Interaction Between the Descending Mesencephalic Locomotor Region Pathway and High Threshold Reflex Pathways in the Control of Lumbar Alpha Motoneurons during Fictive Locomotion in the Cat.

by

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Abstract

The purpose of these studies was to examine the effects produced in lumbar motoneurons by a descending pathway from the brainstem which, when stimulated, can produce locomotion in the decerebrate cat. The interaction of this descending pathway with high threshold cutaneous and muscle segmental reflex pathways was also examined in an effort to determine whether convergence of these two pathways onto common interneurons in the lumbar spinal cord occurs.

Precollicular postmammillary decerebrate cats were used in these experiments; fictive locomotion was produced by electrical stimulation of the mesencephalic locomotor region (MLR) in the brainstem. Intracellular recordings from lumbar alpha motoneurons during control and locomotor periods were made.

The stimuli to the MLR evoked short latency postsynaptic potentials in alpha motoneurons during fictive locomotion. The mean latency of the MLR evoked excitatory postsynaptic potentials (EPSPs) was 5.1 ms, while that of evoked inhibitory postsynaptic potentials (IPSPs) was 6.5 ms. In 35 of 41 motoneurons examined, the MLR evoked an EPSP during thhe depolarized phase of the locomotor drive potential (LDP) and was observed to alternate with a longer latency IPSP observed during the hyperpolarized phase of the LDP. In 5 motoneurons only an EPSP was produced by MLR stimulation. These EPSPs were observed to be modulated in amplitude during the step cycle, being of greatest amplitude during the depolarized phase of the LDP. In one motoneuron

the predominant effect from the MLR stimuli was an IPSP which was of greatest amplitude during the hyperpolarized phase of the LDP. The alternating EPSPs and IPSPs evoked by the MLR stimuli were only observed during stimulation of the brainstem site which was optimal for the production of fictive locomotion, and the alternating PSPs were not observed until the motoneuron displayed the LDPs characteristically observed during fictive locomotion.

Using a condition-test paradigm the presence of convergence of the descending MLR pathway and the high threshold reflex pathways onto shared interneurons within the lumbar spinal cord was demonstrated. The data indicates that both the excitatory and inhibitory pathways from the MLR to motoneurons share interneurons in the lumbar spinal cord with high threshold reflex pathways.

It was also observed that the afterhyperpolarization (AHP) following an action potential is decreased in amplitude in half of the motoneurons examined during fictive locomotion. In addition to the reduction in the AHP, an EPSP immediately preceding the action potential was observed in 4 of 6 motoneurons examined using spike triggered averaging. This data suggests that perhaps the intrinsic membrane properties of the motoneuron alone do not determine the firing pattern of all motoneurons during fictive locomotion, but rather that appropriately timed EPSPs from the locomotor central pattern generator may play a role in both the production of the depolarized phase of the LDP and

the generation of action potentials in motoneurons during the step cycle.

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Introduction

Based on the work done over the last 75 years, it is known that pathways from various supraspinal centers can activate the spinal neuronal organization within the lumbar spinal cord which is responsible for the production of locomotor activity (for review see Grillner, 1975). It is also clear that afferent information from the periphery can influence and modify locomotion by way of spinal circuitry (Grillner, 1975). It is the goal of this thesis to examine one descending pathway from the brainstem which when stimulated can produce locomotion, addressing specifically the effects produced by this descending pathway at the motoneuronal level and how such effects are influenced by high threshold cutaneous and muscle afferents.

I. Concept of the Spinal Locomotor Central Pattern Generator

It has been established that the vertebrate spinal cord has the neuronal organization necessary to produce several different rhythmic motor behaviors including swimming, scratching and walking (Stein, 1984). The present discussion will emphasize only stepping behavior, focussing specifically on work using the cat model since many of the relevant spinal reflex and supraspinal descending systems have already been characterized in the cat.

With the investigations on mammalian stepping behavior at the turn of this century (Sherrington, 1906; Brown, 1911), the concept of an intrinsic spinal cord organization

capable of producing the rhythmic alternating movements characteristic of stepping began to evolve. The concept of a central pattern generator, or CPG, centered around the idea that the actual behavior in its "purist" form could be produced at the level of the spinal cord without the requirements of phasic afferent feedback from the moving limb (Grillner, 1975; Stein, 1984) or descending input from supraspinal centers (Grillner, 1975).

It has been demonstrated that after deafferentation, either by cutting nerves to the hindlimb peripherally (Sherrington, 1913b), or by dorsal root transections (Grillner and Zangger, 1984), many animals are capable of using the deafferented limb with increasing ability with the passage of time (Grillner, 1975). In experiments in which locomotion was produced in the animal following deafferentation, patterns of locomotor activity could be observed in peripheral muscle nerves and limb muscles which did not display a significant deviation from the patterns observed in intact spontaneously locomoting animals (Grillner, and Zangger, 1984; Perret and Cabelguen, 1980; Perret, 1983).

An alternate method for removing phasic afferent information from the moving limb during locomotion is the use of curare-like paralytic agents such as gallamine triethiodide. It is this approach which has been used extensively in recent years to investigate single cell activity during locomotion. The term commonly used for this is "fictive locomotion" (Stein, 1984), in which the rhythmic

hindlimb activity associated with locomotion is not monitored by measuring muscle activity (electromyograms) since activation of the muscle is blocked, but rather by monitoring nerve (electroneurograms) or motoneuron activity at the level of the ventral root or the motoneuron soma.

II. Interactions Between Peripheral Afferents and the Locomotor Central Pattern Generator

With the results of the deafferentation experiments and the subsequent development of the concept of an intrinsic spinal cord organization responsible for the production of locomotor activity, the means by which peripheral afferents interacted with the CPG to modify reflexes and the locomotor act itself became an issue to be addressed. Examination of a variety of afferent information from the limb during walking, as well as the reflex pathways activated with electrical stimulation, yielded some information which would aid in conceptualizing the spinal organization controlling the movement of the limbs (Grillner,1975).

Several studies have investigated the role of proprioceptive input during locomotion. Engberg and Lundberg (1969) demonstrated that activity in ankle extensor motoneurons began prior to the actual foot contact with the ground, implying the activation of the ankle extensor muscles independent of afferent information about placement of the paw and passive stretch of the ankle extensor muscles themselves. This was in agreement with work by Graham-Brown

(1914), which demonstrated that much of the pattern of activity in various muscle groups was centrally programmed and not simply a chain of reflexes as Sherrington (1913b) first hypothesized. However, it was also hypothesized that during the yield part of the stance phase when the limb must deal with the weight of the animal, that extensor stretch reflexes could aid the limb in dealing with the load placed on it (Lundberg,1969). Using a treadmill locomoting cat and monitoring muscle activity in the triceps surae muscles of one hindlimb, Akazawa et al. (1982) found that reflexes evoked by short stretches of this muscle group could produce a variable reflex response depending on which phase of the step cycle was currently underway. The evoked reflex was largest during the stance phase and smallest during the swing phase of the step cycle. Other investigators (Schomberg and Behrends, 1978), using intracellular recordings with a limited number of motoneurons, reported that in addition to the monosynaptic EPSPs, there were observations of polysynaptic Ia effects which appeared during the depolarized phase of the motoneuron membrane oscillations during the step cycle. This finding raised the possibility that polysynaptic Ia effects mediated by an excitatory interneuron(s) associated with the spinal cord locomotor organization may exist. Further investigations using the fictive locomotion preparation and electrical stimulation of group I fibers in peripheral nerves revealed no significant change in the amplitude of the monosynaptic EPSP nor evidence for polysynaptic reflexes from Ia

afferents during the fictive step cycle (Shefchyk et al., 1984). This data supported the hypothesis that the monosynaptic EPSP was simply superimposed on the membrane potential oscillations during the depolarized phase of the step cycle. The motoneuron membrane potential with the EPSP superimposed was more likely to cross the threshold for firing and thereby contribute to the recruitment of the motoneuron into the stretch evoked reflex response. During the hyperpolarized phase of the step cycle, the membrane potential was displaced away from the threshold level and the superimposed EPSP was less likely to produce spiking in the motoneuron. It is unlikely that any convergence of Ia afferent information onto elements of the locomotor CPG account for the change in stretch reflexes during locomotion, but rather it appears that the Ia evoked responses are simply superimposed on the basic rhythm established in the motoneurons by the locomotor CPG.

Sherrington (1909,1910) originally demonstrated that after severing the cutaneous nerves of the hindlimb one could still observe stepping movements, and several studies have further examined the role of cutaneous information from the limb during locomotion. Clearly, cutaneous afferent information from the paw can play a role in providing feedback to the central nervous system about the changing terrain. It has been demonstrated that spontaneously walking intact animals (Duysens et al.,1980; Forssberg,1979), decerebrate walking animals (thalamic and

mesencephalic preparations) (Duysens, 1977), and chronic spinal animals (Forssberg et al., 1973) can respond in a variety of ways to natural or electrical stimulation of peripheral cutaneous nerves during locomotion depending upon when in the step cycle the stimuli are delivered. In general, if the stimuli to the pad or plantar surface of the paw occur during the extension phase of the cycle, activity in the extensor muscles is facilitated, resulting in an augmentation of the extension phase. If on the other hand, the stimuli occur during the flexion phase, the flexor activity is augmented. This response has been termed the "stumbling corrective response" (Forssberg, 1979). In addition, the effects of high intensity cutaneous stimulation (from a variety of hindlimb regions) during locomotion not only produced phase dependent reflex responses in the stimulated limb but also phase dependent reflexes in the contralateral limb (Gauthier and Rossignol, 1981). Clearly, there is an interaction between cutaneous reflex effects and those events determining the motoneuron activity during locomotion. However, the degree to which this interaction involves convergence of the cutaneous afferents onto components of the CPG remains to be determined and is an issue addressed in the present study.

III. Supraspinal Activity during Locomotion and Descending Influences to the Lumbar Spinal Cord

There are numerous supraspinal regions of the brain which have been identified as having monosynaptic or polysynaptic influences onto neurons in the spinal cord

(Kuypers, 1981; Wilson and Peterson, 1981; Asanuma, 1981). Since several of these descending systems have connections to alpha motoneurons associated with the limbs, they were targets for investigation as to their potential influence on the pattern of activity in the cat hindlimb during stepping (Orlovsky,1972). The vestibulospinal tract (VST) originating from the lateral vestibular (Dieters') nucleus is one such system. The VST is involved in the relay of information required to make postural adjustments in response to changing vestibular input and plays a role in the maintenance of balance during locomotion. Neurons in Dieters' nucleus have been shown to be rhythmically active during treadmill locomotion (Orlovsky, 1972), and electrical stimulation of Dieters' nuclei during walking can influence extensor activity during the step cycle (Russel and Zajac, 1979). However, bilateral lesions of Dieters' nucleus do not prevent the initiation and maintenance of treadmill stepping produced by electrical stimulation of the mesencephalic locomotor region (MLR) (Jell et al.,1984).

A second brainstem area that has been studied is the reticulospinal system which descends via the ventrolateral funiculus of the spinal cord (Kuypers, 1981). Regions in the medial reticular formation have been shown to receive projections from brainstem sites capable of producing locomotion (Steeves and Jordan, 1984) and the ventrolateral quadrant of the spinal cord has been demonstrated to be the region of the cord which must be intact in order for

stimulation to produce locomotion (Steeves and Jordan,1980). Cells in the reticular formation are rhythmically active during treadmill stepping (Orlovsky, 1970) and the pattern of activity of some reticulospinal cells can be correlated with activity in muscle groups of one or more limbs active during a particular time of the step cycle (Drew et al.,1983). Recently, electrical stimulation of the medullary reticular formation during locomotion in thalamic cats spontaneously stepping on a treadmill revealed phase dependent responses in limb muscles during locomotion (Drew et al., 1982) The evidence indicates that structures in the reticular formation may function to relay commands for the initiation of locomotion from higher brainstem structures to the spinal CPG.

Other supraspinal regions that have demonstrated rhythmic activity during locomotion include the red nucleus (Orlovsky and Shik, 1976), and the motor cortex (Armstrong and Drew, 1984). However, the presence of rhythmicity within a region of the brain does not demonstrate its necessity for the production of the movement since lesions of these regions, or transection of the neuroaxis, as will be described below, do not prevent the production of stepping activity by the spinal cord.

IV. Brainstem Areas Capable of Initiating Locomotor Activity

Transection of the neuraxis at a variety of levels in the cat does not affect the ability of the spinal cord to produce stepping, but can change the manner in which locomotion can be initiated. The transected brainstem

preparation provides a tool with which investigators can systematically examine, in a controlled fashion, the descending systems and spinal organization involved in controlled treadmill or fictive locomotion.

If the brainstem is transected at a level extending dorsally from the rostral border of the superior colliculi to the optic chiasm ventrally (termed a precollicular preparation), the animal is capable of spontaneous stepping activity in all limbs (Orlovsky, 1969). In addition to the spontaneously occurring episodes of stepping, controlled locomotion can be induced using electrical stimulation of the subthalamic nucleus (Orlovsky, 1969).

If the transection is made from the rostral border of the superior colliculi to a level which is postmammillary, a preparation with marked decerebrate rigidity is produced which will not spontaneously walk. This is called the mesencephalic preparation, and locomotion can be produced with electrical (Orlovsky,1967; McCrea et al., 1980) or chemical (Garcia-Rill and Skinner, 1984) stimulation of a region in the brainstem corresponding anatomically to the cuniform nucleus and referred to as the mesencephalic locomotor region (MLR) (Steeves and Jordan, 1984). The MLR is also implicated as a relay site in the production of locomotor activity from higher centers since it is necessary for the production of locomotion using stimulation of corticofugal fibers and pyramidal tract stimulation in which the pyramidal tract caudal to the level of stimulation was

sectioned (Shik et al., 1968). The MLR also receives projections from other regions of the brain which are implicated in the production of locomotion (Garcia-Rill et al., 1983; Kettler and Jordan, 1984).

Since the MLR is presently the best defined brainstem site which can produce locomotion when electrically stimulated, it is the mesencephalic preparation induced to walk with stimulation of the MLR that has been selected for use in the experiments presented in this thesis.

V. Half-Center Hypothesis for the Locomotor CPG

Since the work of Sherrington at the turn of the century (Sherrington, 1906) the concept of reciprocity in neuronal organization in the spinal cord has developed steadily and was incorporated into the locomotion literature with the work of Graham-Brown (Graham-Brown, 1911). More recently these ideas have been expanded to provide a conceptual theory for the organization of the locomotor central pattern generator by the Swedish investigators working with L-DOPA treated acute spinal cats (Anden et al.,1966; Jankowska et al., 1967).

