TONIC AND PHASIC DEPRESSION OF Ia EPSPs DURING FICTIVE LOCOMOTION.

BY

SIMON GOSGNACH

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

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TONIC AND PHASIC DEPRESSION OF Ia EPSPs DURING FICTIVE LOCOMOTION

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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

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ABSTRACT

Intracellular recordings from hindlimb α-motoneurons were used to examine the effects of fictive locomotion, evoked by stimulation of the midbrain locomotor region (MLR), on group Ia monosynaptic excitatory postsynaptic potentials (EPSPs). The EPSPs were evoked from homonymous or close synergist afferents by electrical stimulation of peripheral nerves at group I strength (≤2T). The intracellular recordings were used to examine differences in EPSP amplitude between the locomotor and non-locomotor state as well as the modulation of EPSP amplitude between the flexor and extensor phases of locomotion. The present study is the first to investigate the change of composite group Ia monosynaptic EPSP amplitude between the locomotor and non-locomotor states.

Ninety of the 101 monosynaptic EPSP recordings were decreased during locomotion compared to their pre-locomotor amplitude (mean depression=32%). Only four EPSPs were increased during locomotion ($\bar{x}$=33%) and 7 EPSPs remained within 5% of their pre-control values during locomotion. In some cases a phasic modulation of EPSP amplitude between the flexor and extensor phases of locomotion was superimposed on this tonic depression. This modulation was inconsistent and usually minor in comparison to the locomotor-induced tonic EPSP depression. The recovery of EPSP amplitude was delayed following cessation of locomotion. Only 8 of the 11 EPSPs examined had recovered within a 5 minute period after the cessation of locomotion ($\bar{x}$=114s). Motoneuron membrane resistance was compared between locomoting and non-locomoting periods. Decreases in motoneuron membrane resistance occurred in all cases in which EPSP amplitude decreased during locomotion ($\bar{x}$=39%) however this decrease was weakly correlated with EPSP depression (r^2=0.32), suggesting that decreases in motoneuron membrane resistance are not the primary mechanism responsible for monosynaptic EPSP depression during locomotion.

In order to investigate group I synaptic activity in the vicinity of the α-motoneurons, extracellular field potential (EFP) amplitudes recorded in the ventral horn were examined before, during and after locomotion. Fifteen of 22 EFPs were decreased during locomotion ($\bar{x}$=22%). One was augmented (12%) and 6 remained within 5% of

control values. Eight of the 15 EFPs examined recovered within 5 minutes after the cessation of locomotion ($\bar{x}$=137s).

Taken together, the decrease in monosynaptic EPSP amplitude, the poor correlation between motoneuron membrane resistance and EPSP decreases, and the decreases in group I EFPs during locomotion are consistent with the hypothesis that during fictive locomotion there is a specific regulation of synaptic transmission from group Ia afferents to motoneurons. This inhibition along with increased excitability of motoneurons and phase dependant disynaptic excitation during locomotion reported in other studies likely contribute to the gain modulation of the stretch reflex during locomotion.

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INTRODUCTION

Although we take the act of walking for granted, it involves the execution of an extremely complex series of events by the spinal cord. The debate concerning the mechanism responsible for locomotion began in the early 1900's during which there were 2 alternative hypotheses to account for locomotion. Charles Sherrington (1910) demonstrated that afferent feedback from limb proprioceptors activates muscles which result in a locomotor-like pattern of activity. He argued that input from limb proprioceptors supports the genesis of the basic locomotor rhythm. The second hypothesis contends that certain centres within the nervous system form a "central pattern generator" (CPG) which activates and coordinates the muscles involved in locomotion. Graham Brown (1914) demonstrated this through his observation of maintained locomotor activity following the transection of dorsal roots which carry afferent input from the periphery to the spinal cord. Examples of locomotor-like activity in the absence of afferent input have since been shown in monkeys (Taub, 1976), in neonatal rats (Kudo and Yamada, 1987) and in cats (Grillner and Zangger, 1974). There are a number of hypotheses attempting to describe the structure and organization of the locomotor CPG (Brown, 1914; Miller and Scott, 1977; Bergman, Burke and Lundberg, 1969; Szekely, Czeh and Voros, 1969; Pearson 1976; Perret and Cabelguen, 1980).

Although the basic locomotor pattern likely results from a spinal CPG, not surprisingly there is evidence that afferent input can alter the locomotor step cycle. Shik, Severin and Orlovski (1966) demonstrated that the mesencephalic cat matches its locomotor speed with that of the treadmill, presumably as a result of afferent input from the limbs. It has been postulated that afferent feedback through actions on the spinal cord controls both the timing of the transition between the phases of locomotion and the adjustments to the locomotor pattern required by the environment (reviewed by Pearson,1995). The way in which afferent input can reflexively regulate CPG operation is currently under investigation in several laboratories.

I. Modulation of reflexes during locomotion

Reflexes consist of a reflex arc which can be thought of as a basic unit of integrated neural activity. The arc consists of a sensory receptor, an afferent neuron, one or more synapses in the spinal cord, an efferent neuron and the effector muscle. In the non-locomoting subject, muscle stretch produces a well documented pattern of muscle activation and inhibition. As will be shown below, during activation of the spinal locomotor circuitry some of the reflexes which occur in the non-locomoting subject are replaced or supplemented by a different set of reflexes. Locomotor reflex modulation has been shown to occur for cutaneous, group II, and group I afferent reflexes including the stretch reflex (discussed below).

Modulation of cutaneous reflexes

Cutaneous reflexes are perhaps the most complicated and least understood segmental reflexes. There appear to be several general principles of organization (see McCrea 1992). The first is that cutaneous reflexes can be highly specialized producing detailed patterns of excitation and inhibition. Such reflexes are particularly prominent in the foot and ankle (LaBella, Kehler and McCrea, 1989; Hongo, Kudo, Oguni and Yoshida, 1990). In 1989, Schmidt, Meyers, Tokuriki and Burke investigated the modulation of transmission in short latency, distal hindlimb cutaneous (superficial peroneal (SP), sural, saphenous and plantar) reflex pathways during fictive locomotion. They found that oligosynaptic EPSPs in flexor motoneurons were consistently enhanced in amplitude during the flexion phase. From this observation they suggested interactions between the locomotor CPG and excitatory cutaneous reflex pathways depend on both the source of the afferent input and on the identity of the target motoneuron population.

Cutaneous reflexes have also been shown to affect muscle activation during locomotion on a larger scale. Following stimulation of the superficial peroneal (SP) nerve (innervating the dorsum of the paw), Forssberg, Grillner and Rossignol (1977) demonstrated that in chronic spinal cats walking on a treadmill, stimulation of the SP nerve in the swing phase of locomotion evoked a flexion response with a concomitant crossed extension, whereas in stance it caused increased ipsilateral extension. Forssberg (1979) as well as Wand, Prochazka and Sontag (1980) investigated the same reflex in the

intact cat walking on a treadmill and found that a detailed pattern of muscle activation occurred. Forssberg (1979) referred to this pattern of reaction to stimulation of the SP nerve during locomotion as the stumbling corrective response and suggested that it is evoked in an attempt to avoid tripping when obstacles contact the dorsal surface of the paw during locomotion. We have recently shown that this organization persists and can be investigated during MLR-evoked fictive locomotion (McCrea, Quevedo, Fedirchuk, and Gosgnach, 1998)

An example of the reorganization of a cutaneous reflex in humans was described by Zehr, Komiyama and Stein (1997) who demonstrated that tibial nerve stimulation results in variable output depending on when in the locomotor cycle it is stimulated. If it is stimulated during late stance the result is dorsiflexion, however if it is stimulated late in the swing phase of locomotion the result is plantar flexion.

Modulation of group II reflexes and FRAs

A further organization scheme for cutaneous reflexes involves cutaneous evoked flexion reflexes. Stimulation of any of the flexor reflex afferents (FRA) including the group II afferents produces ipsilateral flexion and contralateral extension in the low spinal animal (Eccles and Lundberg, 1959). There is also strong evidence for supraspinal control of spinal FRA pathways (Holmqvist and Lundberg, 1961). In the low spinal cat, injection of L-DOPA changes the effects of stimulation of the FRA from a short latency flexor reflex to longer latency reflexes. Since these longer latency actions occur in conjunction with alternating excitation of flexor and extensor motoneurons, it has been proposed that after L-DOPA, FRA stimulation can activate interneurons in the spinal locomotor CPG (Jankowska, Jukes, Lund and Lundberg, 1967). This finding led to studies on the modulation of the group II reflexes during locomotion. Perreault, Angel, Guertin and McCrea (1995) induced fictive locomotion in the decerebrate cat by electrical stimulation of the mesencephalic locomotor region (MLR) and showed that the effects of stimulation of different hindlimb flexor nerves at group II strength depended on the phase of locomotion. Stimulation of the flexor nerves TA, PBSt and Sart during flexion terminated activity in flexor nerves and initiated activity in extensors. On the

other hand, when same three nerves were stimulated in the extensor phase, only Sart stimulation had an effect on the locomotor cycle, it prolonged the extensor phase. This finding is perhaps more interesting when it is compared to the observations by Schomberg, Petersen, Barajon and Hultborn (1998) who observed that during DOPA-induced fictive locomotion, stimulation of flexor muscle nerves at group II strength resets the step cycle to flexion. Further, Hiebert, Whelan, Prochazka and Pearson, (1996) observed that in the decerebrate cat walking on a treadmill stimulation of the EDL nerve at group II strength increased the duration of flexion. These findings show that group II reflex responses during locomotion are preparation dependant.

The Group I reflexes during locomotion

One of the first observations that group I reflex actions might be very different during locomotion was by Duysens and Pearson (1980). In premammillary cats walking on a treadmill they demonstrated that loading of the hindlimb extensor muscle triceps surae inhibits the onset of flexion in that limb. This was thought to reflect the need to prevent the initiation of swing phase when hindlimb extension is required to support the weight of the animal. Conway, Hultborn and Kiehn (1987) further demonstrated the reorganization of group I reflexes during locomotion. They showed that stimulation of group I extensor afferents at rest causes inhibition of ipsilateral extensor activity but the same stimulation during the extension phase of locomotion caused inhibition of flexor burst initiation and therefore a prolongation of extensor activity. Extensor nerve stimulation at group I strength during flexion has been found to terminate the flexion phase and initiate extension (Conway et al., 1987; Guertin, Angel, Perreault and McCrea, 1995). Also, it has been shown that the non-reciprocal inhibition of group I reflexes occurring at rest is replaced by a disynaptic excitation of hip, knee and ankle extensor (McCrea, Shefchyk, Stephens and Pearson, 1995; Angel, Guertin, Jimenez and McCrea, 1996) and flexor (Degtyarenko, Simon, Norden-Krishmar and Burke, 1998; Quevedo, Fedirchuk, Gosgnach and McCrea, 1998) motoneurons during locomotion. At first it was thought that the alteration of the step cycle was evoked by group Ib stimulation only (Conway et al., 1987), however recently it has been shown (Guertin et al, 1995) that

group Ia afferent activation also alters the step cycle. Therefore in order to avoid confusion the term group I is used to describe actions produced by both Ia and Ib afferents.

Cutaneous, group Ib and group II afferents all synapse on spinal interneurons which in turn directly or indirectly excite or inhibit motoneurons. The effects of afferent input can therefore be gated by these interneurons before reaching the motoneurons. The group Ia reflex is different because of its monosynaptic excitation of motoneurons (LaPorte and Lloyd, 1952). This is arguably the most studied reflex in mammals including man (i.e. the H-reflex). Thus we know that the Ia afferent fibers originate from muscle spindles with dynamic sensitivity to muscle stretch. The discharge from Ia fibers monosynaptically excites motoneurons to homonymous and synergist muscles and excites inhibitory Ia interneurons which inhibit antagonistic motoneurons. The detailed pattern of group Ia monosynaptic excitation an disynaptic inhibition has been described (Eccles, Eccles and Lundberg, 1957; 1958)

There is substantial evidence that modulation of the group Ia monosynaptic reflex occurs during locomotion. One might expect that during movement, neural pathways which mediate muscle receptor signals would be augmented to ensure reliable transmission of detailed information to the central nervous system to fine tune the locomotor pattern. The opposite has been found to occur. As will be discussed, during locomotion there is a decrease in the gain of the monosynaptic reflex. During a movement such as locomotion it has been estimated that the net Ia afferent input to the triceps surae motoneurons is 15,000 action potentials per second (Prochazka, Trend, Hulliger and Vincent, 1989). Obviously, such intense afferent input could lead to a disrupted motor system whereby reflexes could disturb the central motor program to an unacceptable degree. This makes the phenomenon of a reduction of monosynaptic input from Ia afferents to motoneurons during locomotion less surprising.

