBSt stimulation (dashed trace) the antidromic response is increased by 40% compared to control (solid line).

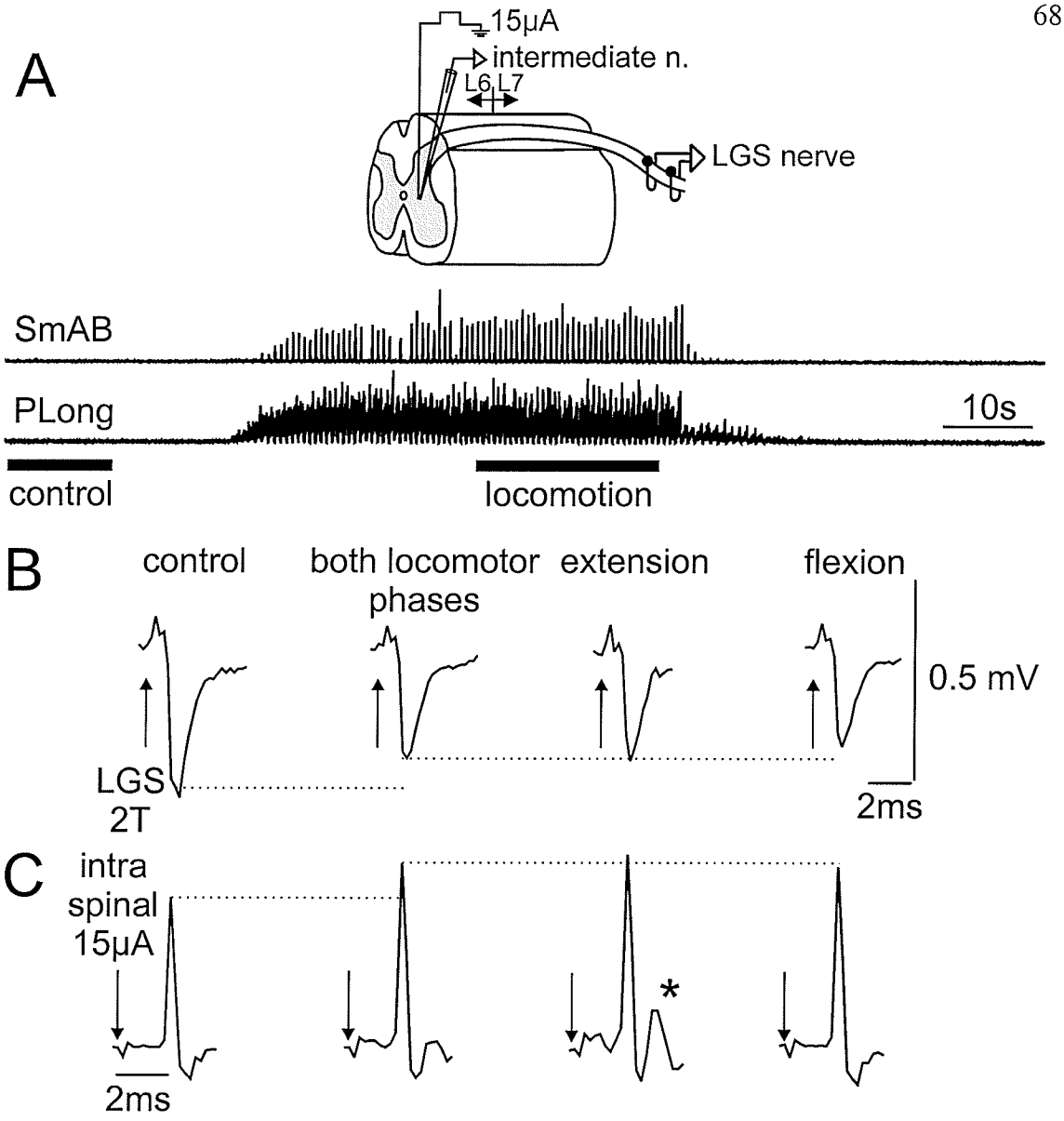

Figure 2. *Locomotion can result in a depression of group I field potentials and a PAD*. **A** Stimulating and recording electrodes located within 200μm of each other in the caudal L6 segment (depth 1.8mm). Antidromic response in the LGS nerve is evoked by intraspinal stimulation (15μA). Group I field potentials are evoked by LGS nerve stimulation (2T, 5Hz). Below, rectified, integrated ipsilateral ENGs from extensor (SmAB) and flexor (PLong) peripheral nerves before and during and after a run of MLR evoked locomotion. **B** Averages of group I field potentials taken from the indicated time period in panel A are illustrated before (control) and during (both locomotor phases) fictive locomotion. During locomotion the field potential is depressed by 28% (32% during flexion, 27% during extension). **C** Antidromic response of LGS nerve to intraspinal stimulation is increased by 24% during locomotion (23% during flexion, 27% during extension).