In the acute spinal cat no stepping activity below the lesion has been observed without pharmacological manipulation (Grillner, 1975). Immediately after spinal transection, the animal can be induced to produce stepping movements with intrathecal injections of noradrenalin (Omeniuk and Jordan, 1982) or with intravenous injections of L-DOPA (Jankowska et al.,1967) or the noradrenergic agonist Clonidine (Forssberg and Grillner, 1973). The intravenous

use of L-DOPA, presumably functioning as a precursor to noradrenalin (Anden et al. 1966), revealed a number of reflex changes in segmental reflex systems in the acute spinal cat, and these changes were subject to extensive examination. One of the significant reflex changes observed after L-DOPA was the suppression of the short latency flexor reflex afferent (FRA) effects and the appearance of longer latency, long lasting effects (Anden et al., 1966; Jankowska et al.,1967). Flexor reflex afferents included high threshold muscle afferents, high threshold cutaneous afferents, and joint afferents, and appear to share a common reflex pathway, (Baldissera et al., 1981). The experiments demonstrated that ipsilateral FRA stimulation produced late long lasting excitation of the ipsilateral flexor motoneurons and similarly late long lasting inhibition of the ipsilateral extensor motoneurons (Anden et al., 1966; Jankowska et al., 1967). Stimulation of the contralateral FRA produced the late excitation of the ipsilateral extensors and inhibition of the ipsilateral flexor motoneurons (Jankowska et al., 1967). Using a conditioning-test paradigm with varying intervals between the ipsilateral and contralateral FRA stimulation, Jankowska et al. (1967) demonstrated that activation of the ipsilateral FRA pathway after L-DOPA could effectively inhibit the effects produced by the contralateral FRA pathway and visa versa. This data was taken as support for a reciprocal organization within the spinal cord coordinating the FRA reflex pathways after

L-DOPA. Since there was no evidence for a postsynaptic action of L-DOPA on the motoneurons themselves, nor a presynaptic effect on the incoming sensory fibers, the presence of interneurons mediating these effects was hypothesized (Jankowska et al,1967). These investigators proceeded to identify interneurons in lamina VII of the lumbar spinal cord which appeared to be responsible for these reciprocal effects (Jankowska et al., 1967b).

The fact that L-DOPA produced these reciprocal reflex changes in the acute spinal animal, paired with the fact the the L-DOPA acting through noradrenergic mechanisms could produce stepping movements in the acute animal, led to the hypothesis that perhaps this reciprocal organization revealed during the L-DOPA experiments reflected the spinal organization for stepping (Jankowska et al., 1967). Further support for the link between the two systems came when Grillner and Shik (1973) demonstrated that in the mesencephalic preparation, electrical stimulation of the MLR not only produced locomotion but also produced the same changes in the short and long latency FRA evoked responses as observed in the L-DOPA treated acute spinal cats. This reinforced the hypothesis for a reciprocal organization of the spinal locomotor CPG first proffered by Graham-Brown (1914) and expanded on by Lundberg and the Swedish investigators (Anden et al., 1966; Jankowska et al., 1967).

VI. Activity of Identified Spinal Neurons During Fictive Locomotion

Turning now to the lumbar spinal cord specifically, the

work over the last 20 years has revealed the details about only a few of the neurons within the lumbar spinal cord and only recently have the identified cells been examined during locomotion (Jordan, 1981). One of these neuron groups was the Ia inhibitory interneuron (IaIN) (Hultborn et al., 1976). Although the concept of reciprocal organization in the spinal cord had been around for almost 80 years (Sherrington, 1906) and intracellular records from motoneurons showed that Ia afferents produced excitation in homonymous and agonists along with longer latency inhibition of antagonists (Eccles, 1957), the interneurons mediating the inhibition had remained unidentifed. In a series of experiments, the peripheral reflex connections (Hultborn et al., 1976), the supraspinal influences (Hultborn et al., 1976), and finally the monosynaptic connections between the Ia interneuron and motoneurons were shown (Jankowska and Roberts, 1973).

It has been demonstrated that IaINs are rhythmically active during either the extension or flexion phase of the fictive step cycle (Jordan, 1981,1983). Althoughh their period of activity during the step cycle is consistent with their having a role in inhibiting antagonist motoneurons appropriately during the step cycle, the pharmacological removal of their glycine mediated inhibitory action onto motoneurons did not prevent the production of locomotion (Jordan, 1981,1983). In these experiments, the glycine antagonist used was strychnine and after intravenous

administration oof the drug, the animals continued to display fictive stepping and the motoneurons displayed rhythmic depolarizations and firing during each cycle without the normally occurring interburst hyperpolarization. Although the IaINs contribute to the pattern of activity in motoneurons during locomotion, they do not appear to be essential for the generation of the activity and hence are not an integral part of the CPG.

Another interneuron group within the spinal cord that has been documented extensively are the Renshaw cells (RCs) (Eccles et al., 1961). RCs are activated monosynaptically from motoneuron axon collaterals and polysynaptically from some peripheral afferents and descending systems (for review see Baldissera et al., 1981). They are known to inhibit alpha motoneurons (Eccles et al.1961), IaINs (Hultborn et al., 1971), and other RCs (Ryall, 1970). Initially it was thought that RCs were completely inhibited during locomotion (Shik and Orlovsky, 1976) but that proved to be incorrect (McCrea et al.,1980). RCs are rhythmically active during the step cycle and their phase of activity corresponds with the motoneuron groups responsible for their activation (Jordan,1983). The primary excitation of RCs during the step cycle comes from motor axon collaterals (Noga et al., 1982) and it appears that, during locomotion produced by MLR stimulation, a weak descending inhibition also influences RCs (Noga et al., 1982), but is insufficient to prevent their activation during the step cycle. Investigations using the nicotinic antagonist, mecamylamine, have

demonstrated that blocking the motor axon collateral activation of RCs during locomotion does not prevent treadmill locomotion or rhythmic motoneuron activity during fictive locomotion (Jordan, 1983). The results indicated that RCs may function to limit motoneuron and IaIN firing during each burst of activity in a step cycle , but that the role of RCs was not one of an integral part of the spinal CPG.

In conclusion, although both of the inhibitory interneurons current defined in the spinal cord , the IaINs and RCs, may play a role in limb movement during locomotion, their participation as an integral part of the locomotor rhythm generating CPG seems unlikely.

VII. Activity of Alpha Motoneurons During Fictive Locomotion

The activity of alpha motoneurons has been examined during a number of reflex and motor behaviors, including locomotion. Basically, motoneurons innervating single joint muscles are depolarized during either the flexion or extension phase of the step cycle as would be expected from the pattern of muscle activity known during the step cycle (Engberg and Lundberg, 1969). Intracellular examination of single identified motoneurons during fictive locomotion has revealed that during the step cycle the motoneuron membrane potential displays a rhythmic depolarization and hyperpolarization (Perret, 1983; Shefchyk and Jordan, 1985). These oscillations have been termed locomotor drive potentials (LDPs)(Shefchyk and Jordan, 1985) and are

observed in all motoneurons active during locomotion. During the depolarized phase of the LDP, the motoneuron membrane potential may or may not reach threshold for firing. It has also been determined that motoneurons active during locomotion do not display a significant change in membrane input resistance from the depolarized phase to the hyperpolarized phase of the LDP, a fact consistent with the presence of synaptic input from the spinal CPG to all motoneurons active during locomotion during all phases of the step cycle (Shefchyk and Jordan, 1985).

Activation of the muscle fibers from the spinal cord only occurs if the motoneuron reaches threshold and produces an action potential. As mentioned above, a motoneuron may or may not fire during the depolarized phase of the step cycle but when it does fire, the factors determining its rate of firing will be of fundamental importance in determining the extent of muscle contraction and force generation (Stein and Parmiggiani, 1981). For some time various investigators have been examining the factors intrinsic to the motoneuron which regulates the motoneuron's firing pattern (Schwindt and Calvin, 1972; Schwindt, 1973). It has been established the time course of the potassium current producing the afterhyperpolarization (AHP) is the determining factor for the rate of firing of motoneurons during current evoked motoneuron firing (Gustaffson, 1974). Zajac and Young (1980) examined the firing pattern in single ventral root filaments during fictive locomotion, and their results were consistent with the afterhyperpolarization duration being the likely

factor determining the firing pattern within each burst of motoneuron activity during the fictive step cycle. Their conclusions were based on data from extracellular recordings from motor axons exiting the spinal cord, with no attempt to examine the events actually occurring in the motoneuron. On the other hand, intracellular records from motoneurons during fictive locomotion show that the afterhyperpolarization was not as large as that produced during depolarizing current injection. This raises the possibility that mechanisms other than the intrinsic membrane properties of the motoneuron determine firing patterns during the fictive step cycle (Jordan and Shefchyk, 1984).

VIII. Goals of Present Work

It has already been pointed out that electrical stimulation of the MLR can activate the lumbar locomotor CPG. Using MLR stimulation, the effects from the descending MLR pathway via elements of the CPG, will be characterized in a variety of alpha motoneurons. This will provide information as to the nature of the elements within the CPG which mediate the descending MLR effects and thereby determine the specific synaptic events underlying the LDPs in the motoneurons. It is hypothesized that the effects from the MLR onto motoneurons share premotoneuronal elements in the spinal cord with high threshold cutaneous and muscle afferents as suggested by the work on the acute spinal animal treated with L-DOPA. By employing intracellular

recording from identified alpha motoneurons and testing for spatial facilitation in the descending and peripheral afferent pathways, the presence of interneurons common to both pathways, and related to the locomotor CPG can be examined. It is also hypothesized that the motoneuron firing pattern during locomotion is not determined solely by the time course of decay of the AHP, but rather reflects an interaction between the AHP and excitatory synaptic events impinging onto motoneurons from the spinal CPG during the depolarized phase of the LDP.

I. General Surgical Preparation

A total of 20 cats of either sex were used for these experiments. The mean weight of the animals was 3.0 kg (range 2.3 to 4.0 kg).

Halothane (Halocarbon, Malton, Ontario, Canada) carried in a mixture of oxygen and nitrous oxide was the anaesthetic used. Initial induction was achieved using 4% halothane. Anaesthesia was maintained using 0.8% to 2.5% halothane delivered via a face mask and later through a tracheal catheter. Once the animal was anaesthetized a subcutaneous injection of atropine sulfate (.2cc; 0.6mg/cc) was given . During the surgery on the dissection table the animal's body temperature was maintained at 37 ^{o}C using a heating pad.

The right common carotid artery was cannulated using silastic tubing filled with a lactated Ringer's and heparin solution (4:1). The catheter was attached to a pressure transducer to permit the continuous monitoring of the blood pressure with a polygraph. The left common carotid artery was dissected free from the vagus nerve and surrounding connective tissue and a loose tie placed around the artery. By gently pulling the ends of the tie the carotid could be temporarily occluded if necessary during later surgical procedures such as the craniotomy and decerebration.

The right external jugular vein was cannulated using polyethylene tubing filled with either lactated Ringer's or a saline solution (0.9% sodium chloride). This intravenous

line was used for the i.v. administration of a variety of fluids (saline, lactated Ringer's, dextran) or drugs when required throughout the experiment. Once the cannula was in place, 1-2 ccs of dexamethasone (4mg/ml) was immediately administered as a prophylactic measure against excessive swelling in the brainstem and spinal cord area during the experimental manipulations. In some cats the cephalic vein of the right forelimb was also cannulated. It served as a pathway for a constant 5% glucose sodium bicarbonate infusion (5 ml per hour) which was used to replace fluid loss during the experiment as well as a means with which to maintain a normal pH balance in the animal.

II. Peripheral Nerve Dissection

A variety of hindlimb peripheral nerves were dissected and used for either recording and/or stimulating procedures. The nerve branches to the left vastus lateralis, intermedius and medialis (together referred to as quadriceps, abbreviated Q) were cut, tied together and placed into a plexiglass cuff for stimulation. The rectus femoris branch of the quadriceps nerve was not included since it does not function as a primary knee extensor as do the other three branches (Eccles et al., 1957). In addition, the nerves to the gracilis (Grac) and sartorius (Sart) muscles of the left hindlimb were cut and placed in similar cuffs. The cuff apparatus was made of a piece of plexiglass in which 1 or 2 tunnels had been drilled and through which the nerve could be threaded. Two 25 gauge

silver wires attached at 90 degree angles to the direction of the tunnel shaft were placed in each tunnel. These wires were attached to insulated flexible wires which were led out of the animal and to the appropriate recording or stimulating apparatus.

The left sciatic nerve and many of its branches were also dissected, isolated from the surrounding tissue and muscles, and cut. The nerves routinely used in the experiments included posterior biceps (PB) and semitendinosus (St), semimembranosus and anterior biceps (SMAB), lateral gastrocnemius-soleus (LG-S), medial gastrocnemius (MG), plantaris (Plant), tibialis anterior (TA), flexor digitorum longus (FLD), and both medial and lateral branches of the sural nerve. The remaining branches of the common peroneal and posterior tibial nerves were cut and the entire nerve network was isolated from the surrounding tissue. Later when the cat was placed in a Transvertex frame (Transvertex, Stockholm,Sweden) the nerves were lifted from their position in the hindlimb and placed into a specially designed plexiglass container behind the animal. This left the rest of the hindlimb free to hang downward in its normal position while the nerves were isolated in the mineral oil filled bath. The procedure was identical for the right hindlimb except for the isolation of fewer nerves (PBSt, LG-S, MG, and TA). The nerves were placed on bipolar hook electrodes in the mineral oil bath and were attached to either a preamplifier for recording procedures or to the stimulator. The nerve pools were

heated with an infrared lamp suspended over the animal. Stimulation intensity to the peripheral nerves was expressed in multiples of threshold to electrical stimulation (0.1 or 0.2 ms duration square wave, frequency of stimulation 5-30Hz). Threshold (abbreviated T) was defined as the stimulus intensity required to activate the lowest threshold fibers in the nerve. The threshold levels were determined during the experiment by examining the cord dorsum records. Since the condition of the nerves could change during the course of the experiment, the nerve thresholds were checked routinely and adjusted when necessary.

III. Laminectomy and Mechanical Support

A laminectomy extending from the junction of the first sacral and seventh lumbar vertebrae to the fourth lumbar vertebra was performed. The opening in the spinal canal was widened sufficiently to provide good access and to permit visualization of the left side of the spinal cord and the exiting spinal roots. Cautization of surrounding muscles was employed to stop any bleeding into the area of the spinal cord. The exposed spinal cord, with dura intact, was covered with a thin layer of gauze soaked in warm saline so as to maintain a moist environment until such time as a heated mineral oil pool around the spinal cord could be arranged.

A vertebral clamp was placed around the spinal canal at the L4 or L3 cord level and served to help suspend the animal from the Transvertex frame later in the experiment.

A second clamp was placed at the mid-thoracic level holding the spinous process tightly and functioned to support the upper region of the animal's body once placed in the support frame.

The animal was moved to the Transvertex frame and the head was placed securely into a stereotaxic headholder. The hindlimb region of the animal was secured with metal pins placed tightly against the iliac crests. When in place, the pins secured the lower section of the spinal column yet left the hindlimbs free to move. The region of the lumbar enlargement in the spinal cord was further secured by positioning two L-shaped metal clamps, one on either side of the column. The skin of the back was tied in such a manner as to create a mineral oil pool over the exposed spinal cord. A thermistor placed in the pool was used to provide feedback to a heater which controlled an infrared lamp suspended over the spinal cord which maintained the temperature of the mineral oil pool at 38 degrees Celsius.

IV. Craniotomy and Decerebration

The animal's head was secured in the stereotaxic headholder, a scalp incision made, the muscle overlying the skull reflected and the bone covering the parietal and temporal cortex removed. A blunt spatula was used to remove the two cerebral hemispheres and to transect the brainstem. The brainstem transection extended from the rostral edge of the superior colliculi on the dorsal surface to the caudal edge of the mammillary bodies ventrally, producing a

precollicular, postmammillary mesencephalic preparation. All brain tissue rostral to the transection was removed and the floor of the cranium packed with absorbent hemostat. Once the transection was completed the anaesthetic was terminated and a slow i.v. infusion of saline and dextran was administered until such time as recovery of the blood pressure had occurred. The exposed brainstem was covered with a 4% Agar in saline solution to prevent drying and deterioration of the remaining brain tissue during the recovery period that followed the decerebration.