Capaday and Stein (1986, 1987) reported that the amplitude of the monosynaptic Hoffman reflex (H-reflex; observed by stimulating a muscle nerve at group I strength and recording its output) of the human soleus muscle was decreased during locomotion when

compared to quiet standing. The reflex gain of the triceps surae reflex has also shown to be greatly reduced during locomotor activity in the decerebrate cat (Bennett, DeSerres & Stein, 1996). Akazawa, Aldridge, Steeves and Stein (1982) investigated the amplitude of the monosynaptic reflex in cat ankle extensors during locomotion and found that is modulated during the step cycle and reaches a peak in early extension. Yang, Fung, Edamura, Blunt, Stein and Barbeau, (1991) demonstrated in paretic patients when this reduction of reflex gain does not occur during locomotion, there is clonus and spasticity.

One factor which may lead to the reduction of the monosynaptic reflex during locomotion is a modulation of the monosynaptic EPSP generated in the motoneuron. Studies investigating the modulation of the group I monosynaptic EPSP during locomotion have resulted in different observations depending on the preparation used for evoking locomotion. In 1978, Schomberg and Behrends recorded Ia EPSPs during DOPA induced fictive locomotion in spinal cats and observed that monosynaptic EPSP amplitude was larger during the active phase of the motoneurons. They also noted the presence of Ia-evoked polysynaptic EPSPs during the active phase of the motoneuron. Shefchyk, Stein and Jordan (1984) investigated monosynaptic EPSP amplitude during fictive locomotion induced by MLR stimulation and found that the EPSP amplitude was larger in the active phase in less than half of the motoneurons analysed. They observed very few examples of Ia-evoked polysynaptic EPSPs. Monosynaptic EPSP amplitude during MLR-evoked fictive locomotion was also investigated by Gossard et al. (1996). This is the only study which compared group Ia monosynaptic EPSP amplitude during periods of non-locomotion and locomotion. Although they had a limited number of recordings (4 flexor motoneurons, 2 extensor motoneurons) they observed that the unitary monosynaptic EPSP evoked during the flexor phase of locomotion were always larger than those evoked during extension or during periods of non-locomotion. This thesis will more closely examine the modulation of monosynaptic reflexes during locomotion by comparing Ia monosynaptic EPSP amplitudes in control and locomoting conditions.

II. Possible causes of the depression of the group Ia monosynaptic reflex during locomotion

It has been shown that a group of interneurons synapse on the terminal of primary afferent fibers. The release of transmitter from these interneurons onto the afferent terminal can depress transmitter release from the terminal. This is referred to as presynaptic inhibition and coincides with a depolarization of the primary afferent terminals (primary afferent depolarization, PAD). A historical overview of this topic will be provided after first discussing evidence for the existence of presynaptic inhibition during locomotion. Thus far, there are three lines of evidence which suggest that inhibition of group Ia monosynaptic reflexes during locomotion occurs due to a synaptic mechanism. First there is a decrease in threshold for firing of group Ia afferent terminals during locomotion. Using a modified Wall technique (Wall, 1958), Duenas and Rudomin (1988) documented the existence of a tonic reduction of the intraspinal threshold of populations and single Ia afferent fibers during fictive locomotion. This decrease began at the onset of locomotion and returned to baseline levels shortly after locomotion. These findings are in conflict with those of Bayev and Kostyuk (1982) who described a tonic decrease in the excitablility of the afferent fibers. This finding has never been substantiated and has yet to be explained physiologically.

The second line of evidence suggesting a locomotor related presynaptic inhibition is that there is a primary afferent depolarization (PAD) of group I afferents during locomotion (Gossard, Cabelguen and Rossignol,1991; Menard, Leblond and Gossard, 1999). By impaling muscle spindle primary afferent fibers a rhythmic depolarization of the afferent fibers during locomotion was observed. The afferents were depolarized maximally in the flexor phase and underwent a smaller depolarization during extension. Due to a limitation in recording techniques these studies were unable to examine whether there was a tonic PAD like the tonic excitability increase observed by Duenas and Rudomin (1988). The finding of a maximal depolarization during flexion by Gossard et al. (1991) suggests that Ia EPSPs should be reduced most during flexion. This is in apparent conflict with the findings of Angel et al. (1996) who found the monosynaptic EPSPs to be larger sometimes during the active phase of the motoneuron and sometimes during the inactive phase.

Another line of evidence suggesting that the decreased gain of the group Ia monosynaptic reflex during locomotion may be due to a synaptic mechanism is the observation that extracellular group I field potentials (EFPs) recorded in the intermediate nucleus are substantially decreased throughout locomotion (Perrault Shefchyk, Jimenez and McCrea, submitted). Since the EFPs represent synaptic current flowing into the population of interneurons located at the tip of the microelectrode, EFP depression is thought to represent a decrease in transmission from primary afferent terminals. It is important to note that in the Perreault et al. study (submitted) no unambiguous distinction between EFPs produced by Ia or Ib afferents could be made and it is well known that both types of group I afferents end in the intermediate area (see Jankowska, 1992). This mechanism is referred to as synaptic rather than presynaptic since it is possible that EFP depression may occur due to either a decrease of transmitter from the presynaptic terminal or modification of postsynaptic receptors.

Since presynaptic inhibition in the form of PAD is thought to be primarily responsible for the decrease of the monosynaptic reflex during locomotion a brief history of presynaptic inhibition of group Ia afferents is provided

III. History of presynaptic inhibition of group Ia afferents

The idea of presynaptic inhibition suggests that the effectiveness of a given afferent input to the motoneuron is controlled through a regulation of the amount of neurotransmitter released from the primary afferent terminal. The observations that led to the discovery of presynaptic inhibition of group I afferents began in 1925 by Ballif, Fulton & Liddell who observed that long lasting inhibition of the knee jerk reflex resulted when single shocks were delivered to ipsilateral hindlimb nerves. At the time this was attributed to the release of an inhibitory substance in the spinal cord. The next major finding in this field was by Barron & Matthews (1938) who noted that following electrical stimulation of a neighbouring dorsal root, the amplitude of the resulting discharge from the ventral root evoked by stimulating the dorsal root in the same segment 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 were correlated with the reduction of the ventral root recording. Barron and Matthews attributed the DRPs to current spread from the nearby discharge of afferents and interneurons. They suggested that DRPs were due to branchpoint failure (the failure of an afferent impulse to invade all of the branches of its dividing axon).

Howland Lettvin, McCulloch, Pitts and Wall (1955) used field potential mapping techniques to investigate where the reduction of the group I afferent volley due to conditioning stimulus occurs. They observed a reduction of the fields in the medial dorsal horn of the spinal cord where the primary afferent terminals are located. Based on this finding, the DRP was thought to be due to a decrease in transmission from the primary afferent terminal. In 1957 Frank and Fourtes discovered that the group Ia monosynaptic reflex was reduced following conditioning stimuli with a time course that was too long, and a distribution incompatible with, reciprocal inhibition. They proposed that a post-synaptic mechanism was responsible for this inhibition. Since they were unable to detect any postsynaptic excitability changes in the motoneuron it was referred to as "remote inhibition" and assumed that the inhibition occurred at distal dendrites (an electrically remote site). Although at first this remote dendritic inhibition was postulated for the decreased Ia reflex, Frank (1959) later recanted, suggesting that presynaptic factors were responsible for the long lasting inhibition.

The debate about the relative contribution of postsynaptic conductances in motoneurons to the long lasting conditioned EPSP depression continued for some time. Edie, Jurna and Lundberg (1968) found that the Ia EPSP was depressed by a conditioning stimulus without a measurable change in membrane potential change and that the time course of the conditioned and unconditioned EPSP were almost identical. Other evidence came from Kuno (1964) who applied quantal analysis to Ia EPSPs evoked from the triceps surae motoneuron before and after conditioning stimuli and found that the number of quanta released from the presynaptic terminal was reduced by conditioning stimuli. Carlen, Werman and Yarri (1980) observed motoneuron input resistance changes in GS motoneurons following conditioning stimuli of the PBST nerve and suggested that this membrane resistance decrease could account for all of the EPSP depression. However in

1990 Carlen discounted this suggestion when McCrea, Shefchyk and Carlen (1990) discovered large decreases in group Ia monosynaptic reflexes following conditioning stimuli in the absence of a detectable decrease in motoneuron membrane resistance. The argument that motoneuron membrane resistance decreases were responsible for inhibition of group Ia monosynaptic reflexes following conditioning stimuli was also weakened by the work of Edwards, Redman and Walmsley (1976). They demonstrated, through the use of computer simulations that group Ia EPSPs will not be "substantially" altered by post-synaptic membrane conductance increases unless the decrease in membrane resistance is approximately 10 times less than the resting resistance. This was later reinforced by McCrea et al. (1990) who, using similar modelling techniques, found that large motoneuron membrane conductance increases were required to account for decreases in group Ia EPSP amplitude.

In summary it appears that there is primarily a presynaptic locus to the reduction of Ia EPSPs following conditioning stimulation. In the next section we will discuss the neurotransmitter and ionic mechanisms thought to be involved in presynaptic regulation of transmitter release from Ia afferents.

PAD due to increases in extracellular K^+

Upon discovery that DRPs were likely due to presynaptic inhibition, it was first thought that a non-specific liberation of K^+ ions into the extracellular area surrounding the primary afferent terminal and a resulting depolarization of the terminals was responsible (Barron and Matthews, 1935; Eccles and Malcom, 1946). More specifically, this theory contends that 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 leads to a buildup of K^+ in a restricted area surrounding the axon and results in the depolarization of nearby axons simply by altering the $K^+_{outside}/K^+_{inside}$ ratio.

While it is possible that K^+ accumulation may be one of the mechanisms by which PAD occurs, and has even been shown in the neonatal rat that depolarization comparable

in amplitude to DRPs can be produced by elevation of extracellular K^+ levels (Kremer and Lev-Tov, 1998), there are many difficulties in the acceptance that extracellular K^+ accumulation is solely responsible for PAD of group I afferents. One difficulty is the slow time course of K^+ accumulation. Extracellular K^+ accumulation has been shown to take approximately 20-30 seconds to develop. This is much too slow to account for the inhibition of the group Ia monosynaptic reflex. It has also been observed that pharmacological agents can act on PAD and extracellular K^+ in opposite manners. For example, barbituates prolong DRPs but decrease extracellular K^+ flow and the $GABA_A$ receptor antagonist picrotoxin abolishes the DRP while increasing extracellular K^+ (Bruggencate Lux and Liebl, 1974). In some cases it is possible to evoke DRPs with the complete absence of extracellular K^+ accumulation. Nicoll (1979) demonstrated this in the frog. Also, Jimenez, Rudomin, Solodkin and Vyklicky (1984) found that conditioning stimuli considerably reduced the threshold of individual Ia afferents without any significant increase in extracellular K^+ and that cutaneous fiber stimulation causes very little depolarization of group Ia fibers despite substantial increases in extracellular K^+. In summary, while extracellular K^+ accumulation may play a role in the production of PAD it is unlikely that it is the primary mechanism.

PAD of group I afferents due to GABA-mediated depolarization

As outlined below it is thought that PAD occurs due to the activation of a set of GABA-releasing interneurons which synapse on the axons of the primary afferent terminals. The present theory which has been proposed to explain PAD contends that GABAergic interneurons form axo-axonic synapses at the central terminals of the primary afferent fibers. This has been supported by the recent observation that GABA-immunoreactive axo-axonic appositions are present on group I fiber terminals in the lumbrosacral spinal cord (Lamotte d'Incamps, Destombes, Thiesson, Hellio, Lasserre, Kouchtir-Devanne, Jami and Zytnicki, 1998). When these GABAergic interneurons are activated, they release GABA and bind to GABA receptors on the primary afferent terminal. Two subtypes of GABA receptors ($GABA_A$ and $GABA_B$ receptors) are involved in presynaptic inhibition.

The first pharmacological investigations of presynaptic inhibition were performed by Eccles, Schmidt & Willis (1963). They showed that the PAD was eliminated when the $GABA_A$ receptor antagonist picrotoxin was applied and that topical GABA application to the spinal cord evokes DRPs. Barker and Nicoll (1972) later found that another $GABA_A$ receptor antagonist, bicuculine, blocked PAD following a conditioning stimulus, but other convulsive agents such as strychnine did not have the same effect. This led to the belief that GABA was the substance responsible for the PAD and involved the $GABA_A$ receptor type. $GABA_B$ receptors have also been shown to play a role in the production of PAD. Monosynaptic reflexes elicited by the stimulation of group I fibers have been shown to be depressed after intravenous injection of low doses of the $GABA_B$ receptor agonist baclofen (Kato, Waldman and Murakami, 1978; Curtis, Lodge, Bornstein and Peet, 1981). In addition, Stuart and Redman (1992) showed that there was a reduction in PAD in group I afferents when the $GABA_B$ receptor antagonist saclofen is administered.