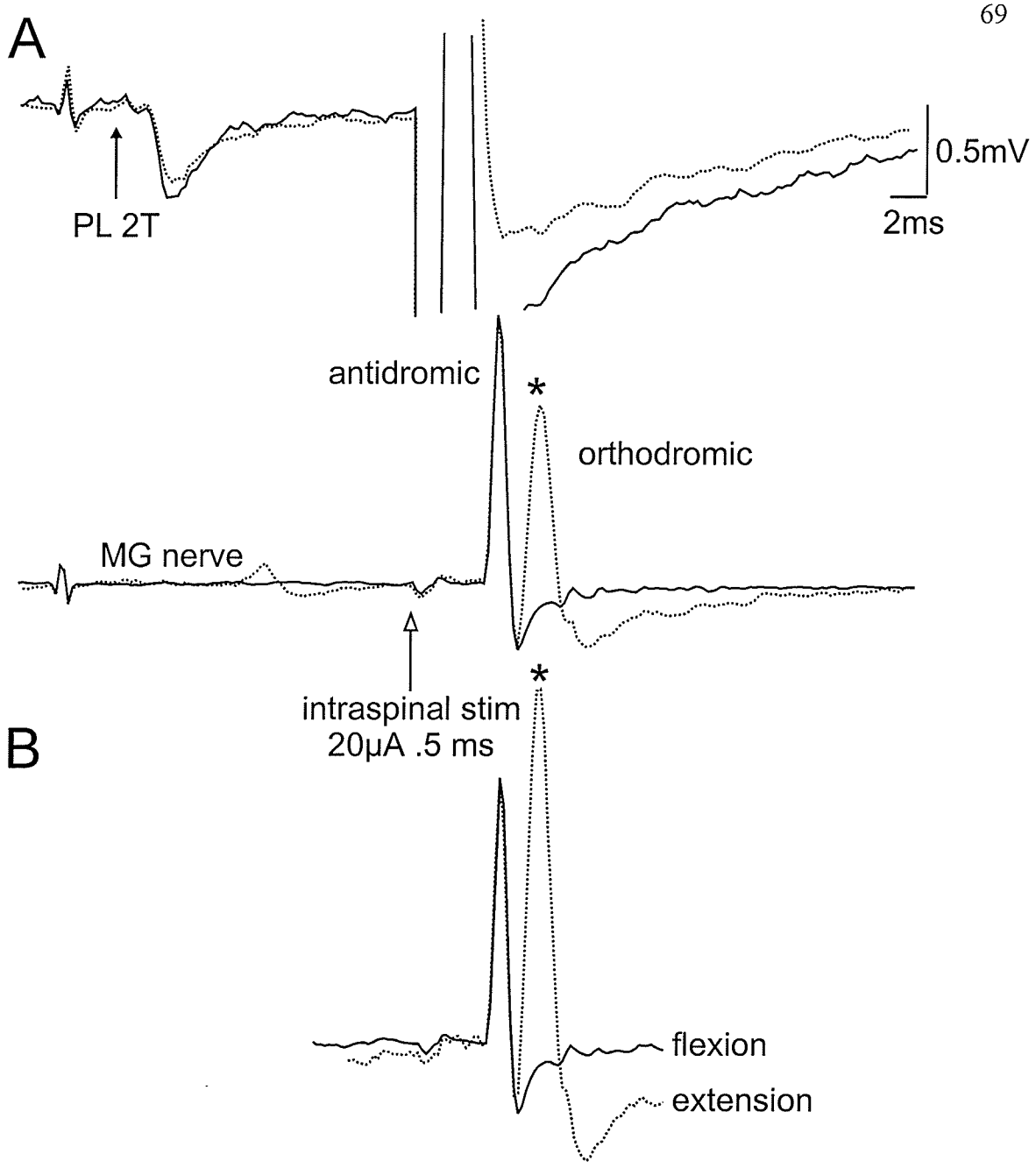

Figure 3. *Group I field potential depression during locomotion can occur without a change in afferent fibre polarization.* **A** Group I field potential (upper traces) evoked by PL nerve stimulation (2T, 5Hz) during locomotion. The field potential during locomotion (dashed line) is reduced by 22% compared to control (solid line). Antidromic responses in the MG nerve (lower traces, evoked by 20μA intraspinal stimulation) is similar during control (solid line) and locomotion (dashed line). **B.** Locomotor antidromic response divided into flexion (solid line) and extension (dashed line). Longer latency (*, 2.6ms) response only occurs during extension suggesting it is orthodromic.