V. Spinal Cord Dissection

The dura was cut open along the extent of the exposed spinal cord and gently retracted, revealing the L5 to S1 spinal dorsal roots. Each root was carefully separated and the accompanying ventral root identified. A ventral root filament from the L7 segment was dissected free from the remainder of the the ventral root, cut distally to the cord and mounted on a bipolar hook recording electrode. This filament served as one of the monitors of rhythmic locomotor activity in the cat during the experiment. For some experiments the left ventral roots (VRs) of S1, L7, and L6 were cut and placed on stimulating bipolar hook electrodes.

Cord dorsum recordings were obtained using a silver ball electrode placed on the spinal cord at the segment in which the intracellular recordings were to be obtained. A small hole was made using fine forceps in the arachnoid and

pia mater, for the recording microelectrode to enter the spinal cord.

VI. Fictive Locomotion Preparation

The cats were paralyzed using a 1-2 cc intravenous injection of gallamine triethiodide (8 mg/ml), and the animal was artificially respired. The expired CO_2 was monitored and maintained at 4-6%. In some cats a bilateral pneumothorax was done to decrease respiration artifacts detected during intracellular recording procedures.

The presence of rhythmic activity in the ventral root filament and/or the LG and TA electroneurograms (ENGs) from either or both sides of the animal was used as the monitor of fictive locomotion. Fictive locomotion was induced using electrical stimulation of the mesencephalic locomotor region (MLR) (Shik et al., 1966) (See Figure 1) using an insulated monopolar stimulating electrode (exposed tip 0.25 mm, diameter 0.1 mm). The MLR stimulating electrode was positioned approximately 4 mm lateral to the midline, and 1 mm caudal to the junction of the superior and inferior colliculi. The depth of the stimulating electrode ranged from about 4 to 6 mm from the surface of the inferior colliculi. Square wave pulses (0.5 ms duration) were delivered to the MLR at 10 to 50 Hz with the stimulation strength ranging from 50 to 200 μA.

VII. Intracellular Recording Procedures

Intracellular recordings from alpha motoneurons were obtained using 2 M potassium citrate filled glass

microelectrodes. The electrode tip size was under 2 microns and the microelectrode resistance was less than 10 megohms when measured in the spinal cord.

The alpha motoneurons were usually identified using antidromic activation from identified peripheral nerves. If antidromic activation was not possible, the pattern of monosynaptic synaptic inputs from identified hindlimb nerves was used to characterize the motoneuron (Eccles et al, 1957). In addition, the motoneuron's activity pattern during fictive locomotion was correlated with the activity pattern of the ENGs, thereby identifying it as being active during either the extension or flexion phase of the step cycle. Injections of short depolarizing pulses (60 nA, 200 μs) to produce spikes were used to obtain the afterhyperpolarization of the motoneuron and a 20 to 25 ms, 2 nA hyperpolarizing pulse was often used to obtain the "resting" motoneuron membrane resistance. This same pulse was used to check the bridge balance periodically during the experimental trials in order to rebalance the bridge when necessary. In some experiments, continuous depolarizing or hyperpolarizing current was injected and used to evaluate the effects of membrane potential changes on various aspects of the motoneuron activity during the fictive step cycle.

VIII. Experimental Paradigm and Data Collection

The intracellular records from the motoneuron (both a high gain and low gain record), the cord dorsum recording, the ventral root filament and ENG activity were all

recorded on magnetic tape for later analysis (tape recording bandwidth DC-2.5KHz).

1). Stimulation in and around the MLR:

In several experiments an effort was made to examine areas within the MLR and surrounding brainstem areas to look for the coexistence of stimulation sites that could produce both locomotor activity and discrete postsynaptic potentials (PSPs) in hindlimb motoneurons during locomotion. Since a number of brainstem areas can produce PSPs in alpha motoneurons when stimulated (Hongo et al.,1965; Jankowska et al, 1974; Peterson et al., 1979), this approach was used as an attempt to link the evoked PSPs specifically with descending MLR locomotor pathways.

In order to determine the optimal site for the production of fictive locomotion, sites within the region classically defined as the MLR (L4, P1, H4-6) were systematically investigated. The stimulating electrode was placed at the L4, P1 coordinates and lowered in 0.5 mm increments from the surface of the inferior colliculi in an effort to find the optimum depth for the production of locomotion. If the lateral and posterior coordinates required changes, again a systematic search was done using 0.5 mm increments of change. The site which had the lowest threshold for evoking fictive locomotion was localized and the presence of rhythmic activity in the monitors for locomotion as well as the occurence of stimulus evoked PSPs in the hindlimb alpha motoneurons documented. When the

optimal site the production of fictive locomotion was found, PSPs in motoneurons which were evoked by brainstem stimulation were examined for latency, sign and changes in the PSPs amplitude or sign during the various parts of the fictive step cycle. Stimulation of areas surrounding the MLR was done using the stimulus parameters set at the optimal MLR site, and the presence of evoked PSPs in motoneurons were documented. At the termination of these experiments the brainstem was removed, fixed, sectioned on a cryostat and stained using hematoxylin eosin. The stimulus sites were identified in cross sections of the brainstem and localized using the nearby anatomical landmarks.

2) Facilitation of MLR-evoked PSPs in motoneurons by segmental high threshold cutaneous and muscle afferent polysynaptic pathways to motoneurons:

There have been a number of examples in the literature where the effects of both supraspinal descending and segmental reflex pathways on alpha motoneurons have been evaluated and the presence of facilitation or occlusion of one pathway by the other documented (Hongo et al., 1972; Hultborn et al., 1976). The possibility of two systems influencing the neuron, not simply at the level of the neuron membrane but also by sharing common interneurons projecting to the neuron, can be evaluated using the technique of spatial facilitation (Baldissera et al., 1981). The purpose of some of these experiments was to investigate the possibility of convergence of the descending MLR pathway

and high threshold muscle and cutaneous afferents onto common interneurons in the lumbar spinal cord. MLR stimuli which were sufficient to evoke fictive locomotion and observable PSPs in the motoneuron were used. The high threshold muscle and cutaneous afferent stimulation was at a minimum strength of 5 times threshold , a stimulus strength which included afferents characterized as flexor reflex afferents (FRA) (Baldissera et al., 1981) and was sufficient to produce an observable PSP in the motoneuron. In those experimental trials in which both the MLR stimuli and peripheral nerve stimuli were delivered together, the interval between the two stimuli was adjusted so that the evoked responses in the motoneuron overlapped. In many cases the interval was varied and the effects of the combined stimuli at the various intervals compared to one another. In a second arrangement, the peripheral nerve stimuli were delivered randomly in relation to the MLR stimuli. The reverse paradigm was also used, with the MLR stimuli delivered randomly to the peripheral nerve stimuli. In addition, data was obtained in which the effects of the peripheral nerve stimulation alone were examined in the absnce of fictive locomotion.

IX. Data Analysis

During the experiments filmed records of PSPs evoked in motoneurons by peripheral nerve stimulation, action potentials (orthodromic, antidromic, and current evoked), motoneuron afterhyperpolarizations, and hyperpolarizing long

pulse injections to obtain motoneuron membrane resistance were routinely obtained. In addition, data was recorded on tape as previously mentioned.

The majority of data analysis in this project was accomplished offline using a computer. The high gain intracellular records were digitized at a rate of 7.5 to 15 KHz while the low gain intracellular record and the ventral root filament/ENG records were digitized at up to 3 KHz. In order to analyze PSPs occurring at various points within the fictive step cycle it was necessary to determine the "average" or "normalized" fictive step cycle for each episode of fictive locomotion (Figures 2 and 3) and to sort the PSPs prior to averaging into contiguous divisions based either on time or motoneuron membrane potential level during the fictive step cycle (Figure 3). The "averaged" fictive step cycle for a period of fictive locomotion was determined by dividing each fictive step cycle into as many as ten time segments based upon activity in the ventral root filament/ENG or the membrane potential of the motoneuron. Frames (or sweeps) of the AC coupled intracellular record of 25 to 40 ms duration and time-locked to the MLR and/or peripheral nerve stimulus from many fictive step cycles were then sorted and placed into between four and ten bins corresponding to contiguous segments of time in the cycle. Alternatively, the fluctuations in the motoneuron's membrane potential (locomotor drive potentials, abbreviated LDPs) during the fictive step cycle were divided into four to ten membrane potential (Em) voltage ranges or bins, and frames

of the intracellular record occurring over several LDPs were sorted into the appropriate bins. The intracellular records falling into each bin were then averaged and the resultant PSPs were displayed (Figure 3).

Once the averaged records were obtained a number of manipulations could be executed. Included in these was the weighted sums of waveforms. This ability allowed for the evaluation and quantification of the changes in evoked PSPs during the various phases of the step cycle and under various conditions (i.e. continuous depolarizing or hyperpolarizing currents). This utility was used extensively in the experiments looking for convergence onto common interneurons by the MLR and peripheral afferent pathways. By summing the averaged waveform from the MLR stimulation alone with the averaged waveform from the peripheral reflex pathway alone one obtains a representation of the resulting PSPs recorded in the motoneuron if the two pathways converged independently onto the motoneuron. If one then takes this sum and subtracts it from the waveform obtained when both pathways were stimulated in a condition-test paradigm, the presence or absence of spatial facilitation can be evaluated. A simple arithmetic summation would be expected if the descending and segmental inputs simply converged at the motoneuron membrane, while convergence of the two inputs onto shared interneurons which project to the motoneuron would result in an effect which was larger than the arithmetic sum of the two stimuli

delivered separately (Baldissera et al., 1981).

In addition, the LDPs of the motoneuron during fictive locomotion could be normalized with respect to time in the step cycle, providing a quantification of the LDPs during a specific period of fictive locomotion. This allowed for a quantified comparison of the LDPs between different locomotor trials in which various manipulations had been done (i.e constant injection of depolarizing or hyperpolarizing currents into the motoneuron).

It has long been assumed that the primary determinant of motoneuron firing patterns was the afterhyperpolarization and time course of its underlying potassium conductance (Gustaffson, 1974; Schwindt, 1972; Schwindt and Calvin, 1973). Since excitation of motoneurons was observed during the fictive step cycle, the role of the afterhyperpolarization (AHP) as well as that of discrete excitatory PSPs during the depolarized phase of the step cycle in influencing motoneuron firing patterns during fictive locomotion was further investigated. A spike-triggered averaging technique was used to compare the events before and after motoneuron action potentials. In some cases the action potentials were produced by short pulse current injections during non-locomoting control trials and during locomotor trials while in other cases the action potentials were generated by the depolarization of the motoneuron during the step cycle. This was achieved by using the rising phase of an action potential to trigger a computer averager (spike triggered averaging). The data was

continuously digitized at either 10KHz or 20 KHz and placed in a circular buffer memory. Averages were triggered by the action potentials and consisted of data obtained before and after the trigger signal (pretriggered averaging). Under these circumstance the data selection during the fictive step cycle was limited to periods of motoneuron firing, usually only during the depolarized phase of the step cycle. The averaged records were then either plotted out or displayed on the oscilloscope screen and photographed.

Abbreviations

MLR	mesencephalic locomotor region
LDP	locomotor drive potential
AHP	afterhyperpolarization
IaIN	Ia inhibitory interneuron
CPG	central pattern generator
ENG	electroneurogram
PSP	postsynaptic potential
EPSP	excitatory postsynaptic potential
IPSP	inhibitory postsynaptic potential
FRA	flexion reflex afferent
LG	lateral gastrocnemius
MG	medial gastrocnemius
TA	tibialis anterior
PBSt	posterior biceps- semitendinosus
FDL	flexor digitorum longus
Sart	sartorius
Q	quadriceps
Grac	gracilis
VR	ventral root
L7	seventh lumbar
ms	millisecond(s)
s	second(s)
mV	millivolt(s)
T	threshold
mΩ	megohms
mA	milliamperes
nA	nanoamperes

Results

Intracellular recordings were obtained from 42 lumbar motoneurons. The alpha motoneurons groups sampled included tibialis anterior (n=6), lateral gastrocnemius (n=6), medial gastrocnemius (n=16), semitendinosus (n=2), and posterior biceps-semitendinosus combined (n=8). Three motoneurons which received monosynaptic excitation from only flexor digitorum longus (FDL) but where antidromic activation was not obtained were also included. The resting membrane input resistance for the motoneurons ranged from 0.8 to 3.0 megohms while the resting membrane potential varied from approximately -40 to -80 mV with the mean of -58 mV. Intracellular current-evoked action potentials or the orthodromic/antidromic action potentials were measured and the motoneuron action potential amplitudes ranged from about 40 mV to 80 mV with a mean of 55 mV.

The motoneurons used in this analysis displayed rhythmic depolarization and hyperpolarization of the membrane potential during the fictive step cycle. The magnitude of these oscillations, or locomotor drive potentials (LDPs), observed during this series of experiments ranged from about 2 to 20 mV. The membrane potential oscillations were standardized as illustrated in Figures 2 and 3. This procedure involved the selection of a period of locomotor activity with a fairly constant rate of stepping during which time the LDPs could be examined in respect to time in the step cycle. A specific membrane

potential level was selected to serve as the membrane threshold level at which the start of the step cycle would be denoted (Figure 2A). A time delay after each threshold crossing was used to prevent premature detection of the start of the following cycle threshold crossing. A plot of the membrane potential values during the LDPs for a segment of 15 steps is illustrated in Figure 2B. During each of the plotted step cycles, the 45000 data points representing the membrane potential level during those steps are plotted. This is representative of typical data, with little variability in the duration of the step cycle being observed. The data values occurring in each designated fraction of the step cycle are averaged together, providing a value representing the mean membrane potential level during that fraction of the step cycle. In the example used in Figure 2B, the step cycle was divided into 10 consecutive time segments, hence, 10 averages are shown superimposed on the plot of the raw data.

I.MLR Production of Fictive Locomotion and Postsynaptic Potentials in Alpha Motoneurons

Since the work done by the Russians in the sixties (Shik, Severin and Orlovsky, 1967) electrical stimulation of the mesencephalic locomotor region of the brainstem has been used to produce treadmill or fictive locomotion in decerebrate cats. In the present series of experiments it was observed that the brainstem MLR site which was the most effective for the initiation of fictive locomotion could also produce postsynaptic potentials (PSPs) which were

associated with each of the stimuli to the MLR. Both excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs) could be evoked in both flexor and extensor motoneurons with electrical stimulation of the MLR. The computer analysis revealed that the PSPs were strongly modulated in peak amplitude and sign during the fictive step cycle, with EPSPs predominating during the depolarized phase and IPSPs observed during the hyperpolarized phase. In Figure 4 the pattern of MLR evoked EPSPs and IPSPs in a PBSt motoneuron can be observed. There is a graded transition from the EPSP to IPSP during the course of the step cycle. The latency of the MLR evoked EPSPs is somewhat shorter than that of the IPSP averaging about 5.1 ms with a range of 3.0 to 7.0 ms. In the example illustrated in Figure 3 the latency for the EPSP was 4.7 ms while that of the IPSP was 6.0 ms. The difference in latency between the EPSP and IPSP can also be seen for the LG motoneuron in Figure 5. In this case the delay from the onset of the EPSP to the earliest detectable deflection of the IPSP was 1.2 ms. The onset of the IPSP is more difficult to detect and often it is not possible to do so because of the presence of a covert EPSP immediately prior to and masking the actual onset of the IPSP. Where the onset of the IPSP could be detected with some degree of confidence, the mean latency was 6.5 ms (n=20, range 5.0 to 8.5 ms). The delays from the arrival of the descending volley from the MLR to the PSPs observed in the motoneurons ranged from

about 2.0 to 3.0 ms indicating a segmental delay consistent with the presence of interneurons at the level of the lumbar spinal cord. The examples illustrated in Figures 4 and 5 are representative of the pattern of alternating MLR evoked EPSPs and IPSPs observed in 35 of the 41 motoneurons examined. There was no evidence that the pattern of alternating MLR evoked PSPs was unique to a particular group of motoneurons since the motoneurons sampled came from a variety of hindlimb muscles. In five motoneurons (2 TA, 1 ST, 1 PBST, and 1 MG) the MLR stimulation produced only an EPSP during the step cycle. The evoked EPSP was modulated in peak amplitude during the step cycle with the maximum peak amplitude occurring during the depolarized phase of the step cycle. In one PB motoneuron the evoked PSPs from the MLR included a very small EPSP observed during the depolarized phase and an IPSP during the entire step cycle. Since the primary aim of the experimental trials was to maintain good episodes of fictive locomotion there was no systematic variation in the MLR stimulus strength in an effort to examine the subsequent amplitude changes in the evoked PSPs. With stimuli parameters optimal for producing fictive locomotion, (strength 50 to 170 μA) the peak amplitude for both the evoked EPSPs and IPSPs was approximately 3.0 mV.