Upon binding to the $GABA_A$ receptor, GABA opens Cl^- channels on the primary afferent terminal thus allowing Cl^- to pass out of the cell down its concentration gradient causing terminal depolarization. This results in a decreased firing threshold for the terminals, therefore the action potential arriving from the periphery has a smaller amplitude which results in a decreased activation of high-threshold Ca^{2+} channels and a lowering of intracellular Ca^{2+} (Dunlap, 1998). This results in decreased transmitter release. GABA binding to a $GABA_B$ receptor causes a reduction in the duration of the action potential in the absence of a depolarization or a detectable change in membrane conductance (Curits et al., 1981; Lacey and Curtis, 1994; Curtis and Lacey, 1998). The reduction of action potential duration is thought to be due to a reduction in the inward Ca^{2+} currents through the N and P/Q type Ca^{2+} channels (Rudomin, 1994). In the lamprey, $GABA_B$ receptor-mediated synaptic suppression in the spinal cord has been shown to be caused by a G-protein coupled second messenger system linked to the $GABA_B$ receptor (Alford and Grillner, 1991). Activation of either type of GABA receptor thus results in a decrease in the activation of high threshold Ca^{2+} channels.

When an action potential invades the afferent terminal, the reduced Ca^{2+} entry decreases neurotransmitter release and thus decreases the amplitude of the group Ia monosynaptic EPSP recorded in the motoneuron.

In summary, it appears that at the present time, presynaptic inhibition of the group Ia monosynaptic reflex results, for the most part, from the activation of GABAergic axo-axonic synapses on primary afferent terminals This does not rule out the possibility that other transmitter systems can act presynaptically to affect Ia EPSPs in motoneurons during locomotion.

Location of the interneurons involved in the PAD of group I input

Although they have yet to be precisely located, there are some indications of the location of the interneurons responsible for PAD. Eccles, Kostyuk and Schmidt (1962) recorded from a group of interneurons located at the base of the dorsal horn which he 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 of the spinal cord produces DRPs (Jankowska McCrea, Rudomin and Sykova, 1981) and a monosynaptic PAD of both group Ia and group Ib afferents (Solodkin, Jiminez and Rudomin, 1984), suggesting that this is the probable site of the PAD interneurons. Another line of evidence for the involvement of intermediate laminae (V, VI) interneurons comes from the work of Ljungdahl and Hokfelt (1973) who reported that a group of small neurons containing GABA existed at the laminae VI-VII border.

IV. Other possible causes of depression of the group Ia monosynaptic reflex during locomotion

Although a large body of evidence suggests that the reduction of the group I monosynaptic reflex during locomotion is due to presynaptic inhibition via a PAD mechanism, there has been some research which claims that this is not the case. In 1996, Gossard recorded from 6 group Ia afferent-motoneuron pairs and observed that in both flexor and extensor pairs the unitary monosynaptic EPSP was larger in the flexor phase than in either the extensor or the non-locomotor state. Since this is not complimentary to

the modulation of the monosynaptic reflex (Akazawa et al., 1982) he suggests that locomotor-related PAD does not contribute significantly to the modulation of this reflex.

There are three potential explanations, other than presynaptic inhibition, for the decrease of the Ia monosynaptic reflex during locomotion. The first is a reduced number of activated afferents due to a reduced gamma drive to the muscle spindles during locomotion. It is unlikely that this occurs since it has been shown that gamma drive is not substantially reduced during locomotion (Prochazka, Westerman and Ziccone, 1976; Murphy, Stein and Taylor, 1984; Prochazka and Gorassini, 1998).

Another mechanism which could be responsible for the down-regulation of the group Ia monosynaptic reflex during locomotion is post-synaptic inhibition of the motoneurons. One mechanism that could decrease the monosynaptic reflex during locomotion is a decrease in motoneuron membrane resistance (increased conductance). This would reduce EPSP amplitude by shunting of synaptic activity. Shefchyk and Jordan (1985) investigated this and observed membrane resistance decreases during the locomotor periods in almost half of the motoneurons studied.

The third mechanism which may cause an alteration of the group Ia monosynaptic reflex during locomotion is the regulation of ionotropic neurotransmitter -gated ion channels by protein phosphorylation. It has been shown in the hippocampus that phosphorylation of glutamate channels is an important mechanism for modulation of ion-channel function (see Smart, 1997). Although it has not been demonstrated that this process affects spinal cord motoneurons during locomotion, the phosphorylation of ion channels may effect EPSP amplitude by modulating the efficacy of a given synaptic input.

V. Objectives

Thus far it has been shown that PAD occurs tonically (Duenas and Rudomin, 1988) and phasically (Gossard et al. 1991, Menard et al., 1999) during locomotor activity. We hypothesise that the tonic PAD will cause a locomotor state dependant decrease in monosynaptic EPSP amplitude. We will also examine the change in monosynaptic group I extracellular field potentials recorded in the motor nucleus of the ventral horn during

locomotion. Motoneuron membrane conductance will also be assessed in both states in an attempt to see if it is sufficient to account for the amplitude modulation.

METHODS

Preparation

Experiments were performed on 23 cats of either sex weighing 2.1- 4.5 kg. For the surgery the cats were anesthetized with halothane delivered in a mixture of 30% oxygen and 70% nitrous oxide. The external jugular vein and a hindlimb vein were cannulated in order to administer drugs and fluids. A carotid artery was cannulated to monitor blood pressure. A tracheotomy was performed. Atropine (0.05mg/kg s.c.) and dexamethasone (2mg/kg i.v.) were given at the beginning of the surgery and a 5% glucose and bicarbonate solution was delivered intravenously throughout the experiment at a rate of 5ml/hr. Supplemental saline and dextran infusions were given as required to maintain blood pressure. Selected left hindlimb nerves were dissected and cut in preparation for electrical stimulation and monitoring locomotion. The dissected nerves included the left semimembranosus (Sm) and anterior biceps (AB), taken together as SmAB, posterior biceps (Pb), semitendinosus (St), sartorius (Sart), quadriceps (Q, vastii and rectus femoris) taken together or separately, plantaris (Pl), medial gastrocnemius (MG), lateral gastrocnemius and soleus (LGS), flexor digitorum hallucis longus (FDHL) which is a branch of the popliteal nerve that includes innervation of the flexor digitorum longus and flexor hallucis longus (sometimes taken separately and FDL and FHL) as well as branches to the interosseous, tibialis posterior and popliteal muscles. Extensor digitorum longus (EDL), extensor digitorum brevis (EDB), tibialis anterior (TA), peroneous longus (Plong), peroneous tertius and brevis (Per T+B) were also dissected. The Q and Sart nerves were placed in a bipolar cuff electrode for stimulation and recording. In the right hindlimb, the AB or SmAB nerve was dissected to monitor contralateral ENG activity. Other femoral, sciatic and obturator nerve branches, as well as tendons around the hip, were cut bilaterally. Following an L4-L7 laminectomy, the cat was stabilized in a rigid frame, the brain was exposed by craniotomy and a precollicular-postmammillary decerebration was performed. Both corticies and all tissue rostral to the transection were

removed. Anesthesia was discontinued and the cat was paralysed with gallamine triethiodide (Flaxedil, 2-3 mg $kg^{-1}h^{-1}$) and artificially ventilated. Skin from the back, left hindlimb and right hindlimb were fashioned into pools and filled with warm mineral oil. The remaining dissected nerves were placed on bipolar electrodes for stimulation and recording.

Stimulation and Recording

The dura of the spinal cord was opened to expose the lumbar segments and small holes were made in the pia for microelectrode insertion. Glass microelectrodes filled with 50mM QX-314 in 2M potassium citrate (tip diameter 1.8-2.5 μm; resistance 2-3 MΩ) were used for intracellular recording from antidromically identified hindlimb motoneurons. Intracellular diffusion of QX-314 was used to block motoneuron action potentials and permit assessment of the afferent evoked EPSPs in the absence of evoked firing. Extracellular recordings were made using glass microelectrodes filled with 2M sodium citrate. Fictive locomotion was evoked by unilateral or bilateral stimulation of the MLR (80-200μA, 1ms pulses at 12-18Hz; see Guertin et al. 1995). In one experiment clonidine (50-200 μg kg^{-1}) and naloxone (50-100 μg kg^{-1}) were administered intravenously to aid in the initiation of MLR-evoked fictive locomotion. Three EPSP measurements were recorded from this cat.

The stimulation and recording setup is illustrated in Figure 1. The protocol for collection and analysis of intracellular data was as follows. Motoneurons were impaled and identified antidromically. Selected peripheral nerves were electrically stimulated using strengths expressed in multiples of threshold current. Threshold current (T) was defined as the smallest current to produce a detectable extracellular compound action potential volley at the cord dorsum recording electrode. The peripheral nerve stimulation strength (≤2T, 3-5Hz, 100μs constant current pulses) was adjusted to evoked a large monosynaptic EPSP in the motoneurons without orthodromic or antidromic action potentials. EPSPs were collected for 10-20s before the onset of MLR stimulation (pre-locomotor state). MLR stimulation produced fictive locomotion and EPSPs were collected for 1-2 minutes (locomotor state). MLR stimulation was then turned off and

EPSPs were collected at 30s intervals in some cases for up to 5 minutes after the end of MLR stimulation (post-locomotor state). EFP recordings were made using the same protocol except that a Na^+ citrate microelectrode was positioned just outside antidromically identified α-motoneurons in the ventral horn.

Data Analysis

For analysis purposes intracellulary recorded EPSPs were deemed monosynaptic if they had a central latency (time between arrival of earliest component of afferent volley at the cord dorsum and the onset of upward deflection of the earliest component of the EPSP) of 0.6-0.9 ms. In order to avoid contamination by the locomotor dependant disynaptic component of the EPSP (Schomberg and Behrends, 1978; McCrea et al, 1995; Angel et al., 1995; Degtyarenko et al., 1998; Quevedo et al., 1998) amplitude measurements were made on the rising phase of the EPSP, at a fixed latency, just before the peak (see Figure 2). EFP measurments were made at a fixed latency just before the peak of the downward deflection on those EFPs which had latencies of 0.6-0.9ms. Measurements were made from averages of monosynaptic EPSPs or EFPs that were evoked before locomotion (pre-locomotor), during locomotion (sometimes divided into those occurring in the flexion and extension phases), and after locomotion (post-locomotor).

In order to make measurements of motoneuron membrane resistance, short duration depolarizing or hyperpolarizing constant current pulses (0.5ms, 15nA to 40nA) were delivered to the motoneuron throughout the pre-control state, the locomotor state and the post-control state. Typically these pulses preceded peripheral nerve stimulation by 10-30ms. The area of the voltage transient resulting from this stimulation was averaged in each of the three states (pre-locomotor, locomotor, post-locomotor) and the membrane resistance was calculated using the formula :

$$\text{membrane resistance } (M\Omega) = \frac{\text{voltage transient area (mV x ms)}}{\text{current pulse (nA) x duration (ms)}}$$

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

QNX or Linux for analysis).

Statistical Analysis

Following data analysis, means and standard deviations were calculated for EPSP amplitude, EFP amplitude and membrane resistance in the pre-locomotor and locomotor conditions (and in some cases flexion and extension phases of locomotion). In the results section means are expressed ± standard deviation. To test the significance of the results a two-tailed t-test assuming unequal variances was used with an α of 0.05

RESULTS

The amplitudes of 101 monosynaptic EPSPs recorded in 72 α-motoneurons were compared during control (pre-locomotor) and fictive locomotor conditions. EPSPs were evoked by electrical stimulation of peripheral nerves at strengths ranging from 1.2T-2T and the motoneurons sampled consisted of 24 flexors, 31 extensors, and 17 bifunctional motoneurons located in the L4 to L7 segments of the spinal cord. Many of the monosynaptic EPSPs were followed by a disynaptic component (cf. Angel et al., 1996; Quevedo et al., 1998). As mentioned in the methods, the monosynaptic EPSP amplitude was measured under all conditions at the same latency, on the rising phase, just before the peak so as to avoid disynaptic contamination. In 29 cases (22 motoneurons) membrane resistance was assessed from intracellular current injection. Extracellular field potentials (EFPs) were also recorded in the ventral horn.

Group Ia monosynaptic EPSPs are depressed during fictive locomotion.

The principle finding was that 90 of the 101 Ia monosynaptic EPSPs recorded in 72 motoneurons showed an amplitude decrease during locomotion compared to the pre-locomotor period. In these 90 cases (recorded from 61 motoneurons) there was a mean EPSP depression from a control EPSP of 2.6 ± 1.6mV to a locomotor EPSP of 1.8 ± 1.2mV with a mean depression of 32% ± 21% (range 6%-82%). Four EPSPs (recorded in 1 flexor, 2 extensor and 1 bifunctional motoneuron) increased in amplitude (18%-53%, $\bar{x}$=33 ± 16%) following the onset of locomotion. Seven EPSPs (recorded in 4 flexor, 3 extensor motoneurons) remained within 5% of pre-control values during locomotion.

Taken together the amplitude of the 101 monosynaptic EPSPs investigated was significantly decreased ($p<0.01$, $n=101$) on average by 28% ± 21% during locomotion.