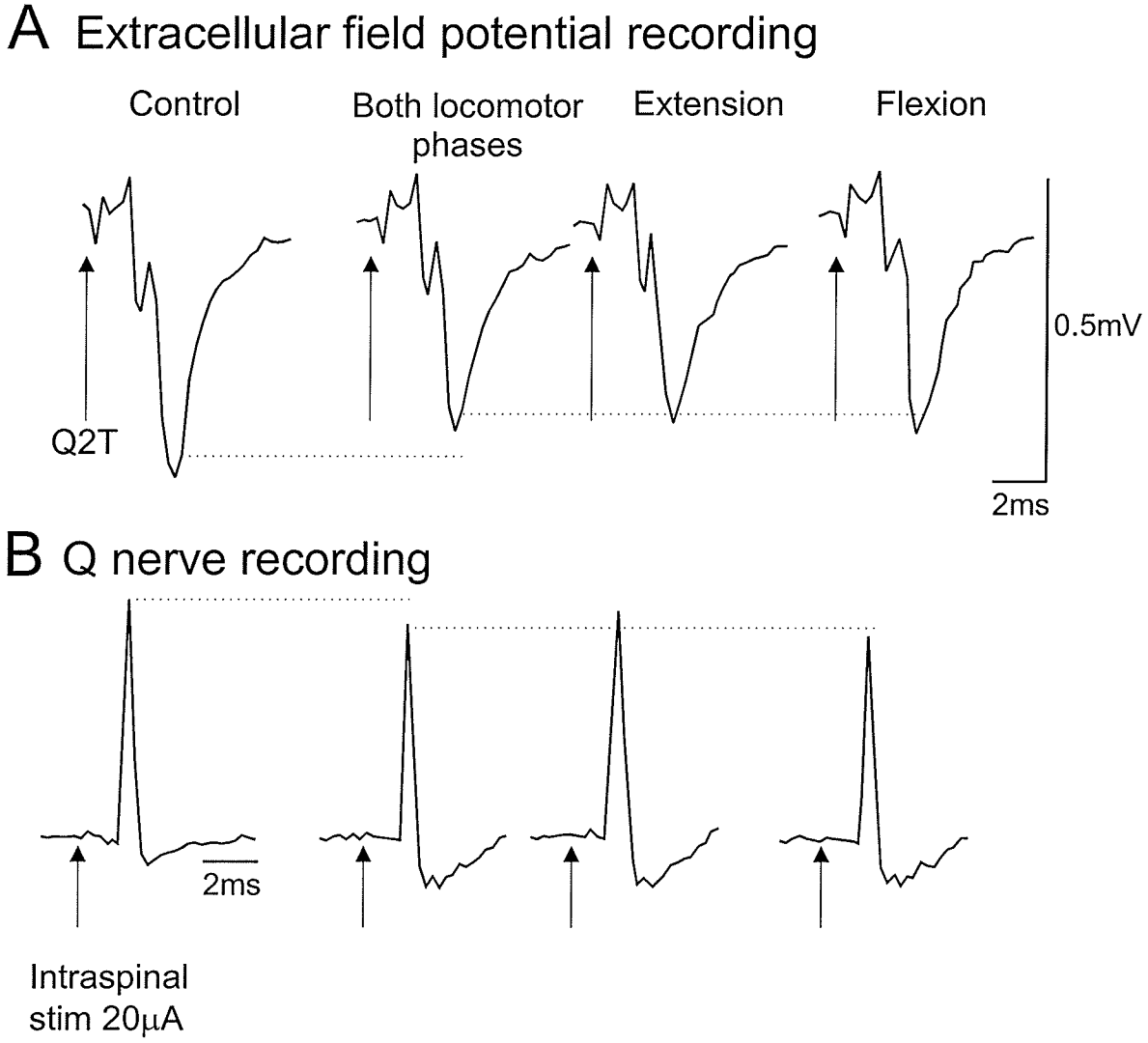

Figure 4. *Locomotion can result in group I field potential depression and a PAH* **A** Extracellular field potentials evoked by Q nerve stimulation (2T, 5Hz) recorded 200μm away from the intraspinal stimulating electrode. Locomotor field potential is 35% reduced compared to control field potential. Field potential amplitude is reduced in both flexion (36%) and extension (31%). **B** Intraspinal stimulation (20μA) evokes an antidromic response in Q nerve. The locomotor response is reduced by 15% compared to control. Antidromic response is decreased in both flexion (19%) and extension (12%).