Since electrical stimulation of many sites in the brainstem can produce PSPs in alpha motoneurons, the relation between the area of the brainstem stimulated to produce locomotion and the PSPs was examined. By

systematically stimulating brainstem sites in proximity to the MLR, the ability of these brainstem areas to produce PSPs in motoneurons was assessed and the evoked PSPs from these sites were compared to the MLR-evoked PSPs during fictive locomotion. This was done in six cats, and the results from the one animal shown in Figure 4 exemplify the results of the brainstem mapping procedure done at various depths at the anterior-posterior and lateral coordinates which were optimum for the production of fictive locomotion. The site at the depth of 5.0 mm below the surface of the inferior colliculus, confirmed histologically to correspond to the cuniform nucleus, was the most effective for inducing fictive locomotion. At this depth, the pattern of alternating MLR-evoked EPSPs and IPSPs could be observed during the fictive step cycle. Although PSPs could be evoked from sites outside the optimum one for the initiation of locomotion, these PSPs were not observed to be modulated in amplitude or sign during stimulation. In such cases, the evoked locomotion required stimulus strengths at least double that required at the optimal sites if locomotion could be produced at all.

From the start of delivery of the the MLR stimulation there was often a delay, ranging from 1 to 10 seconds, until the onset of fictive locomotion. It was also observed that the MLR stimulation during the time prior to the actual production of fictive locomotion could produce small PSPs in the motoneuron and that these PSPs grew in size and

displayed amplitude and sign changes during the step cycle once fictive locomotion and the motoneuron LDPs had been established.

In order to ensure that the changes in the MLR evoked PSPs were not simply a function of the changes in the membrane potential level of the motoneuron during the LDP, the PSPs were sorted according to time in the step cycle as well as membrane potential level. Figure 5 illustrates the results of this procedure. Although bins 1 and 5 occur at comparable membrane potential levels, the MLR evoked EPSP is maximal during bin 5 which corresponded to the end of the depolarized phase of the standardized LDP. Similarly, the MLR evoked IPSPs occurring during the relatively hyperpolarized sections of the standardized LDP differed from each other depending upon the time in the step cycle in which they occurred. In Figure 5 a small EPSP can be seen at the beginning of the PSP in bin #8 which occurs on the rising edge of the depolarized phase of the LDP. In bin 6, which is taken from the falling edge of the depolarized phase of the LDP when the hyperpolarized phase begins, the EPSP can not be seen.

The effects of constant current injection into the motoneuron through the recording microelectrode as illustrated in Figure 6 were used to evaluate the effects of such current injection and the subsequent membrane potential changes on the MLR evoked PSPs. The injection of hyperpolarizing current (Figure 6B) reduced the peak amplitude of the MLR evoked IPSP during the phase of the

cycle in which the IPSP was maximal, while the EPSP was increased in amplitude during some parts of the step cycle. This latter observation, paired with the fact that the EPSP occurred prior to the onset of the IPSP, is consistent with the proposal that the EPSP is due to excitatory synaptic activity and not simply a reversed IPSP. During constant depolarizing current injection (Figure 6C), the IPSP was predominant during more of the fictive step cycle than was observed during control trials of locomotion prior to current injection. The EPSP was decreased in peak amplitude by constant depolarizing current injections, but the interpretation of this effect is more difficult since it could be due to either a direct effect of the current on the EPSP or due to summation of the EPSP with an accentuated IPSP. Similar findings were obtained in 2 other MG motoneurons subjected to this type of analysis.

II. Interaction between MLR Evoked PSPs and High Threshold Afferent Responses in Motoneurons

As described in the previous section, electrical stimulation of the MLR can produce PSPs in alpha motoneurons. Experiments were done to examine the possibility that this descending locomotor pathway and high threshold segmental reflex pathways share interneurons in the lumbar spinal cord.

In three motoneurons (1 MG, 1PBSt, and 1 LG) the PSPs evoked by electrical stimulation of high threshold reflex pathways were evaluated during non-locomoting control and

fictive locomotor trials. Care was taken to include only trials in which the membrane potential of the motoneuron during the control and locomotor trials was similar so that changes in the evoked PSPs could not be attributed to changes in the motoneuron membrane potential level. In the MG and PBSt motoneurons modulation of the PSPs evoked by ipsilateral high threshold afferent stimulation was observed. Figure 7 illustrates the averaged responses in the PBSt motoneuron to electrical stimulation of the ipsilateral sural afferents (5xT) during a prelocomotor period (Figure 7A) and at various times throughout the fictive step cycle (Figure 7B). The normalized motoneuron LDP during the fictive step cycle is shown in Figure 7C. Not only is the response to sural stimulation decreased during episodes of fictive locomotion, but the evoked sural effect is modulated in amplitude within the step cycle. In the MG motoneuron, the PSPs evoked by either high threshold cutaneous or high threshold muscle afferent stimulation displayed this same modulation during the fictive step cycle. No modulation of either the high threshold cutaneous or muscle afferent response was observed in one LG motoneuron. The results are suggestive of changes in the pathway to the motoneurons from the high threshold afferents during MLR evoked locomotion.

Not only were changes in the responses evoked by the peripheral reflex pathways observed during locomotion, but changes in the descending MLR pathway to motoneurons were also observed when high threshold afferents were stimulated randomly during fictive locomotion. In one LG motoneuron

stimulation of the ipsilateral cutaneous sural nerve (5xT,10 Hz) randomly in relation to the MLR stimuli produced a deviation in the pattern of MLR-evoked PSPs during the both the depolarized and hyperpolarized phases of the LDP. Figure 9 illustrates the MLR evoked response when the ipsilateral sural stimuli were being delivered during the depolarized phase of the LDP (left panel, 3nd trace). The usually observed EPSP has been replaced by an IPSP of similar latency. Unfortunately the exact onset of the IPSP is difficult to determine so no exact comparison can be made between its latency and the latency of the MLR-evoked EPSP during control trials. During the hyperpolarized phase (right panel, 3nd trace) a small EPSP can be seen immediately prior to a small IPSP.

In 5 motoneurons the condition-test paradigm described in the Methods section, was used to obtained evidence for convergence of the high threshold cutaneous (sural nerve) or muscle afferents (TA,LG, or MG) onto lumbar interneurons shared with the descending MLR pathway. Pronounced facilitation of the responses from high threshold afferents was observed when the MLR and high threshold afferent stimuli were applied at intervals of between 2 and 3 ms. Four of the motoneurons examined (2 MG, 2 PBSt) displayed facilitation of the evoked EPSPs during combined MLR and high threshold afferent stimulation. In one MG motoneuron the IPSP was also facilitated during the hyperpolarized phase while no change in the evoked IPSPs was observed in a

second MG and 2 PBSt motoneurons during the condition-test trials. The facilitation of the evoked EPSPs is illustrated for high threshold muscle reflex pathways using MG nerve stimulation in Figure 8. When stimulation of the ipsilateral MG nerve at a strength (20xT) sufficient to activate flexion reflex afferents was combined with the MLR stimulus, there was some enhancement of the MLR evoked EPSP during the depolarized phase of the LDP. During the hyperpolarized phase of the LDP, the MG stimulation produced a large EPSP with a latency and time course similar to the EPSP evoked by MLR stimulation during the depolarized phase of the LDP. In this case, high threshold muscle stimulation did not produce an IPSP. Similar results were observed in a second PBSt motoneuron and two MG motoneurons using either high threshold cutaneous or muscle afferent stimulation paired with stimulation of the ipsilateral MLR.

It was also observed that in addition to the facilitation of the short latency MLR-evoked EPSPs by high threshold muscle afferents illustrated in Figure 8 , longer latency effects become prominent during both the depolarized and hyperpolarized phases of the LDP when the MLR and peripheral afferent stimuli are delivered together. In the example shown in Figure 8, a late peak occurring approximately 42 ms after the onset of the early EPSP during the depolarized phase became larger when ipsilateral high threshold muscle afferents were stimulated. During the hyperpolarized phase of the LDP, a large late PSP is present, but appears much earlier at about 17 ms after the

beginning of the early EPSP. During the depolarized phase of the cycle there is a PSP at approximately 17 ms but of much smaller amplitude. The relative contributions of the inhibitory and excitatory events to the shapes of the late PSPs cannot be determined from these records since it is not possible to identify the start or time course of either the EPSPs or IPSPs separately, thereby removing the possibility of sorting out the PSPs producing the observed membrane potential pattern.

In Figure 9, facilitation of the MLR evoked IPSP by stimulation of the ipsilateral sural nerve at 5 times threshold can be seen during the hyperpolarized phase of the fictive step cycle. As described earlier in the Results, the observed EPSP produced in this LG motoneuron by the MLR stimuli during the depolarized phase of the step cycle was profoundly altered when the ipsilateral sural nerve was stimulated during locomotion (Figure 9 left panel third trace). When the MLR and sural stimuli were delivered at an interval of about 2.5 ms, their individual effects occurred at the same time in the motoneuron. The MLR IPSP occurring during the depolarized phase of the step cycle became smaller (Figure 9 left panel), while an increase in the IPSP amplitude was observed during the hyperpolarized phase of the cycle (Figure 9 right panel). This facilitation of the IPSP during the hyperpolarized phase of the LDP with combined MLR stimulation can be seen clearly in the trace labelled DIFFERENCE in Figure 9. The trials were taken at

membrane potentials comparable to one another and did not reflect deterioration of the recording situation. The results from these experiments would indicate that the descending MLR pathway and the high threshold afferent reflex pathways do interact at the level of the lumbar spinal cord and that sharing of both excitatory and inhibitory interneurons by these pathways during fictive locomotion appears likely.

III.Motoneuron firing during locomotion and peri-spike events

The factors which determine the firing properties of motoneuron have been subject to extensive investigation, with the role of intrinsic motoneuron membrane properties being a focal point in these investigations (Gustaffson, 1974; Schwindt, 1973; Schwindt and Calvin, 1974). The presence of excitatory inputs to motoneurons from the CPG during fictive locomotion (Jordan, 1983) and discrete PSPs produced by MLR stimulation suggest that perhaps these excitatory inputs to motoneurons also play a role in determining the firing pattern of the hindlimb alpha motoneurons during the step cycle. During the fictive step cycle a variety of motoneurons display a rhythmic depolarization and hyperpolarization of the membrane potential and often the motoneuron will produce action potentials during the depolarized phase. These action potentials do not appear to have associated with them the large afterhyperpolarizations (AHPs) that are normally observed during spike trains produced with intracellular

current injection (Figures 10 and 11). Figure 10A illustrates action potentials and AHPs in a PBST motoneuron produced with constant depolarizing current injection. The AHPs associated with action potentials produced by short depolarizing pulse injections delivered at a constant rate over several step cycles are shown. In Figure 10C averaged records of the current evoked action potentials during a non-locomoting control period can be compared to those obtained during fictive locomotion. Note that the AHP is smaller during the locomotor cycle than during the non-locomoting control. Since the membrane potential during fictive locomotion was only about 2 mV more depolarized than the control period, it is unlikely that the changes observed in the AHP are due simply to changes in the membrane potential. AHPs decreased during fictive locomotion in 4 of the 8 motoneurons examined.

A decrease in the AHP during fictive locomotion might be indicative of the need for alternate methods with which to determine motoneuron firing patterns during the fictive step cycle. One possible means by which the firing pattern of the motoneuron could be produced is with appropriately timed EPSPs. The presence of such excitatory potentials prior to action potentials was examined using spike triggered averaging as described in the Methods section. Figure 11A illustrates the response of a PBSt motoneuron to depolarizing current injection while panel B illustrates the firing pattern of the same motoneuron during a period of

fictive locomotion. Figure 11C shows the spike triggered average during an period of spiking produced by intracellular current injection. The rising potential preceding the spike used to trigger the average is the AHP of the previous spike. Panel D shows the spike triggered average of the membrane potential during a period of locomotion. Note the small EPSP immediately prior to the action potential. Examination of the raw data shows the individual action potentials produced on EPSPs during the depolarized phase of the LDP. The presence of EPSPs preceding the action potentials were observed in 4 of the 6 motoneurons analyzed with spike triggered averaging. The decrease in the AHP during fictive locomotion, along with the presence of EPSPs preceding action potentials during the depolarized phase of the LDP, suggest that the control of motoneuron firing during fictive locomotion may not be limited to intrinsic motoneuron properties alone.

Discussion

In recent years, the synaptic inputs to motoneurons during the fictive step cycle have been investigated in an effort to determine the types of inputs required to produce motoneuron activity during locomotion. Shefchyk and Jordan (1985) demonstrated that the membrane input resistance of flexor and extensor motoneurons does not change significantly during the depolarized and hyperpolarized phases of the step cycle, a finding which suggests the presence of synaptic input to the motoneuron during both phases of the cycle. In addition, it has been demonstrated that strychnine, a glycine antagonist which blocks the inhibitory action of IaINs, can effectively remove the interburst hyperpolarization observed in the motoneuron during the step cycle without affecting the rhythmic excitation of the motoneuron during the cycle (Jordan, 1981,1983). Taken together, the data indirectly supports the presence of alternating excitatory and inhibitory synaptic inputs to the motoneuron during the fictive step cycle which produces the pattern of membrane potential changes observed during the LDP. The work presented in this study provides direct evidence that an excitatory pathway from the midbrain locomotor region projects to lumbar motoneurons in mammals. It has also been demonstrated that a longer latency inhibitory pathway to motoneurons is activated by the MLR stimulus, and that both types of MLR evoked PSPs are modulated in amplitude and sign during the fictive step cycle. The segmental delay at the

lumbar spinal cord (greater than 1.5 ms) suggests that the descending MLR information is mediated through interneurons in the spinal cord, perhaps components of the central pattern generator itself. The MLR evoked IPSP appears to be delayed by at least 1 synapse after the onset of the MLR evoked EPSP, and in some cases a longer delay appears likely. The effects of constant hyperpolarizing and depolarizing current injections into the motoneurons show that neither the EPSP nor the IPSP is actually a reversed PSP of the opposite sign. The PSPs are evoked by stimulation of brainstem areas which also produce fictive locomotion; stimulation of areas outside the site optimal for the production of locomotion only produce PSPs that do not change in amplitude and sign during the fictive step cycle. The MLR-evoked PSPs are further linked to the initiation of locomotion by virtue of the fact that the PSPs often appear to increase in magnitude and display changes in amplitude and sign at the same time as the commencement of LDPs and fictive locomotion produced by the brainstem stimulation. It is hypothesized that the neurons in these excitatory and inhibitory pathways to motoneurons from brainstem sites which can initiate locomotion also produce the rhythmic membrane potential oscillations, or LDPs, in motoneurons active during fictive locomotion.