The depression of the monosynaptic EPSP is believed to be due to locomotion rather than simply MLR stimulation since in all 5 cases in which there was a delay between the onset of MLR stimulation and the onset of locomotion, EPSP amplitude was not decreased until locomotion began. This is illustrated in Figure 2. This figure shows a 35s period of data collection. The top three waveforms are ENGs from an extensor (GS) and flexor (TA and EDL) peripheral nerves. They show that rhythmic activity begins well after (12s) MLR stimulation is initiated (represented by the filled black bar). The lower 3 traces represent the average amplitude of the EPSPs recorded from a SmAB motoneuron evoked by stimulation of the SmAB nerve (2xT, 4Hz) for the 10s time period directly above each trace. Before MLR stimulation, EPSP amplitude is 2.9mV. In the period of MLR stimulation in the absence of locomotion EPSP amplitude is 2.8mV. In the period of MLR-induced fictive locomotion the EPSP is reduced to 1.3mV.

The typical pattern of EPSP depression which was observed is illustrated in Figure 3. This figure shows a continuous 120s period of data collection. During this period the LGS nerve was stimulated at 1.2 T and 4 Hz while recording intracellularly from an LGS motoneuron. The top two traces in panel A are ENGs from flexor (Sart) and extensor (SmAB) nerves. They show that activity begins in the peripheral nerves at 17s, shortly after the onset of MLR stimulation (represented by the black bar). The third trace shows a DC intracellular recording from an LGS motoneuron (note the LDP, see Jordan, 1983). In this, and all motoneurons, action potentials were blocked by diffusion of QX-314. The plotted line in Fig 3A is the continuous EPSP amplitude (5 point smoothing) recorded during the 120s run. At the onset of data collection (pre-locomotor period) the EPSP amplitude is fluctuating around 3.8 mV. As soon as locomotor activity begins (shortly following the onset of MLR stimulation) the EPSP amplitude decreases sharply and levels off at an average of 2.9 mV. Further into locomotion, the average EPSP amplitude falls further, in this case to an average of 2.7 mV. Note that the period of greatest depression coincides with the development of consistent alternation between

the flexor and extensor ENG bursts (Figure 3C, 3D). Following cessation of locomotion, EPSP amplitude does not return to baseline levels as quickly as it was depressed. The recovery following locomotion will be discussed further (below). The average EPSP recorded during the 17s pre-locomotor period (n=68) and throughout the 83s locomotor period (n=338) are overlaid in figure 3B showing a depression from 3.8 mV to 2.8 mV (26%) during locomotion. Other than the lack of a disynaptic component in the EPSP during locomotion, this example is representative of our data set in that the EPSP depression (26%) is similar to the mean depression (28%) of 101 EPSPs, the amplitude decreased as soon as locomotion started and recovery was delayed. The number of EPSPs used to construct averages in the pre-locomotor and locomotor states is also representative of the other EPSPs examined.

Phasic EPSP modulation is small and inconsistent

To examine whether there is a phasic EPSP depression during fictive locomotion, the amplitude of those EPSPs evoked in the flexion phase of locomotion were compared to the amplitude of those evoked in the extension phase in 13 flexor motoneurons, 15 extensor motoneurons and 6 bifunctional motoneurons. The results are illustrated in Figure 4. Figure 4A shows averages of the data from the locomotor EPSP illustrated in Figure 3B separated into flexion and extension and overlaid. In this LGS motoneuron the EPSP amplitudes during the flexor phase and extensor phases are almost superimposable. Figure 4B shows the ratio of the average EPSP amplitude evoked during extension to that evoked during flexion for the 34 motoneurons mentioned above. In Figure 4B data from flexor (circles), extensor (triangles) and bifunctional (squares) motoneurons are plotted separately.

The points below the 1.0 line represent the motoneurons in which average EPSP amplitude was larger during flexion and those above the 1.0 line represent those motoneurons in which the average EPSP amplitude was larger during extension. It is evident that no clear trends emerge concerning phasic modulation of EPSP amplitude. Of the 34 EPSP ratios in Figure 4B, 9 were larger during flexion, 10 were larger during extension and 15 were close to equal (within 5% of each other) in both phases. If one

(arbitrarily) excludes those EPSPs within 5% of the unity line, then the majority of EPSPs in flexor motoneurons were larger during extension and the majority of EPSPs in extensor motoneurons were larger during flexion. In all flexor motoneurons, however the EPSP amplitude is not significantly modulated (p=0.3). EPSP amplitude is larger during flexion in 2 motoneurons, larger during extension in 5 motoneurons and approximately equivalent (± 5%) between the two phases in 6 motoneurons with an average ratio of 1.02. EPSP amplitude modulation is also non-significant for extensor motoneurons (p=0.1, 6 larger during flexion, 2 larger during extension and 7 similar in both phases, average 0.93) and for bifunctional motoneurons (p=0.5, 1 larger during flexion, 3 larger during extension and 2 similar in both phases, average ratio 0.99).

The only other comparison of Ia EPSP amplitude before and during fictive locomotion of which we are aware is a sample of 6 unitary EPSPs reported by Gossard (1996). That study found unitary monosynaptic EPSP evoked in flexion to be slightly larger than those evoked during control and control EPSPs to be larger than EPSPs evoked during extension in all 4 of the TA motoneurons studied (Gossard, 1996). Of the 34 composite EPSPs analysed here, (13 flexors, 15 extensors, 6 bifunctionals) in Figure 4B, only 2 (both in EDL motoneurons) were larger during either phase of the step cycle than during the pre-control state. In both cases the EPSPs were larger (12 and 21%) during the flexion phase. The nine measurements made from 5 TA motoneurons were analysed further. Of the eight measurements in which EPSP amplitude was decreased during locomotion, 4 EPSPs were larger during the extensor phase (14-25%, $\bar{x}$=12 ± 4%), 1 was larger during flexion (17%) and 3 EPSP amplitudes which were within 5% of each other in both phases. These results thus differ from those reported by Gossard (1996) for unitary EPSPs.

Recovery of EPSP amplitude following locomotion is delayed

As illustrated in Figure 3A (post-locomotor period) EPSP depression during locomotion did not recover immediately. In the short post-locomotor period (10-20s) examined in the majority of recordings, the EPSP remained substantially depressed. Therefore to better estimate monosynaptic EPSP amplitude recovery, EPSP recordings

were made for up to 5 minutes after locomotion in 9 motoneurons (11 EPSPs). Of these 11 measurements, only 8 recovered to within 5% of their pre-locomotor value in the 5 minute period after locomotion ($\bar{x}$=114s). The time taken for the eleven depressed EPSPs to recover is presented in the first row of Table 1. An example of the prolonged recovery of EPSP amplitude after fictive locomotion is illustrated in Figure 5. As in previous figures, the upper two traces are ENG records from flexor (Sart) and extensor (LGS) peripheral nerves. The bar below the ENGs indicates the period of MLR stimulation. The graph charts EPSP amplitude from an LGS motoneuron evoked by LGS stimulation (1.5T at 4Hz) for the 4 min. period illustrated. The values show EPSP amplitudes using 40 EPSPs per average (each point on the graph) for the first 2:10 and 16 EPSPs per average for the remainder of the data collection. The EPSP amplitude decreases at the onset of locomotion and remains reduced until 2 minutes after cessation of the MLR stimulus. This example is similar to the observations summarized in Table 1.

Motoneuron membrane resistance decreases during locomotion

In order to determine whether there is a relationship between EPSP depression and the decrease in motoneuron membrane resistance during locomotion, short duration current pulses were delivered to the motoneuron just before (10-30ms) peripheral nerve stimulation in the pre-locomotor, locomotor and post- locomotor state in 22 motoneurons. The result of this short pulse was a voltage transient from which the membrane resistance was calculated (see methods). 29 measurements from 22 motoneurons were taken, and in all but 2 of these measurements membrane resistance decreased (12%-79%) during fictive locomotion. In the pre-control period the mean motoneuron membrane resistance was $0.77 \pm 0.54 M\Omega$ (0.37-2.37MΩ), during locomotion the resistance was significantly decreased ($p<0.01$) by 39% to a mean of $0.47 \pm 0.35 M\Omega$ (0.15-1.45MΩ). Mean motoneuron membrane resistance was similar ($p=0.2$) in both of the locomotor phases. In the flexor phase the mean was $0.42 \pm 0.35 M\Omega$ (0.15-1.45MΩ) and during extension was $0.50 \pm 0.41 M\Omega$ (0.18-1.28MΩ). The relationship between membrane resistance decrease and EPSP amplitude decrease can be seen in Figure 6 which plots motoneuron membrane resistance during MLR stimulation as a fraction of the pre-locomotor value on the

abscissa. Figure 6 shows that large decreases in membrane resistance are often associated with large decreases in EPSP amplitude. While overall relationship between increased conductance and EPSP depression is significant (r^2=0.31, $p<0.01$) considerable scatter is observed.

The weakness of this relationship is evident from examination of the individual data points. There are five cases in which EPSP amplitude decreased by less than 15% while motoneuron membrane resistance decreased by more than 30%. Other examples in Figure 6 illustrate the generally poor association between conductance and EPSP depression, for example one case showed a 34% decrease in EPSP amplitude and only a 12% decrease in membrane resistance. In 9 cases there was a 5%-30% decrease in membrane resistance. In 11 cases the resistance decreased by 31%-60% and in 9 cases the membrane resistance decreased by 61%-90% of pre-locomotor values.

The phasic modulation of motoneuron membrane resistance was analysed to examine whether phasic changes in EPSP amplitude and motoneuron membrane resistance were related. In only 10 of 29 cases were EPSP amplitude and membrane resistance phasically modulated in the same direction (i.e. greater EPSP amplitude in the phase with higher membrane resistance).

In order to further investigate the relationship between motoneuron membrane resistance and EPSP amplitude decreases during fictive locomotion, recovery time of membrane resistance and EPSP amplitude were compared. In general there seemed to be little correlation between the two processes. In 6 of 16 measurements (from 14 motoneurons) motoneuron membrane resistance returned to pre-control values by the time the EPSP had recovered to its pre-locomotor value. In the remaining 10 cases EPSP amplitude recovered before motoneuron membrane resistance. Although the motoneuron membrane resistance decreases during locomotion, the weak relationship illustrated in Figure 6 and the lack of relationship between EPSP recovery and membrane resistance recovery suggests that increased motoneuron conductance is not the major factor producing the EPSP amplitude decreases during locomotion.

Depression of group I EFPs during locomotion

Extracellular field potentials are a measure of the synaptic current underlying the EPSP in the vicinity of the microelectrode and should not be influenced by motoneuron resistance decreases. Group I evoked EFPs were collected in the ventral horn of the spinal cord to examine the similarity of EFP and Ia EPSP depression during locomotion. Twenty-two EFPs were recorded in 8 locations within the ventral horn of the spinal cord. EFP amplitude was significantly decreased ($p<0.01$) during a locomotion by an average of $22 \pm 9\%$. Eight of the 15 EFPs which were decreased during locomotion recovered within a 5 minute period after locomotion. The mean recovery time of these 8 measurements was 137 ± 45s. Interpretation of this data is complicated by two factors. First, because the recordings were made in the ventral horn, the fields may have had components due to both the antidromic activation of motoneurons and the orthodromic (Ia EPSP) depolarization of motoneurons. Second, it is possible that the antidromic portion of the field was modulated and larger during the active phase of the motoneuron. In an attempt to control for these factors, some locomotor EFPs were recorded just as rhythmic activity in the motoneuron ended, during the first few seconds of post-control. This would remove the problem of fluctuating antidromic activity in the EFP, and because recovery of EPSP depression is quite delayed, allowed measurement of EFP recovery. Seventeen of these measurements were made. Compared to the pre-locomotor values 10 of these 17 measurements were depressed (mean $20 \pm 8\%$) and 8 of the 10 recovered to baseline levels within 5 minutes, the mean recovery time being 114 ± 30s. In order to abolish entirely the antidromic activation component of the EFP, we cut the ventral root in one experiment and recorded 5 measurements from 2 different areas (this was not done in more cases since ventral root activity was required to monitor locomotion). In these 5 measurements all of the EFPs were depressed during locomotion (average $24 \pm 6\%$), and they all recovered to baseline levels (average time 96 ± 21s). The recovery times for the different EFP measurements are provided in Table 1.

An example of the amplitude modulation of a group I EFP is illustrated in Figure 7. In panel A waveforms from flexor and extensor peripheral nerves show rhythmic activity beginning at about 20s. The extracellular waveform shows the intraspinal DC

recording. DC potential shifts have been shown to be linearly related to changes in extracellular potassium (Jimenez et al., 1984). At the onset of rhythmic activity there is a negative shift during which the extracellular potential drops by 2.1mV over a 10s period. At the end of rhythmic activity the DC potential returns to its pre-locomotor level after about 15s. The pattern of DC shift observed in this example was representative of our data set. In 7 measurements in which the DC potential was measured, 6 had a DC shift at the onset of locomotion (mean 1.7 ± 0.5mV) which returned to baseline shortly after cessation of locomotion. Figure 7B illustrates averaged EFP traces from the corresponding times in Figure 7A. The Pb nerve was stimulated (1.4T, 4Hz) throughout the data collection period. Each trace is an average of 40 EFPs (i.e. over a 10s period) except for the last one which is an average of 32 EFPs. In the pre-control state the average EFP had an amplitude of 0.67mV. At the onset of locomotion the EFP amplitude decreased to an average of 0.41mV and remained around this amplitude until well after locomotion ceased. The EFP did not return to its pre-control amplitude until 100-130s after locomotion. Based on the quick recovery of the extracellular DC potential and the delayed recovery of the EFP after locomotion, it is evident that extracellular shifts in DC potential are not closely related to EFP depression.