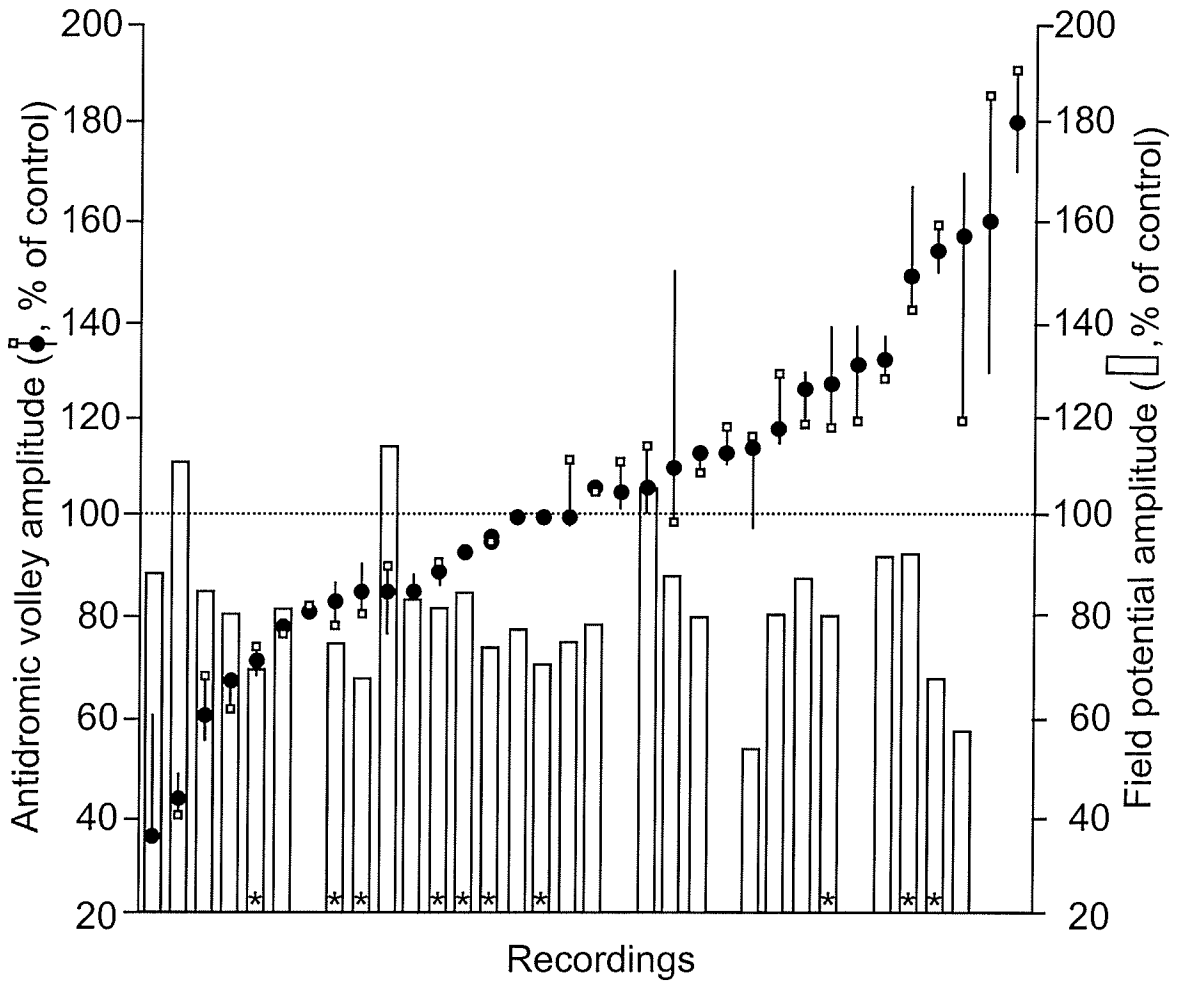

Figure 5. *Relationship between afferent fibre polarization and group I field potential during locomotion.* Antidromic responses to intraspinal stimulation during locomotion are plotted in ascending order on the left ordinate. Circles represent mean polarization during locomotion (as a % of control). Vertical lines represent phasic modulation with open squares indicating the flexor phase. Extracellular field potential amplitude (as a % of control) measured within 200μm of intraspinal stimulation is plotted on the right ordinate represented by the open bars. Those bars which have an asterisk at the base indicate recordings in which the same nerve was used to evoke field potentials and record the antidromic responses.