In addition, the EPSPs from the MLR may have a role in determining the firing pattern of the motoneurons during the depolarized phase of the step cycle. Alpha motoneurons which

are active during locomotion display a large membrane depolarization and hyperpolarization, the LDP, during the step cycle. The motoneuron often produces the action potentials during the depolarized phase of the LDP and it has been assumed that the mechanism underlying this was the displacement of the motoneuron membrane potential across the membrane potential threshold level for the production of action potentials. Zajac and Young (1980) examined the motoneuron firing patterns in ventral root filaments during fictive locomotion and concluded that the firing pattern observed was consistent with the role of the afterhyperpolarization in determining the motoneuron activity. However, intracellular records from motoneurons during fictive locomotion are presented here that indicate a decrease in motoneuron AHPs during fictive stepping. Such a decrease in the AHP amplitude suggests that perhaps the conductances underlying the AHP are diminished during locomotion and that the intrinsic properties of the motoneuron do not function alone to determine the pattern of motoneuron firing during the step cycle. In several mammallian systems the presence of specific neurotransmitter substances can diminish the potassium conductance underlying the AHP and quite effectively remove the AHP during firing of the cells (Haas and Konnerth, 1983; Horn and McAfee, 1980). Such a mechanism may be active during locomotion. If the AHP is removed, or diminished in motoneurons during fictive locomotion, then an alternative mechanism must exist which determines the firing patterns of

the motoneurons during the step cycle. Excitatory input from the CPG could do this and results presented here indicate that EPSPs occur immediately preceding motoneuron action potentials during the depolarized phase of the LDP. These EPSPs could be producing the action potentials during the depolarized phase of the LDP. They could either be mediated by the same cells which produce the depolarizing phase of the LDP, or by a separate population of neurons such as has been suggested in the Xenopus embryo swimming preparation (Roberts, 1983). Another possibility is that these excitatory potentials do not function alone in producing the firing pattern of the motoneuron but rather, that they interact with the intrinsic mechanisms within the motoneuron which regulate motoneuron firing rates. It may be that the AHP conductances are present during locomotion, but interact with the excitatory synaptic conductances occurring during the depolarized phase of the LDP. The voltage deflection associated with the AHP conductance could have the excitatory potentials superimposed and thereby increase the probability of membrane potential reaching the threshold for firing before the completion of the decay of the AHP conductance, as has been described by Fetz and Gustaffson (1983) and Gustaffson and McCrea (1984).

PSPs evoked in lumbar motoneurons by stimulation in the midbrain have been described in the literature (for review see Baldissera et al., 1981), but such observations were not related to the locomoting animal. The possible relay

sites for the MLR descending pathway in the brainstem have been described (Steeves and Jordan, 1984; Garcia-Rill et al., 1983), with the most likely pathway being an excitatory pathway to midline reticulospinal cells in the pons and medulla. Cells from this region are thought to travel in the ipsilateral ventrolateral funiculus of the spinal cord down to the lumbar region (Steeves and Jordan, 1980) and the locomotor CPG. Stimulation of the ventrolateral funiculus (VLF) produces monosynaptic and disynaptic PSPs in alpha motoneurons (Willis et al., 1967). Orlovsky (1969) found that MLR stimulation produced neuronal signals primarily in the VLF, and he showed that midline reticulospinal cells receive monosynaptic activation from the MLR (Orlovsky, 1970). Orlovsky (1970) found that MLR stimulation produced a response in the VLF with a latency of 3.5 ms, while medial reticular formation stimulation produced a response in the same VLF site at a latency of 2.5 ms. Mori and coworkers (Mori et al.,1978) have demonstrated that reticular formation stimulation in this area can induce locomotion and facilitate MLR evoked walking. We have recently shown that cooling of the brainstem along the midline in the probable relay sites for the MLR projection can reversibly abolish MLR evoked locomotion (Shefchyk et al., 1984). This anatomical and electrophysiological evidence suggests the possibility that EPSPs associated with MLR stimulation reported here are produced by a pathway with a minimum of two synaptic delays, one in the medial reticular formation, and one at interneurons in the spinal cord. This latter

hypothesis is supported by the presence of short latency facilitation of the MLR pathway and the segmental high threshold reflex pathway reported here during locomotion.

During periods of fictive locomotion, the PSPs evoked in flexor and extensor motoneurons from electrical stimulation of ipsilateral high threshold afferents were modulated in amplitude and sign during the step cycle. These changes were observed in 3 of 4 motoneurons and are similar to the changes in cutaneous reflex responses observed in high spinal cats during locomotion induced by intravenous administration of L-DOPA (Schomberg,et al., 1981). Of equal importance was the observation in one motoneuron that the MLR-evoked PSPs could be changed from an EPSP to an IPSP during the depolarized phase of the LDP when the ipsilateral cutaneous nerve (5xT) was stimulated during fictive locomotion. The effects on the MLR pathway produced by electrical stimulation of high threshold afferent pathways in this motoneuron were profound and warrant further investigation since such changes indicate that the descending MLR pathway can be altered significantly by high threshold afferent stimulation. Such alterations in the synaptic input to the motoneurons during the step cycle from the descending locomotor pathway will influence the pattern of activity in the motoneuron and hence the final output of the system.

Evidence was obtained from five motoneurons that when delivery of the stimuli to the MLR and ipsilateral high

threshold cutaneous or muscle afferents were separated by an interval of 2-3 msec, a facilitation between the two pathways occurred. The combined stimulation repeatedly (4/5 motoneurons) produced facilitation of the MLR EPSPs while less frequent facilitation of the MLR IPSPs (2/4 motoneurons) was recorded. Facilitation of the PSPs by stimulation of the two pathways does not simply reflect convergence of the two synaptic inputs onto the motoneuron directly. Rather, the PSPS produced by combined stimulation were greter than the algebraic sum of the two synaptic inputs. This indicates tha convergence of the descending MLR pathway and the segmental high threshold reflex pathway onto common interneurons in the lumbar spinal cord which then project to alpha motoneurons. Since both EPSPs and IPSPs were facilitated by combined stimulation of the semgnetal and descending pathways both excitatory and inhibitory interneurons in the spinal cord are shared.

During the depolarized phase of the motoneuron LDP, combined stimulation of the MLR and high threshold afferents commonly produced facilitation of MLR evoked EPSPs. As illustrated in Figure 7, both stimuli give rise first to a short latency EPSP followed by a longer latency depolarization during the depolarized phase of the LDP. This effect might be considered the result of the stimuli activating a portion of the neuronal organization responsible for the locomotor rhythm, thus producing a rapid cycling of the components of the CPG in response to each stimuli. It should be noted that the late effect observed

during the depolarized phase of the LDP is preceded by a small EPSP with about the same latency (17 ms) as the late effect which becomes apparent during the hyperpolarized phase of the LDP (Figure 7 right panel). In this example, the excitatory pathway to the motoneuron normally observed during the depolarized phase of the LDP may be recruited during the hyperpolarized phase of the LDP.

As demonstrated in Figure 6, longer latency IPSPs evoked by MLR stimulation could be facilitated by conditioned stimulation of high threshold afferents. The greatest facilitation of the inhibitory potentials was during the hyperpolarized phase. Predominant IPSPs occurring during the hyperpolarized phase, were not observed during the depolarized phase of the fictive step cycle. This is in contrast to the MLR evoked EPSPs described in the preceding paragraph. It may be that the descending MLR pathway activates the excitatory interneurons in the lumbar cord directly and these in turn project to inhibitory interneurons. This would mean that the recruitment of the excitatory interneurons during the hyperpolarized phase of the LDP is more likely to occur than the recruitment of the inhibitory interneurons during the depolarized phase of the LDP, since excitation of the inhibitory interneurons from the MLR during locomotion requires that the excitatory interneurons be activated first. Since the pathway from the MLR to the lumbar cord initiates locomotion, its effects may be biased to the excitatory interneurons, because they would

facilitate the production of activity in other interneurons and motoneurons, an effect which would be consistent with the production of locomotor activity. On the other hand, activation of inhibitory interneurons would only interfere with the production of locomotion.

The present demonstration of excitatory and inhibitory pathways from the MLR to lumbar alpha motoneurons is interesting because excitatory and inhibitory pathways implicated in the the control of the locomotor rhythm in acute spinal animals treated with L-DOPA have been described from high threshold cutaneous and muscle afferents to motoneurons (Anden et al., 1966, Jankowska et al.,1967), and it is possible to speculate that the components of these same pathways may be involved in the relay of PSPs from the MLR to motoneurons during locomotion. The possibility that interneurons common to both pathways are activated in both preparations is supported by the facts that both the pathways described in the L-DOPA treated acute spinal cat and the mesencephalic cat with MLR stimulation are activated by stimuli which can initiate the locomotor rhythm (Grillner, 1973). The late, long lasting discharges in muscle nerves first described in the L-DOPA preparation (Anden et al., 1967) have been produced by stimulating cutaneous afferents during MLR stimulation (Grillner and Shik, 1973). Our further observation that combined stimulation of the MLR and high threshold afferents leads to spatial facilitation in the PSP pathway from the MLR strongly suggests that the descending MLR pathway and the

high threshold segmental pathways share interneurons in the lumbar spinal cord. The observations presented here indicate further characterization of the high threshold reflex interaction with the descending MLR pathway is warranted. Expansion of the condition-test paradigm to include a greater number of motoneurons, and a systematic examination of the segmental delays and intervals between stimuli which produce the most facilitation in these two pathways, would provide information as to the prevalence of the convergence in these pathways as well as an accurate estimate of the number of synaptic relays within the lumbar cord existing in these pathways to the motoneuron.

Possible candidates for the interneurons which may be the site(s) of convergence of these two systems include the IaINs and the interneurons described by Jankowska et al (1967) in the L-DOPA treated animals. It is known that after L-DOPA the IaINs mediate the late inhibitory effects produced by high threshold afferent stimulation (Fu et al., 1975), However, the IaINs do not appear to be vital for the production of the rhythmic excitation and activity of the motoneurons during locomotion (Jordan, 1980,1983) so, even if the two systems, that from the MLR and the other from segmental high threshold afferents, do share some IaINs, this shared component may not be part of the locomotor CPG itself. The interneurons described by Jankowska and coworkers (1967b) in the lateral region of lamina VII in the lumbar spinal cord are candidates for the excitatory

interneurons projecting to motoneurons. One group of these interneurons is excited by ipsilateral FRA stimulation and inhibited by contralateral FRA stimulation while the second group displayed the opposite pattern; they were excited by the contralateral FRA and inhibited by ipsilateral FRA stimulation. These two groups of interneurons were demonstrated to be reciprocally organized and the firing patterns found in these interneurons were compatible with their mediating the late, long duration FRA evoked discharge observed in motoneurons after L-DOPA. The interneurons described by Jankowska et al. (1967) have not been studied since the original report and may prove to be of renewed importance in light of the present results demonstrating spatial facilitation in the pathways from the MLR and high threshold segmental afferents to alpha motoneurons.

In conclusion, this work has provided the first evidence for a descending excitatory pathway from the MLR to alpha motoneurons during fictive locomotion. This excitatory pathway is hypothesized to produce the depolarized phase of the LDPs in motoneurons and may also participate in the determination of the pattern of action potentials produced in a motoneuron during a step cycle. In addition, an inhibitory pathway from the MLR to motoneurons has been described. Evidence was obtained for convergence of these descending MLR pathways and segmental high threshold reflex pathways onto interneurons in the lumbar spinal cord. The identification of the excitatory interneurons shared by these two pathways would be a significant advancement in the

understanding of the neuronal organization responsible for the production of locomotion in mammals. In order to localize such interneurons in the spinal cord, criteria are required which would aid in their characterization and identification. The patterns of activity in motoneurons produced by stimulation of the MLR and high threshold afferents reported in this study provide the first step in the development of the criteria necessary for the successful identification of these interneurons.

FIGURE 1: The experimental model. The brainstem is transected (dotted line) at the precollicular postmammillary level. The tip of the stimulating electrode is positioned in the MLR, and stimuli of 0.5 ms duration and approximately 50-200 μA in strength are applied at a rate of 10-50 Hz in order to produce fictive locomotion. Activity in a ventral root filament is recorded as a monitor of locomotion (bottom trace). Microelectrode recordings from motoneurons are obtained simultaneously. The upper trace shows an intracellular record from a soleus motoneuron and illustrates the locomotor drive potentials during two fictive step cycles (from Jordan, 1983).