Figure 1: Stimulation-recording technique used in the present study.

In the decerebrate cat fictive locomotion was evoked by electrical stimulation of the mesencephalic locomotor region (MLR). Locomotion was monitored by assessing peripheral nerve electroneurograms (ENGs). Selected peripheral nerves were stimulated at group I strength (<2T, 3-5Hz) and recordings were made either intracellularly from antidromically identified motoneurons (EPSPs) or extracellularly (EFPs).

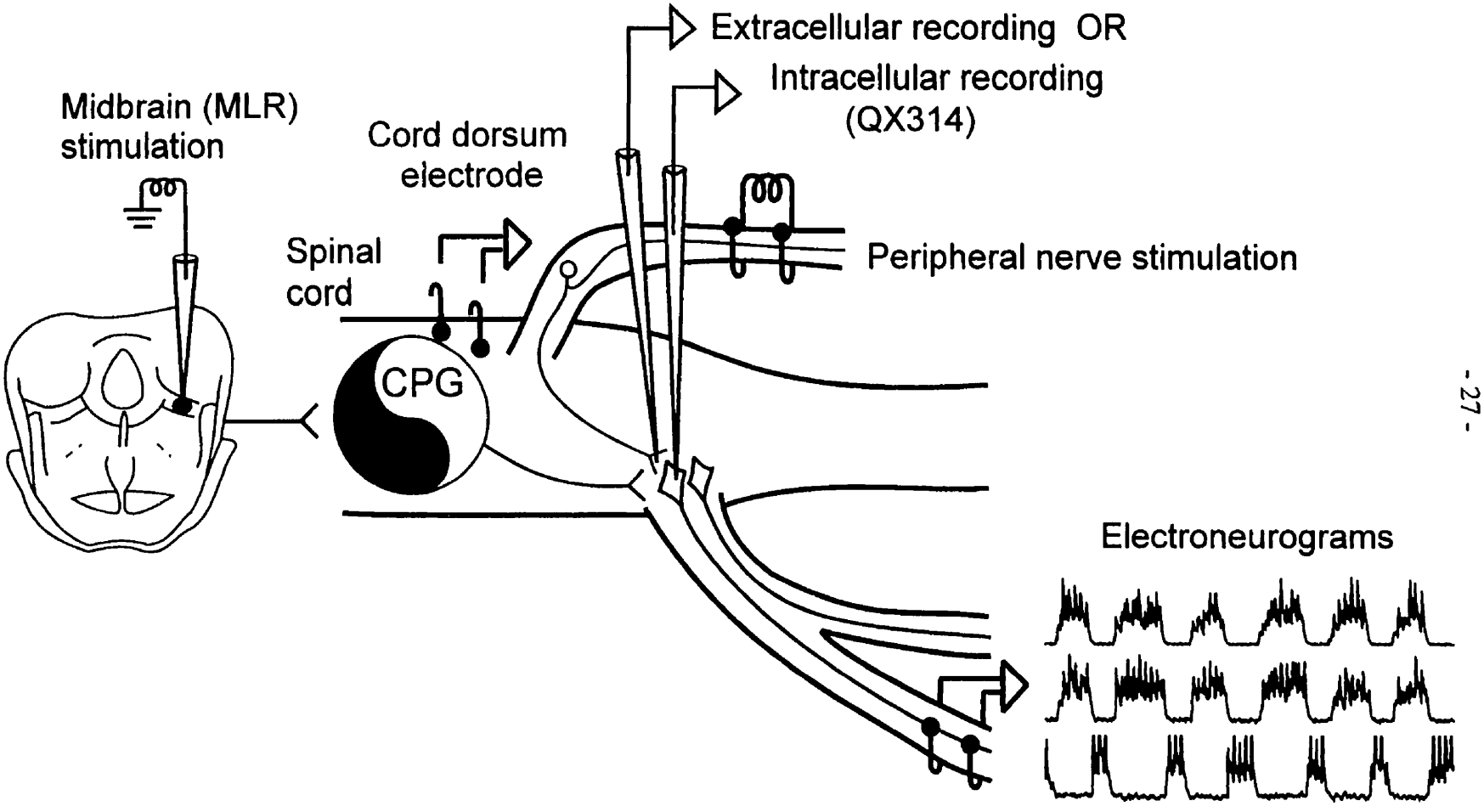

Extracellular recording OR
Intracellular recording
(QX314)
Midbrain (MLR)
stimulation
Cord dorsum
electrode
Spinal
cord
Peripheral nerve stimulation
CPG
Electroneurograms

Figure 2: EPSP depression is caused by locomotion rather than MLR stimulation

Rectified, integrated ipsilateral ENGs from an extensor (GS) and flexor (TA, EDL) peripheral nerves before and during MLR stimulation which begins 13s into the run. Locomotion, represented by activity in the peripheral nerves does not begin until 25s. Bottom 3 traces are averages of 40 EPSPs evoked by SmAB stimulation (4 Hz, 2xT) of a SmAB motoneuron during the 10s time period represented by the bar directly above each. The point at which EPSP amplitude was measured for each trace is depicted by the filled arrow. The empty arrow in the third trace points out the peak of a small disynaptic EPSP. Before MLR stimulation monosynaptic EPSP amplitude is 2.9mV. During MLR stimulation-no locomotion EPSP amplitude is 2.8mV. During MLR stimulation-locomotion EPSP amplitude is 1.3mV.

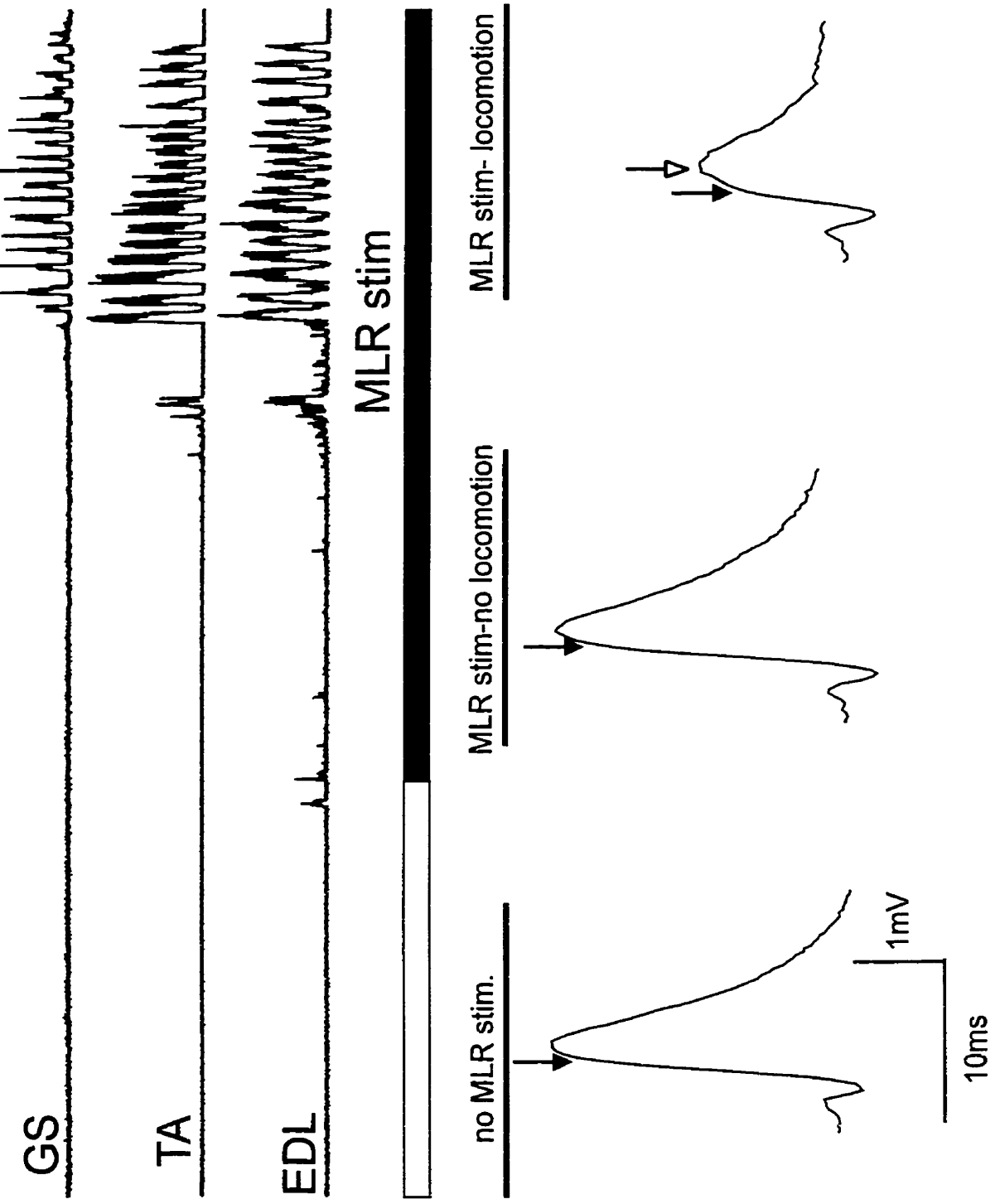

GS
TA
EDL
MLR stim
no MLR stim.
MLR stim-no locomotion
MLR stim- locomotion
1mV
10ms

Figure 3: Group Ia monosynaptic EPSP are depressed during fictive locomotion.

A Upper 3 waveforms are rectified, integrated ipsilateral ENGs from flexor (Sart) and extensor (SmAB) peripheral nerves as well as activity recorded intracellularly from an LGS motoneuron before (pre-locomotor, empty bar), during (MLR on, filled bar) and after (post-locomotor, empty bar) MLR stimulation. Fictive locomotion is represented by alternating activity in the peripheral nerves and rhythmic depolarization of the impaled LGS motoneuron (shown expanded in **C** and **D**). Fourth waveform is plot of the corresponding EPSP amplitude (time scale above) recorded from an LGS motoneuron evoked by LGS (1.2xT, 4Hz) stimulation plotted over time (5 pt. smoothing). EPSP amplitude decreases at the onset of activity in the peripheral nerves and remains decreased during a period of poor locomotion (see expanded time scale in panel **C**). When locomotor activity improves (see expanded time scale in panel **D**) EPSP amplitude is further decreased. **(B)** Averaged monosynaptic EPSP amplitude recorded from the same LGS motoneuron during pre-control period (dashed line, 0-17s in panel **A**) and during a period of fictive locomotion (solid line, 17-100s in panel **A**). The pre-control EPSP amplitude (3.8mV) was 26% larger than during locomotion (2.8mV). **C,D.** Enlarged portions of panel **A** illustrating quality of locomotion in the corresponding areas.