General Discussion

Overview of Results

This thesis characterizes the tonic, centrally generated presynaptic inhibition of group I afferents which occurs during both fictive locomotion and scratch in the decerebrate cat. In the first paper, paired group I extracellular field potential recordings made from the ventral horn and intermediate nucleus demonstrate that there are substantial regional variations in the amount of presynaptic inhibition exerted. This leads us to believe that the centrally generated presynaptic inhibition of transmission in the group I pathway during locomotion is expressed focally in different areas of the lumbar cord. However, due to the similarities in mean field potential depression between these two areas, we suggest that divergence of individual group I afferents onto interneurons, convergence of afferents onto individual interneurons, and convergence of interneurons to motoneurons negates specific regional variations in synaptic transmission resulting in a generalized and tonic gain reduction during fictive locomotion. Also in the first paper, intracellular and extracellular recordings of the same group I EPSPs and field potentials made during both fictive locomotion and fictive scratch demonstrate that transmission in the group I pathway is depressed by a similar amount during both behaviours. Based on this observation, we conclude that the centrally generated presynaptic inhibition is a component of the motor program used for locomotion and scratch, rather than a process evoked by electrical stimulation of the MLR.

The experiments described in the second paper use the Wall technique to investigate whether the locomotor related presynaptic inhibition is due solely to a depolarization of primary afferents (PAD). This does not seem to be the case based on the observation that during locomotion, group I extracellular field potentials can be depressed while afferents are either hyperpolarized, depolarized or unaffected. This leads us to suggest that a mechanism other than PAD must be involved in the centrally evoked presynaptic inhibition during locomotion and that recordings of PAD during locomotion are not an accurate measure of centrally generated presynaptic inhibition.

This section of the thesis is devoted to further development of some of the issues raised in the 2 papers as well as the discussion of certain aspects which have not yet been mentioned.

A comparison of sensory-evoked presynaptic inhibition and centrally evoked presynaptic inhibition.

In addition to the centrally generated presynaptic inhibition acting on group I afferents during both fictive locomotion and scratch described in this thesis, it has been demonstrated that a presynaptic inhibition of group I fibres also occurs following sensory nerve stimulation in the cat at rest (Eccles *et al.* 1962) and can also be evoked during locomotion (Menard *et al.* 2002). This is referred to as sensory evoked presynaptic inhibition and has been described in detail in the General Introduction section of this thesis. Although both types of presynaptic inhibition result in a decrease in transmitter release from group I afferents there are differences between these two processes. The first and most obvious is the manner in which each is evoked. Centrally evoked presynaptic inhibition is assumed to be a component of a motor program and it occurs when the animal partakes in a certain patterned behaviour (i.e. locomotion, scratch). The result is a decrease in group I EPSP (Gosgnach *et al.* 2000), field potential (Perreault *et al.* 1999b; Gosgnach *et al.* 2000, paper 1 this thesis), and reflex (Bennett *et al.* 1996) amplitude. In humans this is manifested as a decrease in group I reflex amplitude during locomotion (Capaday and Stein 1986; 1987). The amplitude decrease occurs immediately following the onset of behaviour (within 1s- see Figure 1 Gosgnach *et al.* 2000) depressing control amplitude by 20-30% (Perreault *et al.* 1999b; Gosgnach *et al.* 2000, paper 1 this thesis). It is sustained throughout the period of locomotion recovering between 10s and 4 minutes after cessation of rhythmic activity (Gosgnach *et al.* 2000). Sensory-evoked presynaptic inhibition, on the other hand, is evoked by electrical stimulation of cortical structures or a sensory afferent neighbouring the one studied. This stimulation results in a substantial (>80% - Curtis and Lacey, 1998) decrease in amplitude of the evoked group I EPSP for a time period in the range of hundreds of milliseconds (see Curtis and Lacey, 1998) depending on the exact stimulus parameters. Maximal EPSP depression occurs 20-30ms following the first inhibitory volley (Curtis and Lacey, 1998). It must be noted that the long term depression, which occurs during centrally evoked presynaptic inhibition can occur following sensory stimulation if the conditioning stimulus train is sufficiently long. Curtis and Lacey (1998) observed that following 40s of conditioning stimulation EPSP amplitude is depressed throughout the stimulation period and

for 3 minutes following stimulation. The implications of this on the possible mechanism of centrally evoked presynaptic inhibition are discussed in the section entitled 'Possible mechanisms underlying centrally evoked presynaptic inhibition'.

What is the origin of the centrally evoked presynaptic inhibition?

Upon reporting that during MLR evoked fictive locomotion group I EPSPs and field potentials were substantially depressed (Gosgnach *et al.* 2000), one concern was that MLR stimulation rather than locomotor activity caused a presynaptic inhibition of group I afferents (see Noga *et al.* 1995). To address this concern we examined whether group I field potential depression, similar to that which occurs during fictive locomotion, also occurs during fictive scratch which is a rhythmic alternating behaviour similar to locomotion which is evoked in the absence of electrical brainstem stimulation (see General Introduction for further description). Our observation that group I field potentials are depressed in both behaviours provides strong evidence that the centrally evoked presynaptic inhibition is due to the motor activity itself rather than electrical stimulation of the MLR. Additional support for this hypothesis could be provided by the demonstration that a centrally-evoked presynaptic inhibition occurs during locomotion in other locomotor preparations.