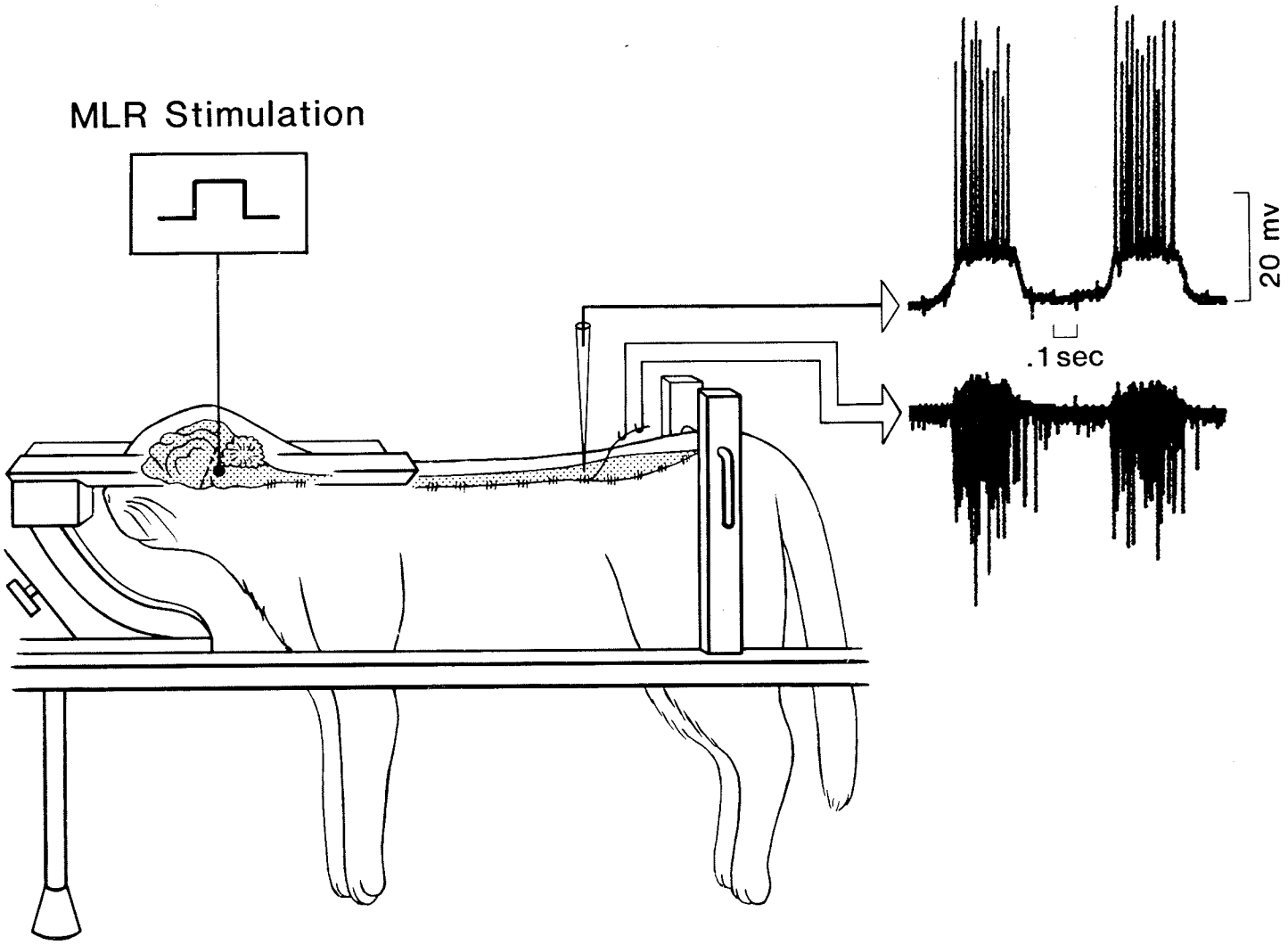

MLR Stimulation
20 mv
.1 sec

FIGURE 2: Illustration of the LDPs in an MG motoneuron during fictive locomotion and the technique used to standardize the LDP with respect to the fictive step cycle. In panel A the low gain intracellular record showing the motoneuron membrane potential during fictive locomotion are shown for a period of 15 steps. In order to provide a rapid display of the membrane potential oscillations during the entire period of locomotor activity, not all the data points are plotted during this graphics routine, and only every n^{th} data point is represented. The horizontal line represents the Em level used as threshold for the determination of the start of the step cycle. The vertical lines represent the start of each cycle. B shows the plot of the data points representing the membrane potential during each step cycle; a total of 45,000 data points representing the membrane potential during the 15 fictive steps comprise the plot. The data points are plotted a second time to represent the cyclic nature of the membrane potential changes. The magnitude of the LDP in this MG motoneuron was about 25 mV while the average step cycle length was about 1 second. The spikes during the depolarized phase have been truncated in this figure. The average Em values for each 1/10 th of the step cycle are represented by black dots with a white surrounding area and are superimposed on the raw data.

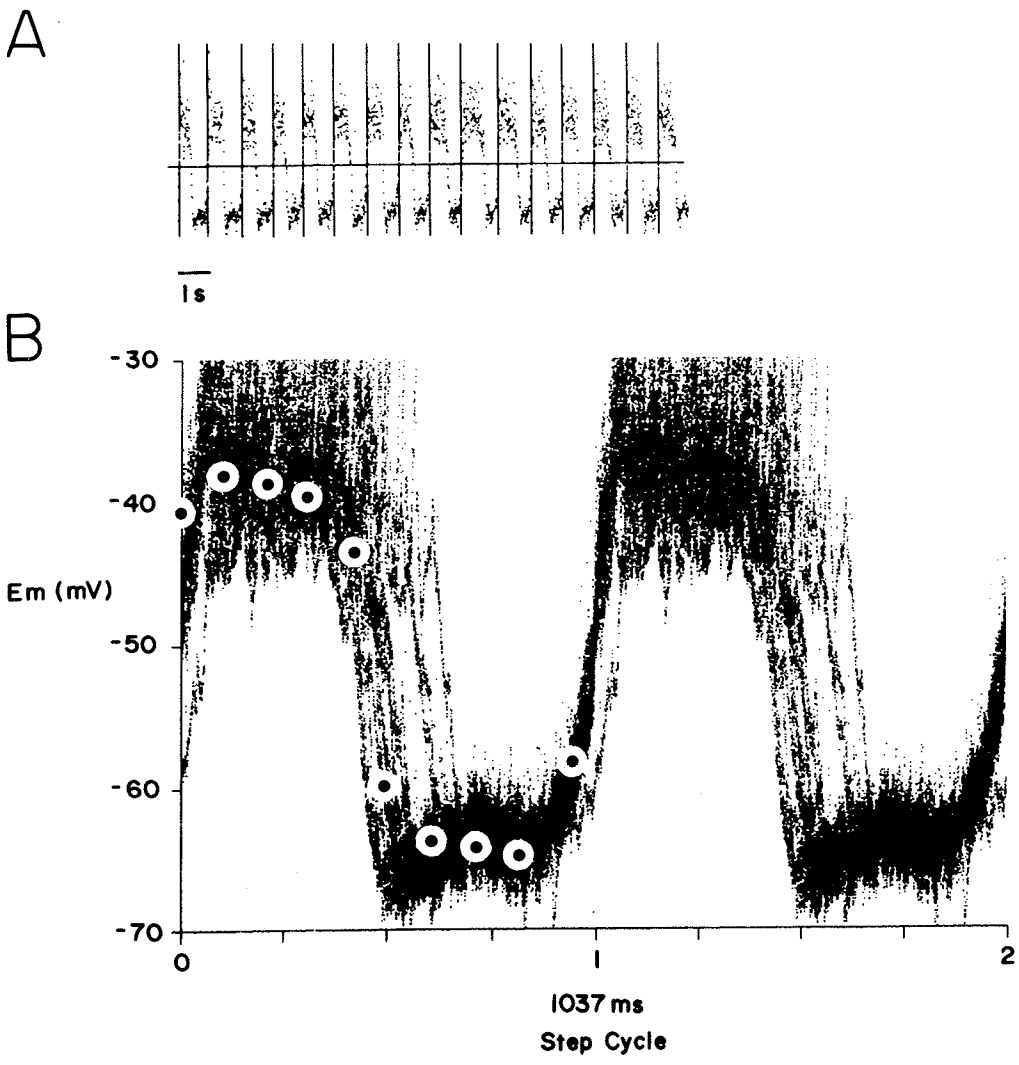

A
1 s
B
-30
-40
Em (mV)
-50
-60
-70
0
1
2
1037 ms
Step Cycle

FIGURE 3: Illustration of the sorting and averaging techniques used to examine the LDPs and MLR-evoked PSPs during the fictive step cycle. In A the DC intracellular record from a PBSt motoneuron (upper trace) and the activity of an L7 ventral root filament (lower trace) are illustrated during a period of fictive locomotion produced by stimulation of the MLR. The LDP seen in the intracellar trace is first examined by determining the membrane potential level throughout the fictive step cycle. For this analysis the beginning of each fictive step cycle was set using a membrane potential level which was approximately halfway between the extremes of the membrane hyperpolarization and depolarization during the LDPs. The duration of each step cycle was divided into 6 segments or bins, as shown along the abscissa in B. The membrane potentials were digitized and those occurring within a particular bin averaged, thus producing 6 averaged membrane potential values for the step cycle. The fast rise time of the LDP seen in A is illustrated by the abrupt transition from the hyperpolarized to depolarized values seen in the plot of the averaged data (B). The magnitude of the LDP was about 10 mV ("resting" membrane potential about -60 mV) and the fictive step cycle length approximately 1 second. The averaged values for each bin are plotted a second time to represent the rhythmic nature of the LDP.

The ipsilateral MLR was stimulated at 10 Hz and the 30 ms segment of a high gain intracellular record following

each MLR stimulus was placed into one of the six bins according to either 1) the fictive step cycle length (1c through 6c as illustrated along the abscissa in B), or 2) according to membrane potential level during the LDP. In the latter method, the voltage range of the LDP was divided into 6 bins of equal voltage size and the traces occurring within each bin were averaged. The averaged MLR-evoked PSPs using the step cycle length method are illustrated in C. The averages obtained using the membrane potential level are shown in D. Each voltage range is displayed to the right of the corresponding average. In both C and D the latencies from the MLR stimulus artifact to the evoked EPSP and IPSP were 4.7 and 6.0 ms,, respectively. The calibration pulse at the start of each average in C and D is 2 mV and 1 ms.

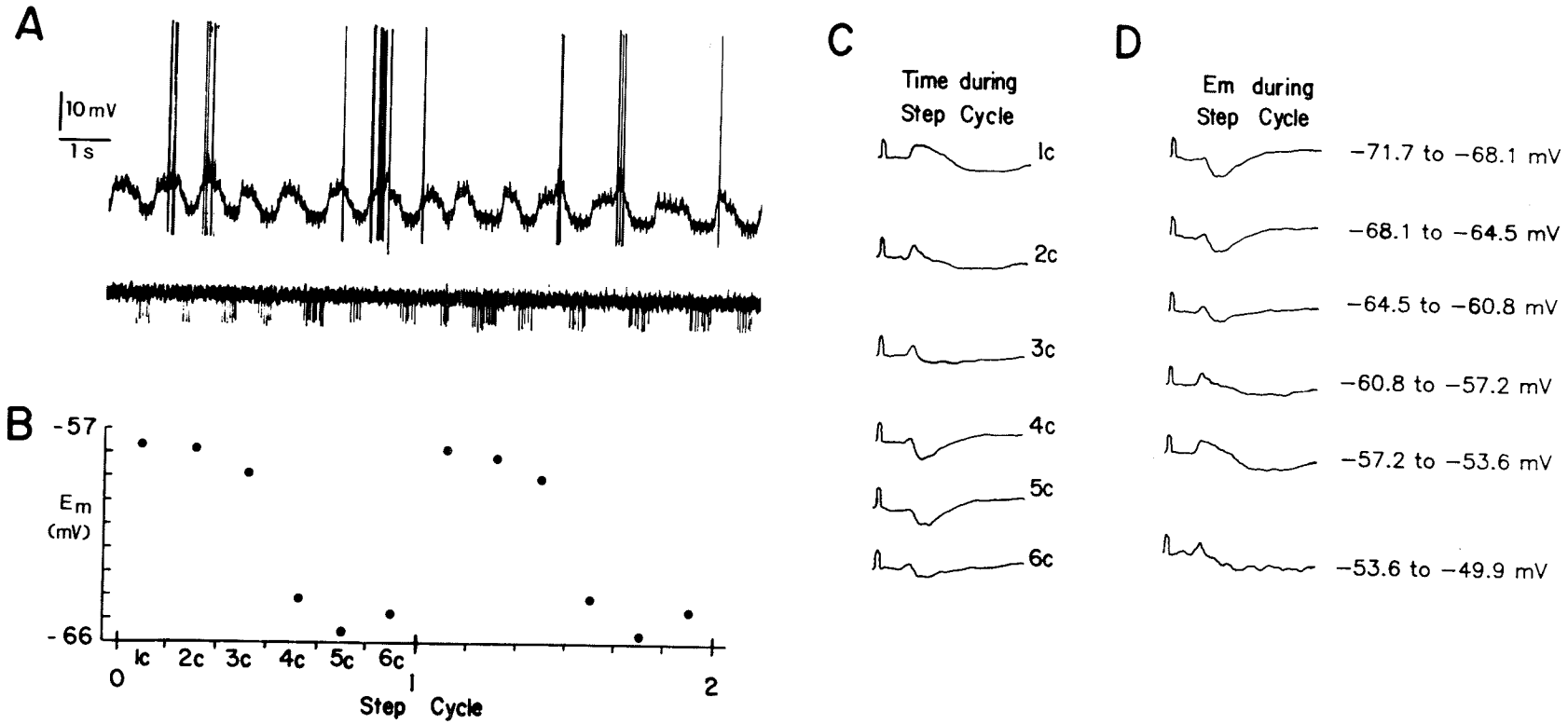

A
10 mV
1 s
B
-57
Em
(mV)
-66
1c 2c 3c 4c 5c 6c
0
1
2
Step Cycle
C
Time during
Step Cycle
1c
2c
3c
4c
5c
6c
D
Em during
Step Cycle
−71.7 to −68.1 mV
−68.1 to −64.5 mV
−64.5 to −60.8 mV
−60.8 to −57.2 mV
−57.2 to −53.6 mV
−53.6 to −49.9 mV

FIGURE 4: PSPs evoked in a PBSt motoneuron with stimulation at various depths within the brainstem in and around the MLR. A cross section of the brainstem at the level of the inferior colliculus is illustrated. The filled circles correspond to sites of stimulation at the L4 coordinate at varying depths. To the right of the cross section are numbers indicating depth from the surface of the inferior colliculus and the intracellular response recorded in the PBSt motoneuron. Stimulation parameters were 150 μA at 10 Hz at all sites. The calibration pulse associated with each trace was 2 mV, 1 ms. Stimulation at the 5.0 mm depth evoked the best locomotor response in the ENGs and VR filament monitors as well as the largest PSPs. The EPSP was observed during the depolarized phase of the LDP while the IPSP was observed during the hyperpolarized phase. The middle trace at the 5.0 mm level shows multiple sweeps including both the EPSP and IPSP. A small EPSP was observed at the 6.0 mm depth, but it was not modulated in amplitude and no rhythmic ENG or VR filament activity was present. Abbreviations: BC, brachium conjuctivum; CB, cerebellum; FTP, paralemniscal tegmental field; IC, inferior colliculus; LC, locus coeruleus; LLD, dorsal nucleus of the lateral lemniscus; LLV, ventral nucleus of the lateral lemniscus; MLB, medial longitudinal bundle; 5N, trigeminal nerve; V4, fourth ventricle.