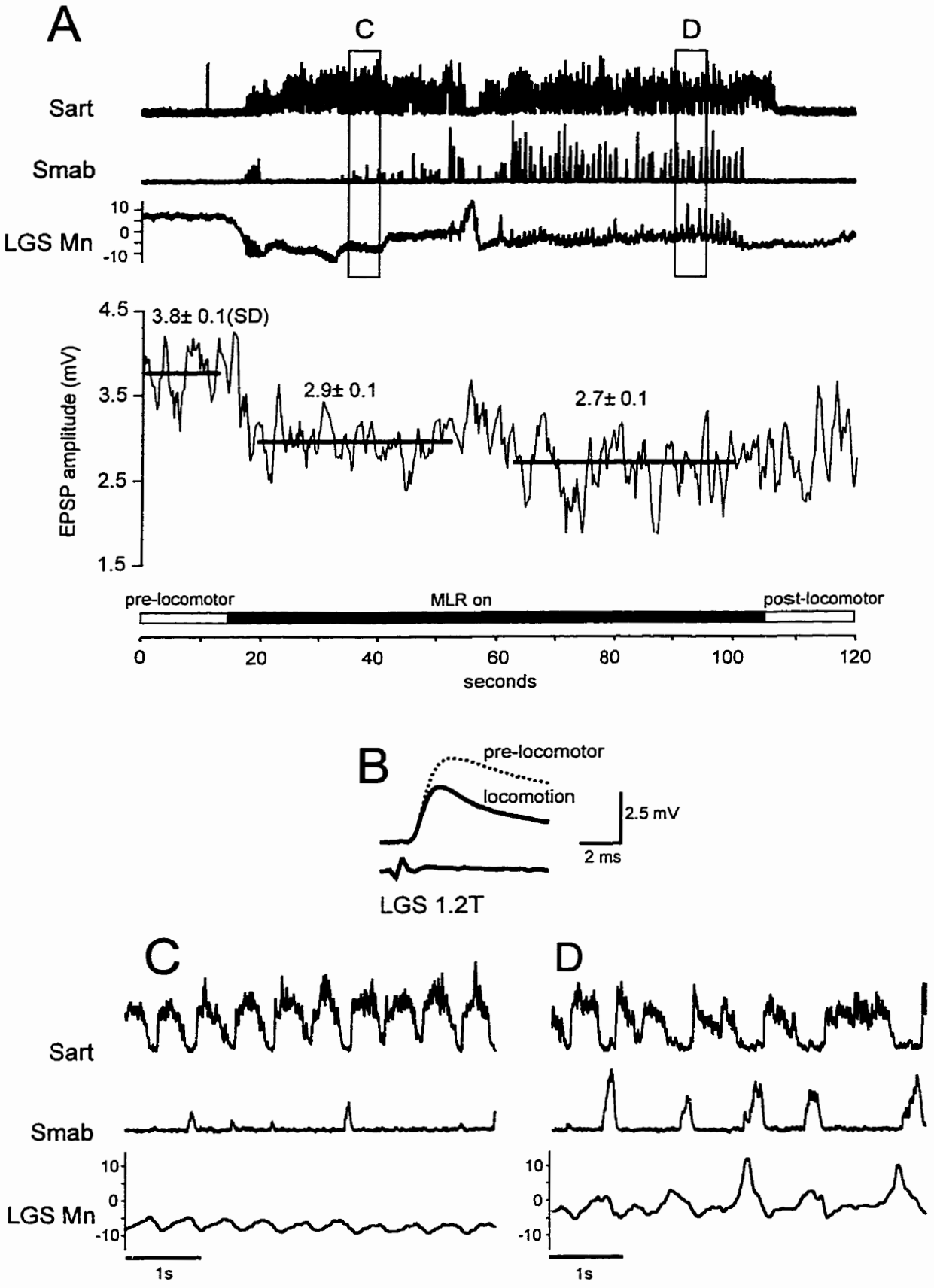
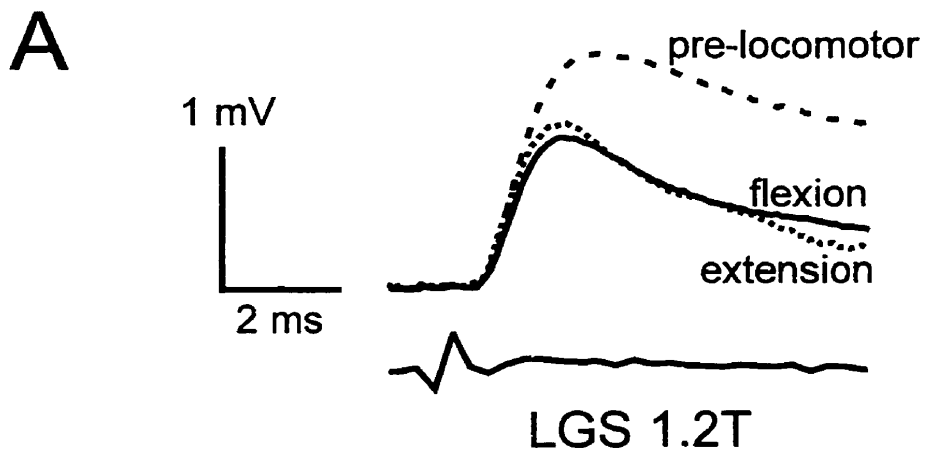

A
C
D
Sart
Smab
LGS Mn
10
0
-10
4.5
3.5
2.5
1.5
EPSP amplitude (mV)
3.8± 0.1(SD)
2.9± 0.1
2.7± 0.1
pre-locomotor
MLR on
post-locomotor
0
20
40
60
80
100
120
seconds
B
pre-locomotor
locomotion
2.5 mV
2 ms
LGS 1.2T
C
D
Sart
Smab
LGS Mn
10
0
-10
1s
1s

Figure 4: Phasic modulation of EPSP depression during locomotion is weak.

(A) Averaged monosynaptic EPSP amplitude during pre-locomotor state (n=68), during flexion (n=252) and during extension (n=86) of EPSPs evoked in an LGS motoneuron by stimulation of the LGS peripheral nerve (1.2T, 4Hz, same cell as Figure 3). **(B)** A plot of the ratio of averaged amplitude of the EPSPs evoked during extension to the average EPSP amplitude of the EPSPs evoked during flexion for 13 flexor motoneurons (circles), 15 extensor motoneurons (triangles) and 6 bifunctional motoneurons (squares). A ratio of 1.0 indicates that the averaged EPSP amplitude was equal in both phases.


A
pre-locomotor
1 mV
flexion
extension
2 ms
LGS 1.2T

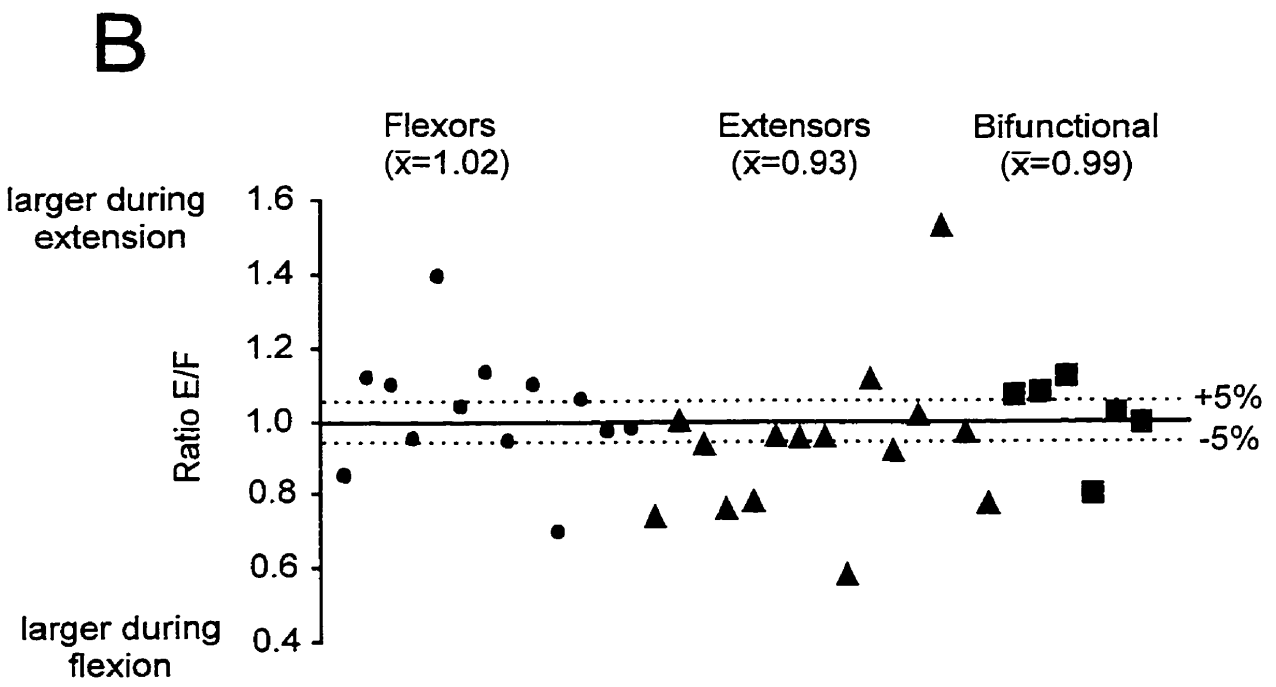

B
Flexors
(x̄=1.02)
Extensors
(x̄=0.93)
Bifunctional
(x̄=0.99)
larger during
extension
larger during
flexion
Ratio E/F
1.6
1.4
1.2
1.0
0.8
0.6
0.4
+5%
-5%

Figure 5: Long lasting depression of the monosynaptic EPSP following locomotion.

Rectified, integrated ipsilateral ENGs from flexor (Sart) and extensor (LGS) peripheral nerves before (pre-locomotor), during (MLR on) and after (post-locomotor) MLR stimulation. Fictive locomotion is represented by alternating activity in the peripheral nerves. EPSPs were evoked by stimulation of the MG peripheral nerve (4Hz, 1.4xT)and recorded in an LGS motoneuron. The plotted points represent the average monosynaptic EPSP amplitude (with standard error bars) for the 10s prior to each point (first 2 minutes 10 seconds), then every 50s until the EPSP had returned to pre-locomotor levels. In this cell the EPSP amplitude does not return to control levels until 2 minutes after the cessation of locomotion.

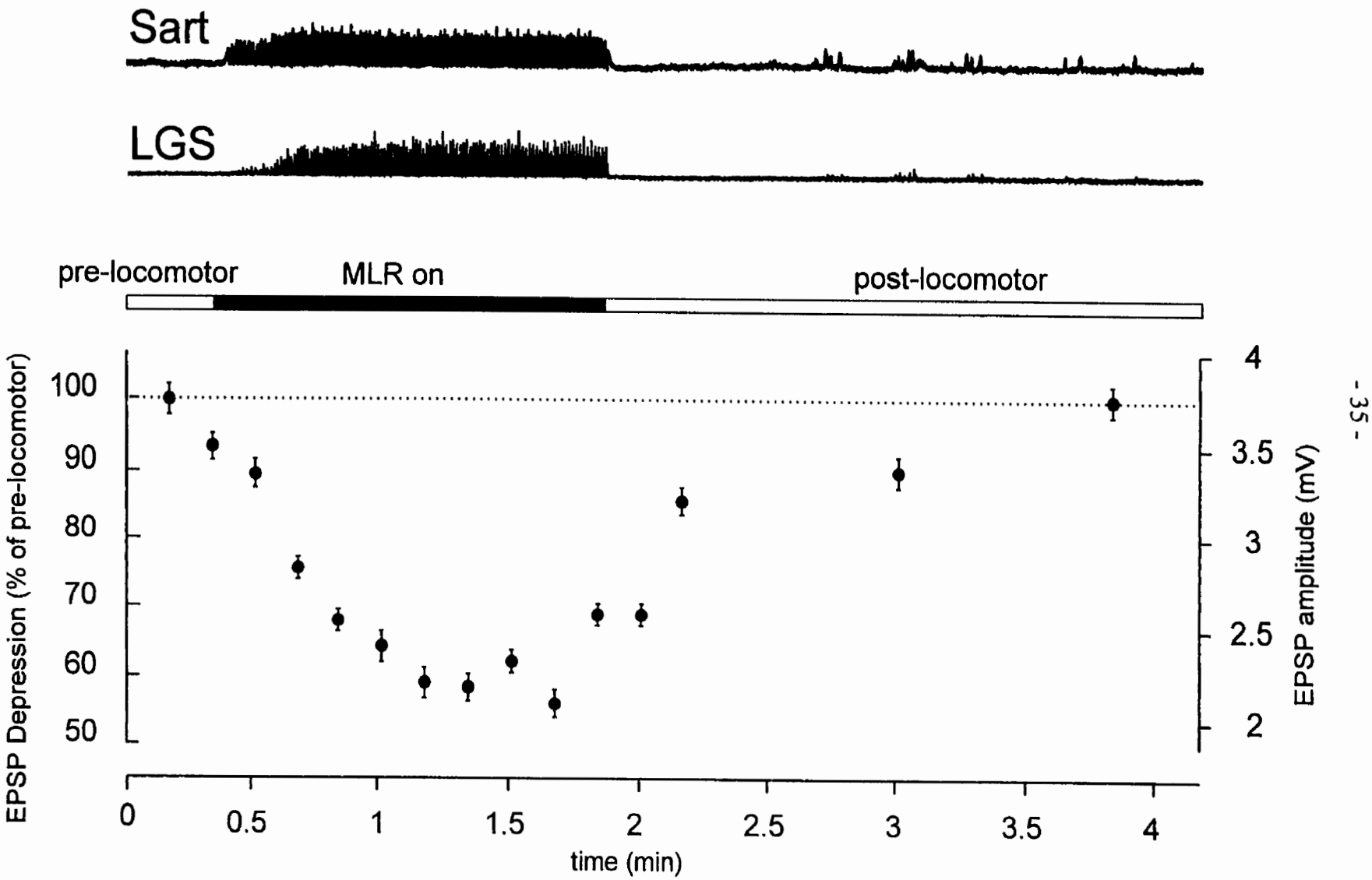

Sart
LGS
pre-locomotor
MLR on
post-locomotor
EPSP Depression (% of pre-locomotor)
100
90
80
70
60
50
4
3.5
3
2.5
2
EPSP amplitude (mV)
0
0.5
1
1.5
2
2.5
3
3.5
4
time (min)

Figure 6: Relationship between EPSP amplitude decrease and motoneuron membrane resistance decrease.