Based on the fact that the centrally generated presynaptic inhibition is evoked by rhythmic activity and it has been shown to be exerted focally in different areas of the lumbar cord (also demonstrated in paper 1), one question that arises is: what is the origin of the centrally evoked presynaptic inhibition which is exerted on the group I fibres during locomotion (and scratch)? Two possibilities immediately come to mind. The first, and most attractive, is that local interneurons exist which are activated by the central circuitry producing locomotion and release transmitter onto group I afferent terminals resulting in presynaptic inhibition. Although there is, as yet, no evidence to support the existence of this mechanism during centrally evoked presynaptic inhibition there is evidence that local interneurons may be involved in sensory evoked presynaptic inhibition (Lomeli *et al.* 1998). Based on the observation in paper 1 that there is a differential control of centrally evoked presynaptic inhibition in adjacent areas of the intermediate nucleus and ventral horn reported in this thesis, one hypothesis is that part of the central locomotor circuitry includes activation of descending systems which regulate the amount of presynaptic inhibition via specific local

interneurons to aid in the locomotor task.

Possible location of local interneurons.

If centrally evoked presynaptic inhibition is a process controlled by the activity of a group of local interneurons, one question that arises is: where are these interneurons? Some insight into this question can be gained by reviewing the current state of knowledge regarding the interneurons responsible for sensory evoked presynaptic inhibition. Although the existence of sensory evoked presynaptic inhibition and PAD has been known about since the middle of the last century, little is known about the location of the interneurons responsible. Studies have shown that microstimulation of a group of cells in laminae V,VI of the lumbar spinal cord produces PAD in group I fibres (Jankowska *et al.* 1981; Solodkin *et al.* 1984; Rudomin *et al.* 1987; Enriquez *et al.* 1996- see General Introduction). Although this information regarding the location of local interneurons responsible for PAD likely pertains to the interneurons responsible for sensory evoked presynaptic inhibition, it may be relevant for centrally evoked presynaptic inhibition since it is assumed that these are groups of GABAergic interneurons and one of our hypotheses regarding the mechanism responsible for centrally evoked presynaptic inhibition (discussed below) involves the activation of a $GABA_B$ receptor which mediates a presynaptic inhibition in the absence of a PAD. It is thus possible that one group of GABAergic interneurons may be responsible for both types of presynaptic inhibition.

PAD cannot be used as a measure of centrally evoked presynaptic inhibition.

Ever since the demonstration of a tonic (Duenas and Rudomin, 1988) as well as a rhythmic depolarization (Duenas and Rudomin, 1988; Gossard *et al.* 1991) of group I primary afferents during locomotion, it has been assumed that this mechanism reflects a presynaptic inhibition of group I afferents. This assumption was based on the analogy to sensory-evoked presynaptic inhibition which is strongly associated with a PAD (Eccles *et al.* 1962) and the observation that the amplitude of PAD occurring during locomotion in the invertebrate is inversely related to the presynaptic spike amplitude and postsynaptic response (Cattaert *et al.* 1992). However it has yet to be demonstrated, during locomotion in the mammal, that either the tonic or phasic PAD results in a presynaptic inhibition. In fact it has been demonstrated that the phasic PAD that occurs during locomotion is not correlated with

postsynaptic group I EPSP amplitude (Gossard 1996). This leads to the suggestion that although PAD occurs during locomotion its presynaptic effects on group I fibres are minimal. This thesis, which is the first study to investigate the association between the tonic presynaptic inhibition during locomotion and PAD comes to the same conclusion, that PAD does not reflect the pattern or degree of depression of monosynaptic transmission from group I afferents. It is clear that a process other than PAD must play a role in centrally evoked presynaptic inhibition, at the very least in those cases in which afferent fibres were not depolarized and field potentials amplitude was reduced.

What is the role of PAH?

The observation of a hyperpolarization of afferents (PAH) during locomotion in some cases led us to consider the effect that this may have on synaptic transmission from group I fibres. Previous investigations have suggested that a hyperpolarization of afferents results in an increase in neurotransmitter release (Mendell and Wall 1964). Most of the support for this theory comes from studies investigating post-tetanic potentiation where positive afterpotentials in a high frequency volley of impulses summate and leave a long lasting hyperpolarization of the terminals which carried the volley (Lloyd 1949; Wall and Johnson 1958). In addition, Eccles and Krnjevic (1959) demonstrated that artificial hyperpolarization of the spinal cord resulted in increased amplitude of afferent spikes and postsynaptic EPSPs. One theory to explain why PAH results in an increase in transmitter release contends that the amount of transmitter release is controlled by presynaptic membrane potential (Takeuchi and Takeuchi 1962), an increase in membrane potential (PAD) being associated with an inhibition of transmitter release and a decrease in membrane potential (PAH) resulting in an increase in transmitter release. Based on the fact that PAH has been shown to enhance synaptic transmission but the PAH during locomotion is commonly associated with a presynaptic inhibition (this thesis) provides further evidence that polarization of afferent terminals plays a minor role in the amount of transmitter release from group I afferents during locomotion.