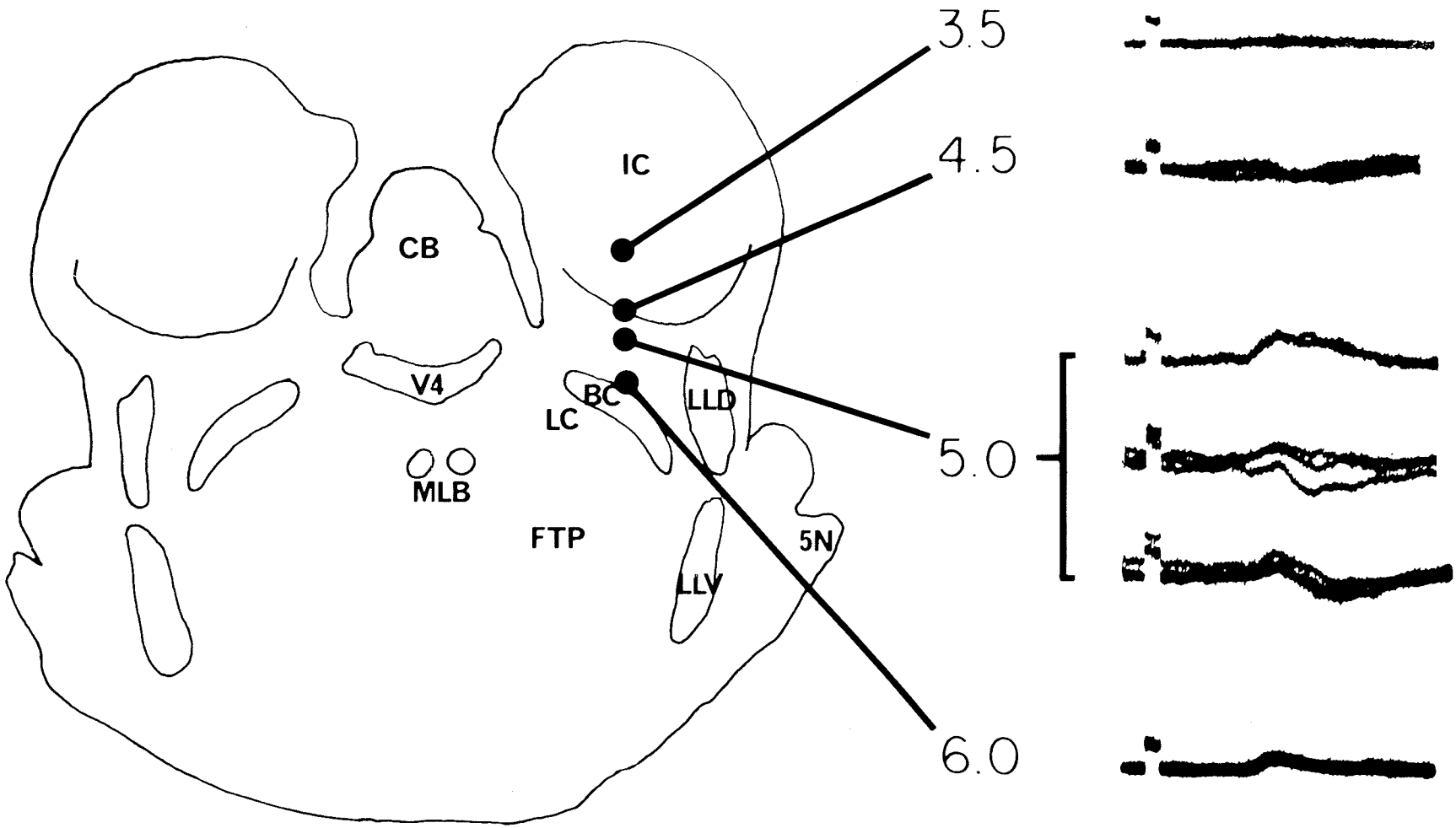

3.5
4.5
5.0
6.0
IC
CB
V4
BC
LC
LLD
MLB
FTP
5N
LLV

FIGURE 5: PSPs in an LG motoneuron evoked by MLR stimulation during the fictive step cycle. A depicts the normalized LDP (determined as in Figure 2) during the step cycle ("resting" Em about -46 mV; LDP magnitude 5 mV; step cycle length 800 ms). In B the 8 averages obtained using a step cycle normalized to time during the cycle are illustrated. The numbers to the left of each average indicate bin numbers and correspond to the numbers in A. The numbers to the right of the averages indicate the number of sweeps used to obtain the averages. The calibration pulse at the start of each average is 2 mV, 1 ms and the small downward deflection immediately following the calibration pulse is the artifact associated with the MLR stimulus. The latency from the MLR stimulus to the evoked EPSP was about 7 ms, while the IPSP occurred about 1.2 ms later. In C the averages of the evoked EPSPs from bin 1 and 5 are illustrated at expanded time and amplitude scales. Although both averages were obtained from samples occurring at similar Em levels, the peak amplitude differs. In D the two averages illustrated (6 and 8) are both taken from samples at a hyperpolarized Em level but at different times in the step cycle.

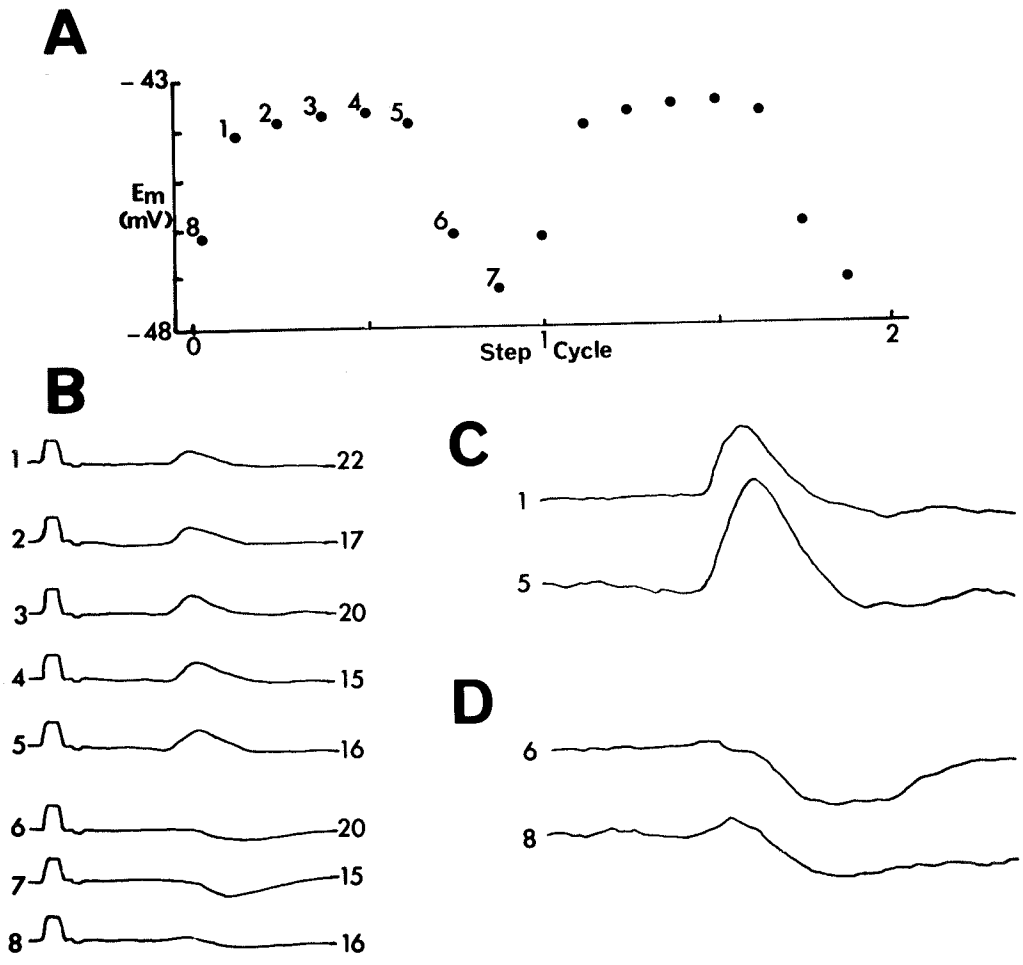

A
-43
-48
Em
(mV)
1
2
3
4
5
6
7
8
0
1
2
Step
Cycle
B
1
22
2
17
3
20
4
15
5
16
6
20
7
15
8
16
C
1
5
D
6
8

FIGURE 6: The effects of constant current injection on the LDPs and MLR evoked PSPs in a PBSt motoneuron during fictive locomotion (same cell as illustrated in Figures 2 and 3). A shows the control condition prior to current injection. MLR stimulating parameters were 150 μA at 10 Hz and were kept constant during all trials. B illustrates the effects of a 10 nA hyperpolarizing current and C a 10 nA depolarizing current. The top traces in A, B, and C are the DC coupled intracellular records (calibration 10 mV, 1 s), and directly below each is the corresponding VR filament activity (same time calibration as the intracellular record). Below the filament activity are the corresponding averages for the control and current injection trials. The averages were based on Em level with the most hyperpolarized level shown in the top average and the most depolarized level shown in the bottom average. Numbers to the right of each average represent the number of sweeps used to make up the corresponding average. The calibration pulse for all averages was 2 mV and 1 ms.

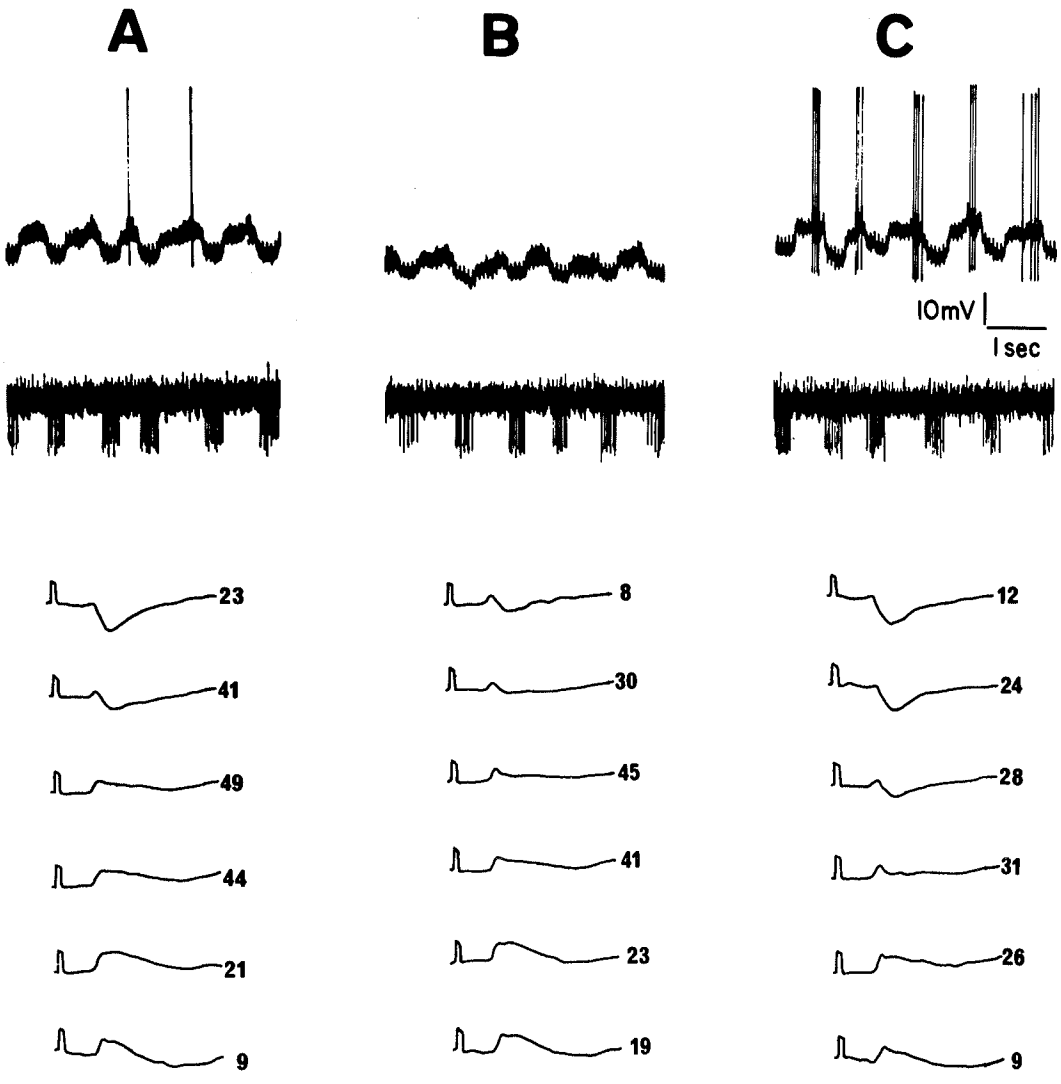

A
B
C
10mV
1 sec
23
41
49
44
21
9
8
30
45
41
23
19
12
24
28
31
26
9

FIGURE 7: Effects of electrical stimulation of the ipsilateral sural nerve at 5xT on a PBSt motoneuron during control and locomotor trials. A illustrates the average of the response recorded intracellularly from a PBSt motoneuron evoked by electrical stimulation of the ipsilateral sural nerve (5xT) during a non-locomoting control period ("resting" membrane potential about -45 mV; 30 sweeps/average). In B the averages of the intracellular record of the ipsilateral sural (5xT) response at various times within the step cycle are illustrated. C shows the average motoneuron membrane potential at the times in the step cycle corresponding to the time during the cycle used in B. The averaged Em values are replotted a second time as in Figure 2. In panels B and C the star indicates the start of the step cycle while the triangle marks the end of the step cycle.

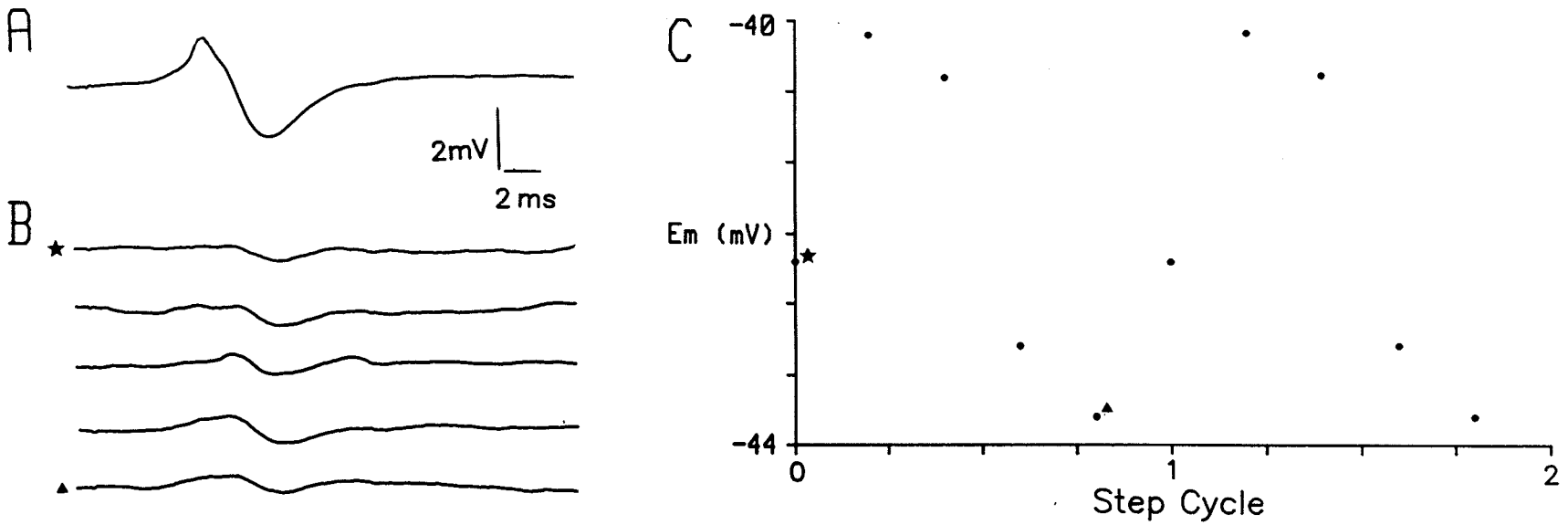

A
2mV
2 ms
B
C
-40
Em (mV)
-44
0
1
2
Step Cycle

FIGURE 8: Effects of high threshold MG muscle afferents and MLR stimulation on a PBSt motoneuron. The panels depict the effects of ipsilateral MG muscle nerve stimulation (20xT) during fictive locomotion, MLR stimulation alone, and combined MLR and MG stimulation (MG stimulus 4.7 ms after the MLR stimulus) during the maximum depolarized phase (left panel) and hyperpolarized phase (right panel) of the step cycle. The calibration bars apply to all traces. The ipsilateral MLR (P1,L4,H5) was stimulated at 10 Hz, 100 μA. The trace labelled DIFFERENCE was obtained through the use of a computer by subtracting the response evoked with MLR stimulation alone and the response from the high threshold muscle afferent stimulation alone during fictive locomotion from the response produced by combined MLR and high threshold MG stimulation.