Locomotor motoneuron membrane resistance as a fraction of the pre-locomotor value is plotted on the abscissa. Locomotor EPSP amplitude as a percentage of pre-locomotor value is plotted on the ordinate. The correlation between the two is (R^2=0.32)

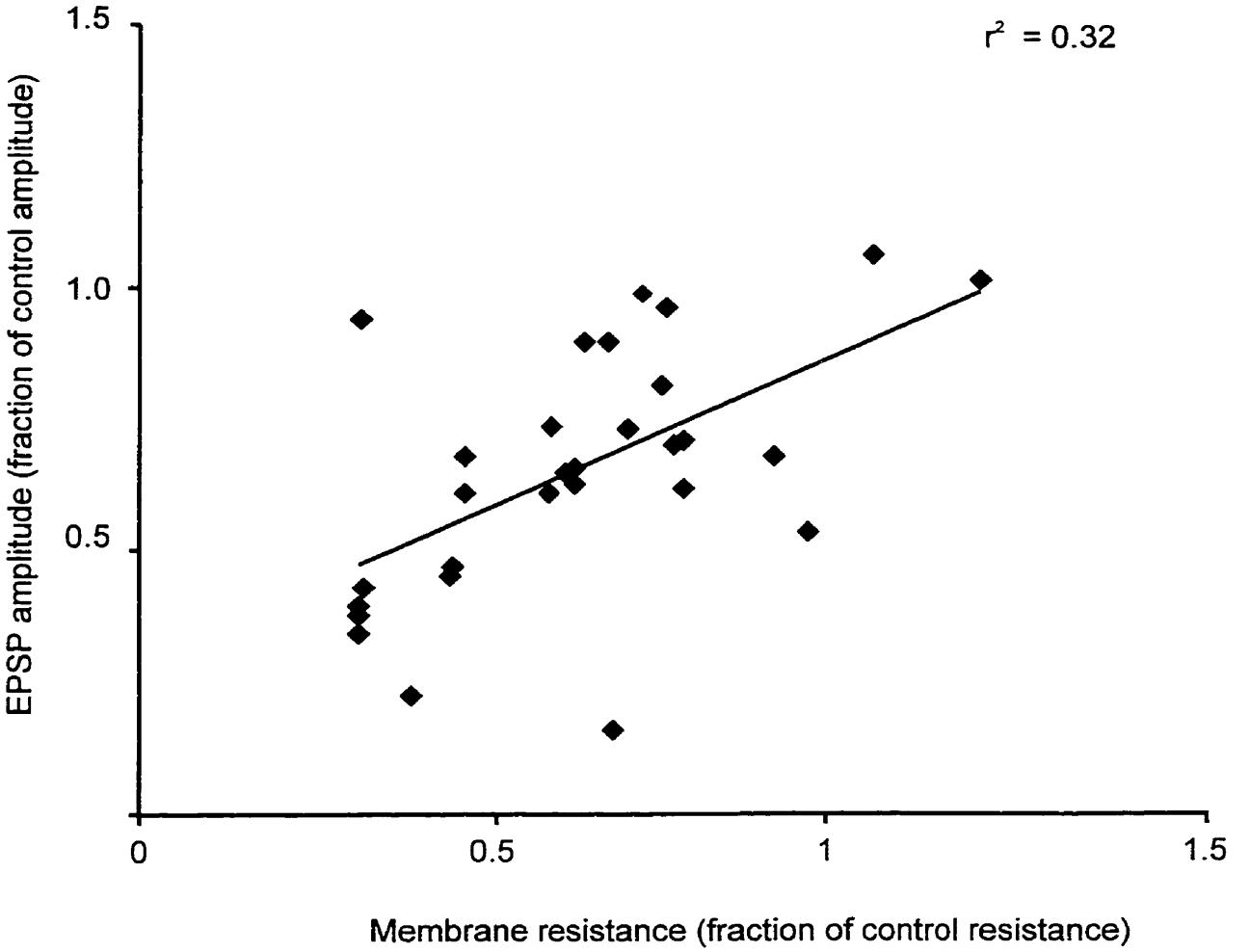
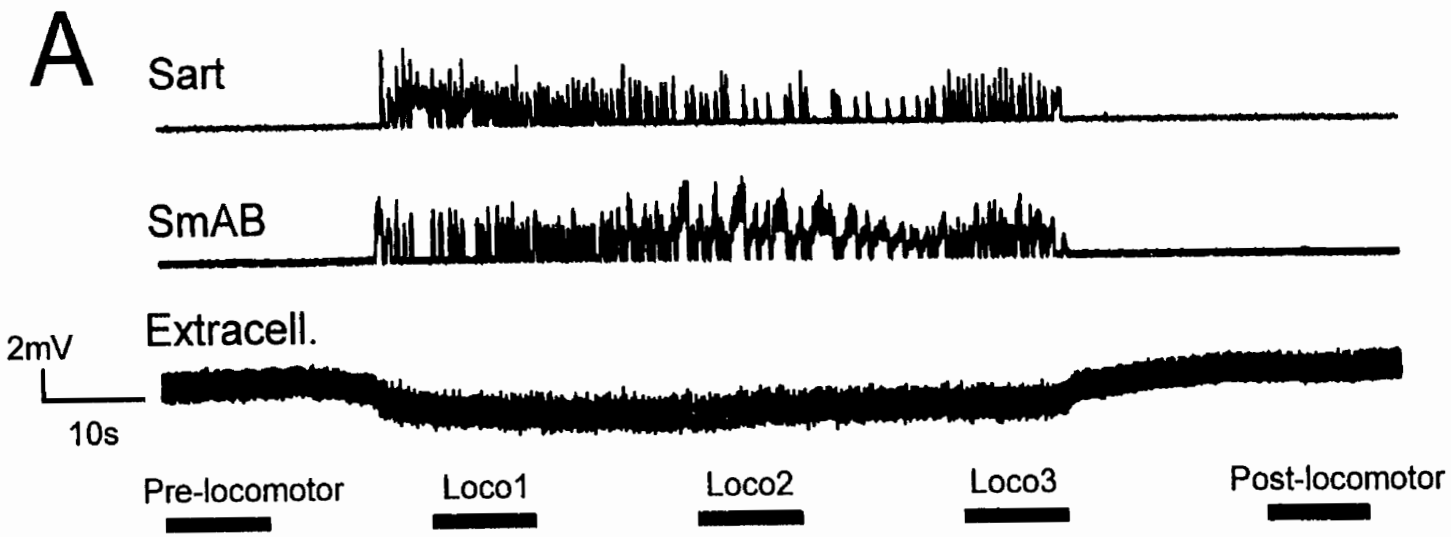

1.5
1.0
0.5
0
0.5
1
1.5
r^2 = 0.32
EPSP amplitude (fraction of control amplitude)
Membrane resistance (fraction of control resistance)

Figure 7: Locomotor modulation of group I extracellular field potentials

A. Rectified, integrated ipsilateral ENGs from an extensor (SmAB) and flexor (Sart) peripheral nerve as well as the recording from the extracellular microelectrode (DC potential) before locomotion, during locomotion (rhythmic activity in peripheral nerves) and after locomotion. A DC shift occurs reaching a peak of 2.1mV 10s after the initiation of locomotion and returns to baseline levels 15s after the end of locomotion. **B** Averaged EFPs from corresponding areas in panel **A**. Each FP is the average of 40FPs except for the last (average of 32 EFPs) EFP amplitude is reduced from 0.68-0.41mV (40%) at the onset of locomotion and remains depressed until 100-130s after the cessation of locomotion.


A
Sart
SmAB
Extracell.
2mV
10s
Pre-locomotor
Loco1
Loco2
Loco3
Post-locomotor

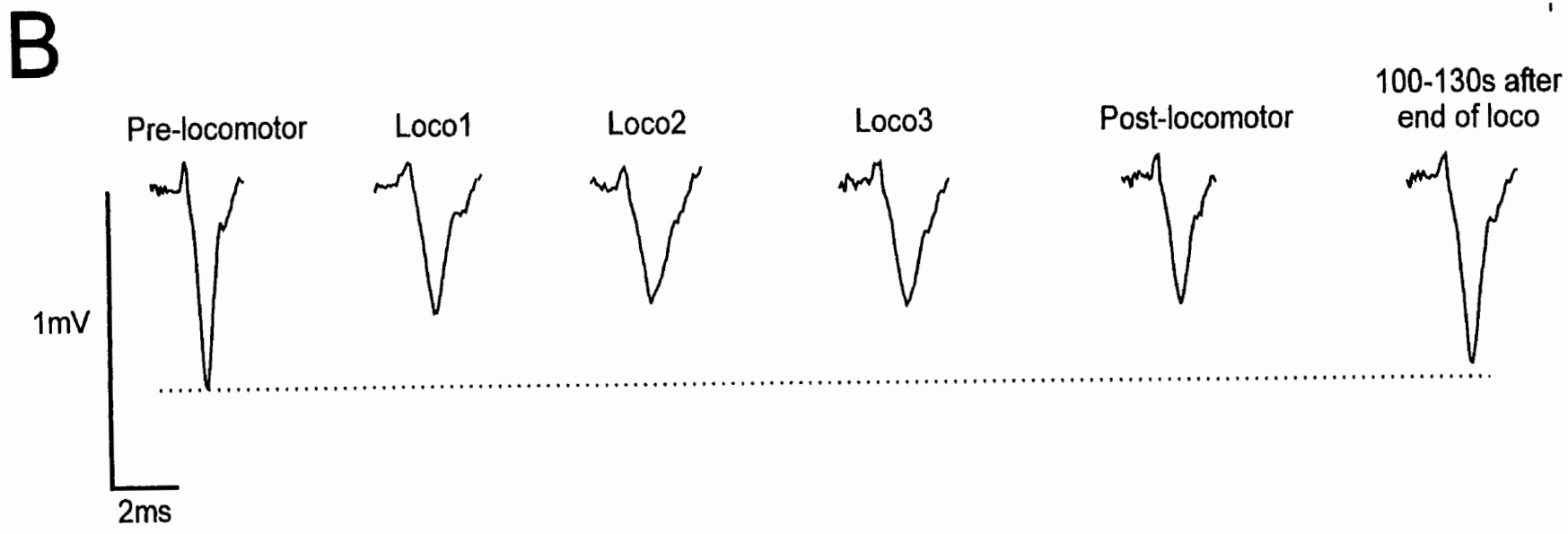

B
Pre-locomotor
Loco1
Loco2
Loco3
Post-locomotor
100-130s after end of loco
1mV
2ms

Table 1: Recovery times after locomotion for EPSPs and EFPs

The time period in which the EPSPs recovered to pre-locomotor levels are given in the first row. Recovery times for the EFP recordings are split up into total recordings (EFPs), those in which the locomotor EFP was measured immediately after locomotion (EFPs loco taken at end) and those in which the ventral roots were cut (EFPs ventral root cut).

Table 1

	Number of measurements	Depressed during loco	Time recovered following locomotor activivty					
			0-59s	60-119s	120-179s	180-239s	240-300s	>301s
EPSPs	11	11	2	2	2	1	1	3
EFPs	22	15	0	2	4	2	0	7
EFPs(loco at end)	17	10	1	4	3	0	0	2
EFPs(ventral root cut)	5	5	2	2	1	0	0	0

DISCUSSION

The main finding in this study is that the amplitude of the group Ia monosynaptic EPSP is decreased during fictive locomotion in the decerebrate cat. This depression occurs at the onset of locomotion and persists beyond the cessation of locomotor activity. Most EPSPs were tonically decreased in amplitude during locomotion with a mean depression to 72% of the pre-locomotor amplitude. In some cases phasic variations in EPSP amplitude during flexion and extension were superimposed on this tonic reduction. Differences in EPSP amplitude between the locomotor phases were, however, often small with maximal decreases in different motoneurons occurring equally in the flexor and extensor phases. Motoneuron membrane resistance was decreased during fictive locomotion, however this depression was weakly correlated with EPSP depression. Following the end of locomotion EPSPs did not immediately return to their prelocomotor amplitude. On average this took close to 2 minutes. Similar observations were made from extracellular field potential data. 15 of the 22 EFPs underwent a tonic decrease in amplitude during locomotion by an average of 22%. The amplitude of the 7 other EFPs were not altered during fictive locomotion.

The present experiments are the first to demonstrate that group I monosynaptic EPSPs are systematically decreased during fictive locomotion. It has previously been demonstrated that activation of descending brainstem sites can cause a reduction of afferent input. Noga, Bras and Jankowska (1992) demonstrated that in anaesthetized animals stimulation of brainstem regions that evoke locomotion in the decerebrate preparation can substantially depress transmission from group II afferents (~29%), however stimulation of these areas reduces transmission from group I afferents to a much lesser extent (<15%). It is therefore possible that stimulation in and around the MLR could depress EPSPs and this depression could be unrelated to locomotion per se. In the present study the group Ia EPSP depression is believed to be due to locomotion rather than simply MLR stimulation since in all 5 cases in which there was a prolonged delay between the start of brainstem stimulation and the onset of locomotion, EPSP amplitude was not reduced until the locomotion began (see Figure 2). It would be useful to measure

the EPSP amplitude during spontaneous locomotion without brainstem stimulation in other preparations to further demonstrate that EPSP depression is due to the locomotor state rather than brainstem stimulation.