Possible mechanisms underlying centrally evoked presynaptic inhibition

Despite the uncertainty regarding the underlying mechanism, the fact remains that there is a centrally evoked presynaptic inhibition of sensory input during locomotion. Two

mechanisms which are active during locomotion and possibly underly the centrally generated presynaptic inhibition observed in the present study are: a descending monoaminergic presynaptic inhibition and a $GABA_B$ receptor mediated presynaptic inhibition.

During locomotion descending pathways releasing 5-HT (raphespinal tract) and noradreneline (coerulospinal tract) are activated (Gerin *et al.* 1995; Fyda *et al.* 1997). These monoamines have been shown to have a variety of presynaptic actions on group I fibres in the lumbar cord including inhibition (Noga *et al.* 1992), no effect (Bras *et al.* 1990) or facilitation (Jankowska *et al.* 2000). Based on these findings it is clear that serotonin and noradrenaline affect transmission from group I afferents. The mechanism by which monoamines affect transmitter release is unknown. Interestingly, it has been demonstrated in deep dorsal horn cells of the rat that 5-HT causes a depression of synaptic transmission that is unrelated to PAD (Lopez-Garcia and King, 1996). The authors suggested that 5-HT was affecting synaptic transmission via 5-HT_1 receptors, the activation of which results in the blockade of a calcium conductance and thus a presynaptic inhibition. Alternatively, a recent study has suggested that 5-HT can presynaptically affect synaptic transmission by activating a G-protein-coupled receptor that binds to one of the proteins responsible for exocytosis (Takahashi *et al.* 2001). Thus, based on the fact that monoamines are released during locomotion and can reduce transmitter release from group I afferents in the absence of a PAD, it is possible that monoamines released in the spinal cord result in the centrally evoked presynaptic inhibition.

Another possibility is that activation of $GABA_B$ receptors during locomotion results in the centrally evoked presynaptic inhibition of group I fibres. The tonic and phasic PAD, which can be recorded in afferent fibres during locomotion, suggests that GABA is released in the vicinity of the afferents during locomotion and interacts with $GABA_A$ receptors. The fact that $GABA_B$ receptors are also located on primary afferent terminals in the spinal cord (Price *et al.* 1984) suggests that these receptors are activated during locomotion. $GABA_B$ receptors have been shown to be metabotrobic and their activation initiates a second messenger cascade (Alford and Grillner 1991; Takahashi *et al.* 1998) which results in a reduction of Ca^{2+} entry into the terminal and a decrease in synaptic transmission in the

absence of a PAD (see General Introduction for further description). Whereas the $GABA_A$ receptor mediated response, being ionotropic, would be immediate upon the release of GABA, the $GABA_B$ receptor mediated response would be delayed until the second messenger cascade was activated and would persist until the termination of the second messenger mediated events. This latter mechanism can account for the characteristics of the centrally evoked presynaptic inhibition that we observe during locomotion in the following way: upon initiation of locomotion, the CPG activates a group of local GABAergic interneurons. The release of GABA from these interneurons binds to $GABA_A$ receptors, which immediately results in a PAD of afferents, and $GABA_B$ receptors, which results in a decreased Ca^{2+} influx and presynaptic inhibition once the number of receptors activated is sufficient for initiation of the second messenger cascade. Following cessation of locomotion, the local GABAergic interneurons would cease to be driven by the CPG and thus the ionotropic effects of GABA on the $GABA_A$ receptors would stop almost immediately. The presynaptic inhibition caused by the activation of $GABA_B$ receptors however would persist due to activation of the second messenger system. This could account for the long time course of recovery of group I EPSPs and field potentials after locomotion.

Support for the theory that prolonged exposure to GABA activates $GABA_B$ receptors which resulting in a powerful presynaptic inhibition comes from work by Curtis and Lacey (1998) who demonstrated that following brief conditioning stimulation, presynaptic inhibition of monosynaptic reflex amplitude can be antagonized by $GABA_A$ receptor antagonist bicuculine but not $GABA_B$ receptor antagonist CGP46381. However, following long term conditioning stimulation (40-60s) there is a sustained reflex depression and following cessation of stimulation, a long term depression (about 3 minutes) of monosynaptic reflex amplitude, both of which can be antagonized by CGP 46381 but not bicuculine. In the discussion section of this paper it is stated that:

"....the very small effect of intravenous bicuculine on the inhibition of reflexes during and after continuous flexor-nerve stimulation suggests that the role of $GABA_A$ receptors may be of relatively minor significance in this form of prolonged inhibition, except for a brief period at the beginning of tetanic stimulation."