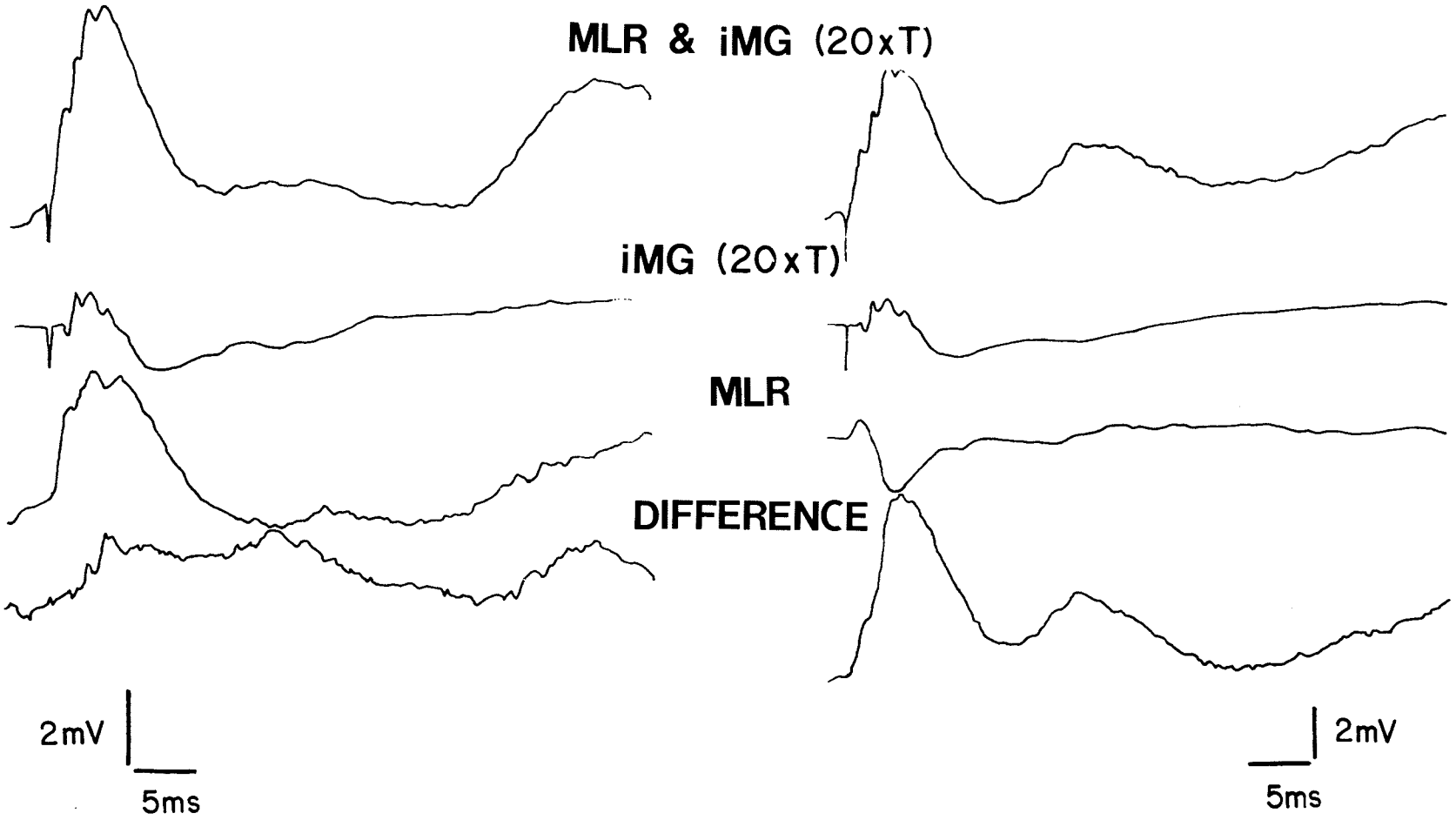

MLR & iMG (20xT)
iMG (20xT)
MLR
DIFFERENCE
2mV
5ms
2mV
5ms

FIGURE 9: Effects of high threshold cutaneous afferents and MLR stimulation on an LG motoneuron. The panels depict the effects of ipsilateral sural nerve stimulation (5xT) during fictive locomotion, MLR stimulation alone, and combined MLR and sural stimulation (sural stimulus following the MLR stimulus by about 3 ms) during the maximum depolarized phase (left panel) and hyperpolarized phase (right panel) of the step cycle. The calibration bars apply to all traces. The ipsilateral MLR was stimulated at 10 Hz, 100 μA. The trace labelled DIFFERENCE was obtained in the same manner as described in Figure 7.

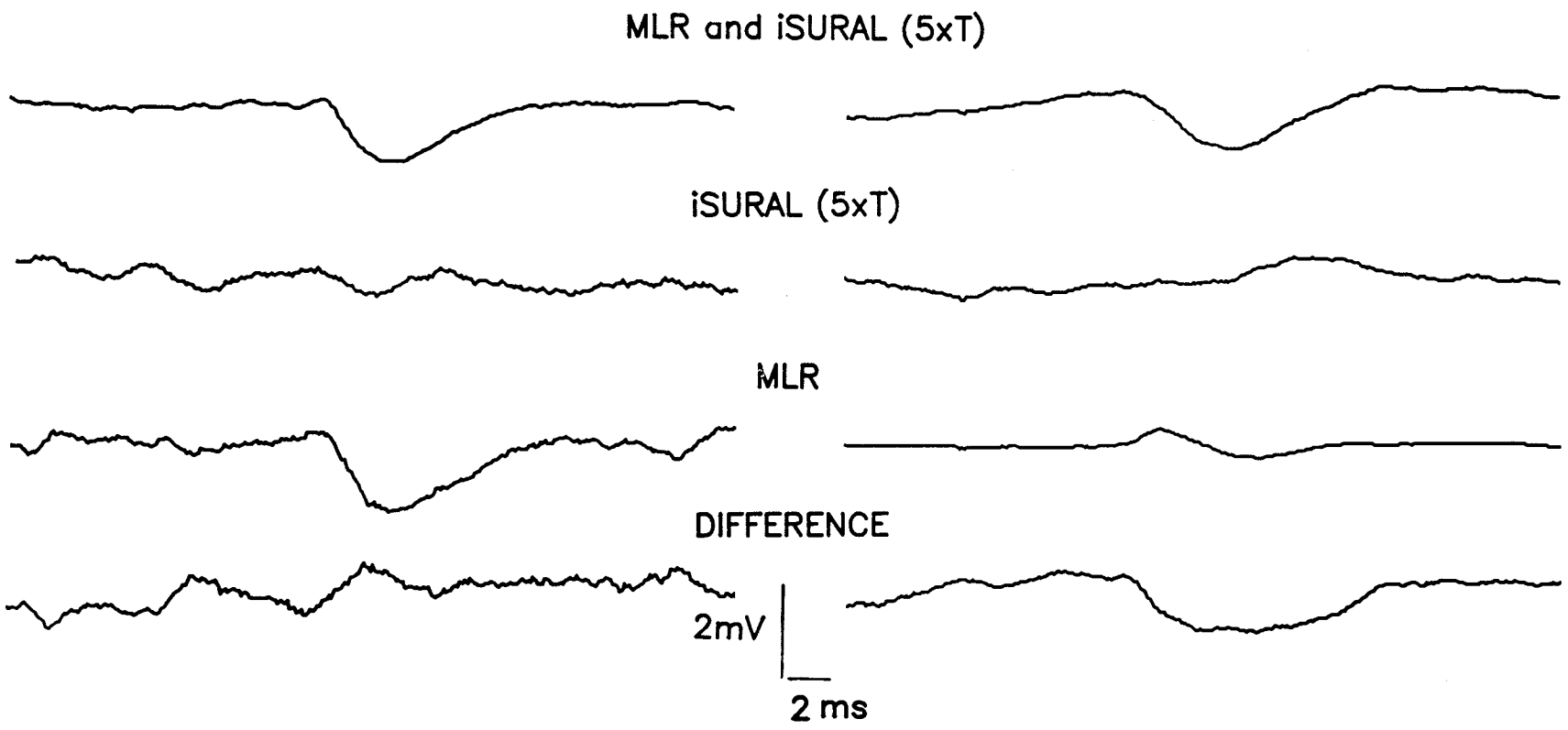

MLR and iSURAL (5xT)
iSURAL (5xT)
MLR
DIFFERENCE
2mV
2 ms

FIGURE 10: Illustration of the afterhyperpolarizations observed in a PBSt motoneuron during depolarizing constant current injections and during locomotion. Panel A illustrates the response of PBSt motoneuron to constant depolarizing current (15nA). B shows the low gain intracellular microelectrode record from the motoneuron (upper trace) and the electroneurogram recorded from the contralateral lateral gastrocnemius nerve (lower trace) during fictive locomotion. Short depolarizing current pulses (60 nA, 200 μs) to produce action potentials were injected via the recording microelectrode at a rate of 10Hz throughout the step cycle. Averages of the spikes evoked by the short depolarizing pulse injections are illustrated in C duirng a pre-locomotor control period (upper trace; 488 sweeps/average), at the start of the step cycle as triggered by the depolarizatioon of the motoneuron membrane potential during the fictive step cycle (middle trace; 81 sweeps/average), and at the end of the step cycle (bottom trace; 58 sweeps/average). In C the square pulse at the start of eachh average is the 2 mV, 1 ms calibration pulse.

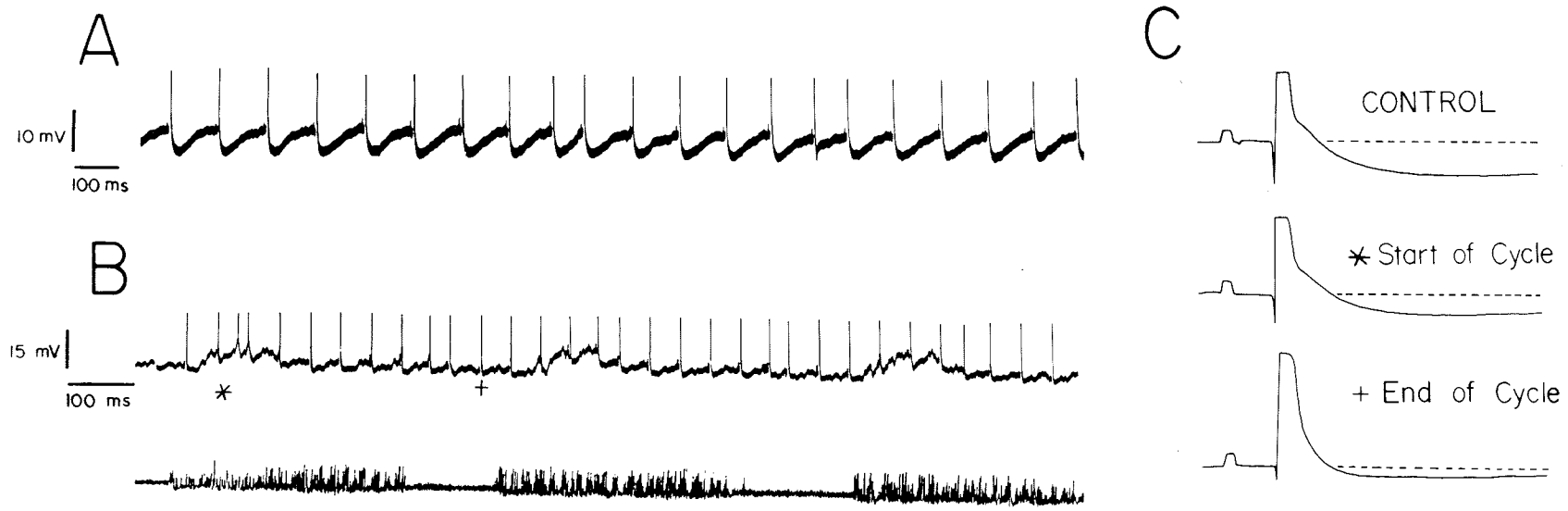
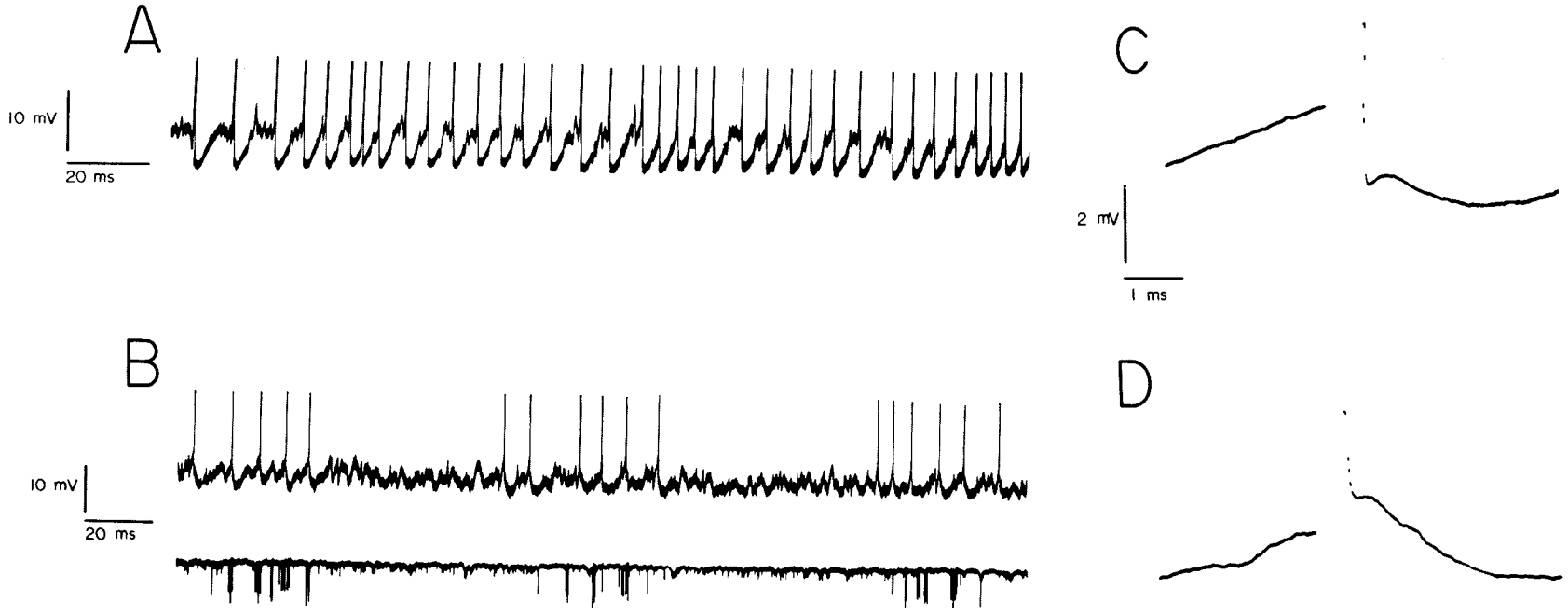

A
10 mV
100 ms
B
15 mV
100 ms
*
+
C
CONTROL
* Start of Cycle
+ End of Cycle

FIGURE 11: Motoneuron firing patterns during current injection and locomotion and the events preceding the action potentials. Panel A illustrates the firing of a PBSt motoneuron during injection of a 30 nA deplarizing current. In B the upper trace shows the intracellular record from the motoneuorn during fictive locomotion while the lower trace is the accompanying activity in an L7 ventral root filament. Spike-triggered averages of the current-evoked action potential (C; 16 sweeps/average) and the action potentials occurring during locomotion (D; 16 sweeps/average) show the events leading to the spike in each case. The calibration for C and D is the same.


A
10 mV
20 ms
B
10 mV
20 ms
C
2 mV
1 ms
D

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