Contribution of a postsynaptic mechanism

The finding of a decreased group I monosynaptic EPSP amplitude during fictive locomotion by itself does not necessarily indicate a presynaptic site for the inhibitory process. Shefchyk and Jordan (1984) observed that motoneuron membrane resistance was reduced during MLR-evoked fictive locomotion in almost half of the motoneurons studied. In the present study membrane resistance decreases occurred in almost all of the cells and in all of those motoneurons in which the monosynaptic EPSP was decreased. The differences between the two studies can possibly be accounted for by the method used for measuring membrane resistance. The Shefchyk and Jordan (1984) study used a longer duration (4-15ms) current pulse, while in the present study a short duration (0.5ms) pulse was given. It is possible that the short duration pulse is a more sensitive measure of membrane resistance changes since a membrane resistance decrease was observed in the vast majority of the cells in the present study. Based on the findings of Shefchyk and Jordan (1984), and the present study which showed an average membrane resistance decrease of 39%, a reduced motoneuron membrane resistance likely plays some role in the depression of the Ia monosynaptic EPSP during locomotion. One relevant question is the relative contribution of this post synaptic membrane resistance decrease to the depression of the monosynaptic EPSP during locomotion. Using modelled data, Clements, Forsythe and Redman (1987) suggested that to account for large decreases in monosynaptic EPSP amplitude following conditioning stimuli, motoneuron membrane resistance must decrease at least 10 fold. Similar results were obtained by Redman and Walmsley (1983) and McCrea et al. (1990) who observed that a 20% decrease in membrane resistance decreased EPSP amplitude by only 4%. It is evident in Figure 6 that decreases in membrane resistance were weakly correlated with decreases in EPSP amplitude (r^2=0.31). Analysis of membrane resistance changes in the flexion and extension phases also suggests that decreases in membrane resistance is only

a partial explanation for EPSP amplitude depression. In 19 of 29 cases monosynaptic EPSP amplitude and membrane resistance phasically modulated in the opposite direction (ie. smaller EPSP amplitude in the phase with higher membrane resistance). Also in most cases analysed (10 of 16), EPSP amplitude returned to pre-control levels following locomotion while membrane resistance remained reduced. Thus, decreases in motoneuron membrane resistance are unlikely to fully account for the Ia monosynaptic EPSP depression.

If increases in motoneuron conductance are unlikely to account for monosynaptic EPSP depression then a decrease in synaptic efficacy from primary afferent terminal to motoneuron is likely. This could be due to either a modulation of the motoneuron's receptor channels or presynaptic inhibition. It has been shown in the hippocampus that phosphorylation increases transmission through glutamate channels and dephosphorylation reduces transmission (see Smart, 1997). It has yet to be demonstrated that there is a dephosphorylation of the glutamate channels of spinal cord α-motoneurons occurs during locomotion. If this were the case however, it would post-synaptically decrease transmission to the motoneuron thus decreasing EFP and EPSP amplitude. Since this mechanism remains a possibility for the observed results, the term synaptic inhibition rather than presynaptic inhibition is preferable when discussing the results.

Contribution of a synaptic mechanism.

In 1988 Duenas and Rudomin observed a tonic decrease in the threshold required for intraspinal activation of the terminals of group I afferents during locomotion. They suggested that this occurred because the primary afferent terminals were depolarized. As discussed in the Introduction, the depolarization of the terminal would decrease EPSP amplitude due to a reduction of Ca^{2+} entering the primary afferent terminal and thus less neurotransmitter release to the motoneuron (Rudomin, 1994).

In the present experiments we observed that the amplitude of group I EFPs recorded in the motor pool of the ventral horn are decreased by 22% during locomotion. This provides strong evidence that a synaptic mechanism is occurring at the group I terminals during locomotion since these EFPs are a measure of synaptic current flowing

into the neurons located near the microelectrode tip. EFPs should not be greatly affected by changes in motoneuron membrane resistance but would be affected by a modulation of motoneuron ion-channel function. The present findings are similar to previous observations by Perrault et al. (submitted) who found that group I EFPs recorded in the intermediate nucleus are decreased during locomotion by 20%. It is tempting to suggest that the observed 28% decrease in group Ia monosynaptic EPSP amplitude during locomotion was the result of a 22% decrease in synaptic transmission (i.e. EFPs decreased 22% during locomotion) and the remaining 6% a minor contribution from decreased motoneuron membrane resistance, however this cannot be substantiated by the present study. The similarities between the group I EFP depression recorded in the intermediate nucleus and motor pool of the ventral horn is interesting when the findings of Lomeli, Quevedo, Linares and Rudomin (1998) are considered. They found that the segmental and ascending collaterals of the same muscle spindle afferent can be differentially affected by presynaptic inhibition in Clarke's column. Since the locomotor depressions of the group I EFPs in the motor pool of the ventral horn and the intermediate nucleus are similar, a differential PAD of muscle afferents does not seem to apply in this situation.

PAD and EPSP depression

Using intraaxonal recording, it has previously been shown that there is a depolarization of primary afferents during locomotion. Gossard et al. (1991) and Menard et al. (1999) found that the fibers are maximally depolarized during flexion and display another but smaller depolarization during extension. If this PAD were to be responsible for the decreased monosynaptic EPSP amplitude observed in the present experiment it would follow that Ia EPSPs should be larger during the extensor phase (minimal PAD). This was not the case and no consistent trend of phasic EPSP modulation could be found (see Figure 4B). A similar observation was obtained in a sample of extensor motoneurons by Angel et al. (1996). Other studies have shown a rhythmic modulation of EPSP amplitude during locomotion. In 1984, Shefchyk, Stein and Jordan observed that the monosynaptic EPSP amplitude was larger during the active phase of the motoneuron

however the phasic modulation was small (21%) and observed in less than half of the motoneurons studied.

In 1996 Gossard measured the transmission from the group Ia afferent during MLR-evoked fictive locomotion in the cat by impaling 6 motoneuron-afferent pairs (4 flexors, 2 extensors). In all 6 cases PAD was largest in the flexor phase but unitary EPSP amplitude was largest in the active phase of the motoneuron (in some cases larger than the pre-control condition). Our findings on the variability of the phase in which the largest EPSP occurs supports Gossard's (1996) statement that the locomotor-related PAD of group Ia afferents does not account for the phasic modulation of Ia monosynaptic EPSPs. However, our findings with composite EPSPs (Figure 4B) are in conflict with the largest unitary EPSP occurring in the active phase of the motoneuron (Gossard, 1996). If a differential pattern of PAD acts on individual afferents, EPSP amplitude could vary with the afferent/motoneuron pair studied. Since the Gossard (1996) study examined such a small sample of motoneurons (4 flexors, 2 extensors) there may have been a sampling bias.

Possible mechanism of synaptic inhibition

Since no pharmacological experiments were performed, the present study cannot give direct evidence for the mechanism causing the decrease of the group Ia monosynaptic EPSP during locomotion. However the delayed recovery to pre-control levels of both EPSPs and EFPs provides evidence that the mechanism is long-lasting.

It has been suggested that increased extracellular K^+ levels produced by repetitive activation of interneurons in the vicinity of primary afferent terminals may account for PAD (Krnjevic and Morris, 1974). In the present experiments, extracellular K^+ was assessed indirectly by looking at the DC potential measured in the vicinity of the extracellular recording electrode (c.f. Jimenez et al., 1984). Jimenez et al. (1984) demonstrated that a 1 mV DC shift accounts for a 1 mmol/l extracellular change in K^+. In 6 out of 7 cases the DC potential shifted to a more negative potential (mean 1.7mV) soon after the initiation of locomotion and returned to pre locomotor levels within 12s after cessation of locomotion. Although this finding suggests that increased extracellular K^+

levels may cause some of the depression of the monosynaptic EPSP during locomotion, the extracellular K^+ accumulates too slowly (see Figure 7) to account for the immediate EPSP depression observed and returns to pre-control levels too quickly following locomotion (see Figure 7) to be the primary mechanism for the Ia monosynaptic EPSP depression.

One possible cause of the monosynaptic EPSP depression which could account for the long lasting depression of the group I monosynaptic EPSP following locomotion is the activation of the $GABA_B$ subtype of the GABA receptor. $GABA_B$ receptor agonist baclofen has been shown to decrease the amplitude of monosynaptic EPSPs when administered intravenously to the cat (Lev-Tov, Meyers and Burke, 1988; Jimenez, Rudomin and Enriquez, 1991). Also, baclofen has been shown to have a depressive effect on the synaptic locomotor drive of motoneurons in the *in vitro* neonatal rat spinal cord while $GABA_B$ receptor antagonist CGP35348 has been shown to enhance locomotor drive (Bertrand and Cazalets, 1999). While the effect of GABA on $GABA_A$ receptors is ionotropic and therefore occurs rather quickly, it has been shown that $GABA_B$ receptors are coupled to a G-protein coupled second messenger system (Dolphin and Scott, 1987; Andrade, Malenka and Nicoll, 1986; Alford and Grillner, 1991) and, therefore, these actions have a much longer time course. The result of $GABA_B$ receptor activation on group Ia afferents is a reduction of the action potential duration at the primary afferent terminal (Curtis and Lacey, 1998). This would reduce of neurotransmitter release from the primary afferent terminal by reducing presynaptic Ca^{2+} influx through voltage gated channels (Curtis and Lodge, 1978) hence decreasing the postsynaptic voltage response.

Physiological implications of EPSP depression

An increased motoneuron conductance (decreased resistance) would by itself make motoneurons less excitable since a given synaptic current would produce a smaller postsynaptic voltage change. There is evidence, however, that motoneuron excitability is increased during locomotion. In the same preparation used here there is a reduced voltage threshold for evoking action potentials in the motoneuron during fictive locomotion and rheobase is lowered (Krawitz 1997). Brownstone et al. (1992) observed

that during fictive locomotion, afterhyperpolarizations (AHPs) were decreased in amplitude compared to AHPs evoked by current injections at rest, and that motoneurons can fire faster during fictive locomotion. Also, Brownstone et al. (1994) demonstrated that the excitatory component of the locomotor drive potentials behaves in a voltage dependant manner, such that the amplitude increases with depolarization (voltage dependant depolarization). Therefore it seems that motoneurons are in fact more excitable during locomotion, especially during the active phase in which they are depolarized (Jordan, 1983).

It has been demonstrated that during fictive locomotion motoneuron excitability is increased (Brownstone, 1992; 1994; Krawitz, 1997), however the gain of the monosynaptic reflex is tonically decreased during locomotion in the locomoting cat (Bennett et al., 1996). The findings in the present study suggest that the increased motoneuron excitability during locomotion is countered by a depression of the monosynaptic EPSP due primarily to a synaptic mechanism thereby resulting in a decrease of reflex gain. The decrease of the reflex gain is beneficial during locomotion for stability. It has been shown that in paretic patients with unusually large reflex gains during locomotion that clonus and spasticity may occur during locomotion (Yang et al., 1991). Monosynaptic reflex amplitude has also been shown to be phasicaly modulated during the step cycle. Akazawa et al. (1982) observed that the stretch reflex of the soleus muscle in the cat was largest at the peak in soleus EMG activity. Capaday and Stein (1986), Faist, Dietz and Pierrot-Deseilligny (1996) as well as Yang and Whelan, (1993) observed that in humans the soleus H-reflex was usually largest during the stance phase of locomotion. These findings on increased extensor monosynaptic reflexes during stance are consistent with the phasic PAD (larger during flexion) of group I afferents observed during fictive locomotion (Gossard et al., 1991; Menard et al., 1999). Present results, however, offer little support for this explanation. As mentioned, cyclic EPSP variations were often small with maximum EPSP depression occurring equally in flexion and extension. Thus we suggest that the increase in extensor motoneuron depolarization (increased excitability) during stance is a more likely explanation for the phasic

modulation in human subjects. Most of the discussion of synaptic EPSP modulation in the literature has focused on the phasic variation in presynaptic inhibition (Crenna and Frigo, 1987; Gossard, Cabelguen and Rossignol, 1991). Duenas and Rudomin (1988) demonstrated that the threshold for group I afferent terminal activation is tonically decreased during locomotion and Perreault et al (submitted) observed a tonic decrease in group I EFP amplitude in the intermediate nucleus during locomotion. These observations and present direct measurement of composite Ia monosynaptic EPSP amplitudes suggest that the tonic synaptic inhibition of group Ia afferents is a more powerful phenomena than the phasic modulation.

During locomotion it has been demonstrated that there is an increased excitability of motoneurons (Brownstone, 1992; 1994; Krawitz, 1997), and a tonic decrease of monosynaptic EPSP amplitude (present study) which may be restored by a phase-dependant disynaptic excitation of motoneurons which has been observed in cats (Angel et al., 1996; Degtyarenko et al, 1998; Quevedo et al., 1998) and in humans (Stephens and Yang, 1996). Complete understanding of the modulation of reflex gain during locomotion requires an understanding of the interactions between these three factors. Unfortunately, such an understanding must await future experiments in which all of these factors are considered.

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