Since the potential stimulus resulting in firing of the GABAergic inhibitory interneurons

during locomotion (CPG activation) is prolonged, the results from the Curtis and Lacey (1998) study suggest that during locomotion activation of GABAergic interneurons would result in a presynaptic inhibition dominated by a $GABA_B$ receptor process.

Where do we go from here?

The results presented in this thesis lead to two obvious questions: First, Is there a group of local interneurons in the lumbar spinal cord which mediate the centrally generated presynaptic inhibition? Second, what is the mechanism responsible for this presynaptic inhibition? One way to investigate the first question would be to examine the groups of putative PAD interneurons supposedly responsible for the sensory evoked presynaptic inhibition (Jankowska *et al.* 1981; Solodkin *et al.* 1984; Rudomin *et al.* 1987; Jankowska and Riddell 1995; Enriquez *et al.* 1996) and determine if these cells are active during locomotion. Since these cells presumably release GABA resulting in a PAD, it is possible that their activation may also result in a $GABA_B$ receptor mediated presynaptic inhibition such as the one proposed to be responsible for the centrally evoked presynaptic inhibition during locomotion (see above). Spike triggered averaging from these interneurons could be used to determine their activity during locomotion and the effect that their firing has on group I field potentials. Although it would be beneficial to know if these interneurons are activated during locomotion, this technique will only give a measure of ionotropic events (i.e. $GABA_A$ receptor mediated) and not those which are metabotropic (i.e. $GABA_B$ receptor mediated) and thus may be of limited usefulness.

The most effective method of determining the neurotransmitter responsible the centrally generated presynaptic inhibition is to use local application (i.e. iontophoresis) of various compounds during locomotion and examine whether this eliminates the tonic extracellular field potential depression during locomotion. A similar experiment has been done to investigate whether $GABA_B$ receptors play a role in sensory evoked presynaptic inhibition (Stuart and Redman, 1992). They studied the effects of the $GABA_A$ receptor antagonist bicuculine and the $GABA_B$ receptor antagonist saclofen on the sensory evoked presynaptic inhibition and observed that bicuculine reduced the presynaptic inhibition by a large amount and saclofen had little effect and thus concluded that $GABA_A$ receptors were primarily involved in sensory evoked presynaptic inhibition . The same could be done during

locomotion, one could locally apply antagonists to $GABA_B$ (e.g. CGP 46381) and $GABA_A$ (bicuculine) to group I afferent terminals during locomotion, observe the effect on group I extracellular field and EPSP depression, and determine the relative amount of inhibition that can be accounted for by the activation of each of these receptors. Local application of 5-HT antagonists would be more difficult due to the large number of 5-HT receptors ($5\text{-}HT_{1A\text{-}F}$, $5\text{-}HT_{2A\text{-}C}$, $5\text{-}HT_3$, $5\text{-}HT_4$, $5\text{-}HT_{5A\text{-}B}$, $5\text{-}HT_6$, $5\text{-}HT_7$), the uncertainty as to which are located on afferent terminals (see Schmidt and Jordan, 2000) and the fact that there has been shown to be considerable heterogeneity in the affinity of the agonists and antagonists for different receptor subtypes (see Honda and Ono, 1999). Even when investigating the effects of blocking $GABA_B$ receptor activation the results obtained from these experiments may be inconclusive since a negative result would be uninterpretable.

Concluding remarks.

During normal locomotion, it has been demonstrated that there is a presynaptic inhibition that reduces the gain of the group I reflex. The decrease of the reflex gain is presumably beneficial for stability during locomotion. It has been shown that paretic patients with an overactive group I reflex during locomotion display clonus and spasticity (Yang *et al.* 1991). The present study provides some insight into the underlying mechanism responsible for this centrally generated presynaptic inhibition. Based on this thesis as well as two previously published papers from the McCrea lab (Perreault *et al.* 1999b, Gosgnach *et al.* 2000), we now know that there is a centrally evoked presynaptic inhibition of transmission in the group I pathway that occurs immediately with the onset of locomotion and continues for a period of time beyond the end. It is exerted focally to different amounts at different areas in the cord and it cannot be completely accounted for by a PAD mechanism. A complete understanding of this process requires that the interneurons responsible are located and the underlying mechanism determined.

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