An Investigation of Descending Brainstem Pathways Involved in the Production of Locomotion

by

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A Thesis

Submitted to the Faculty of Graduate Studies

in Partial Fulfillment of the Requirements for the

Degree of Doctor of Philosophy

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Winnipeg, Manitoba
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December, 1987

ISBN 0-315-44086-4

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Abstract

Experiments were designed to answer key questions regarding the brainstem descending pathways which, when activated, are capable of producing locomotion.

In the first series of experiments we determined the distribution of cells in the medial reticular formation (MRF) and the pontomedullary locomotor strip (PLS) which, when activated, can induce locomotion. Experiments were performed on precollicular-postmamillary decerebrate cats walking on a treadmill. Controlled microinjections of neuroactive substances into the MRF or PLS demonstrated that these areas contained cell bodies capable of producing locomotion. Furthermore, the PLS was demonstrated to be closely related to or synonymous with the spinal nucleus of the trigeminal nerve and is related to the sensory activation of locomotion.

In a second series of treadmill locomotion experiments, the descending pathways from the brainstem locomotor areas were investigated by utilizing reversible (cooling to block synaptic or fiber transmission) and irreversible subtotal lesions of the brainstem or spinal cord. The results demonstrate that the mesencephalic locomotor region (MLR) projects through the MRF and through the spinal cord in the ventrolateral funiculus (VLF). Furthermore, the PLS can produce locomotion by activation of this pathway or by another pathway which descends through the dorsal half of the spinal cord.

In a third series of experiments, we examined the characteristics of short-latency MLR-evoked postsynaptic potentials (PSPs) observed in lumbar spinal motoneurons and their relationship to the production of motoneuron locomotor drive potentials (LDPs) and locomotion. Experiments were performed on paralyzed, precollicular-postmamillary decerebrate cats during intracellular recording from lumbar motoneurons. Evidence is presented that MLR-evoked PSPs are highly related to the process of locomotion and that LDPs and PSPs observed in motoneurons with stimulation of the MLR are produced by a common pathway which relays in the MRF and descends bilaterally through the spinal cord in the VLF. Spinal interneurons that may be related to the production of MLR-evoked PSPs are described.

Acknowledgements

I would like to extend my gratitude and appreciation to my supervisor, Larry Jordan, who has been a true source of inspiration and guidance throughout the years that I have known him. Thank you for showning me how science should be conducted. Can I take back all those "short" jokes?

I would like to thank my fellow graduate students Dean Kriellaars, Rob Brownstone, and Jack Kettler for everything else. Without their talent, knowledge, insight, humour, and their ability to stay awake longer than anyone else I know, this thesis would not have been possible.

I would also like to acknowledge Kim Madec for her help during the experiments and in making the figures (and to the Amazing Randi, who taught Kim such a wonderful disappearing act). I would also like to thank Dan DeJong, Georgina Brown, and Maria Setterbom for their technical assistance througout the course of these experiments (and Maria for always having such a wonderful smile).

I would also like to thank Dave McCrea and Susan Shefchyk for the many wonderful conversations I have had about science and life in general. Dave, maybe someday we can start a jazz band, together. Special thanks also goes to Gilles and Gilbert Detillieux for the development of the most sophisticated, incredible, wonderful, amazing, stimulating and fulfilling data capture and analysis program I have ever seen (and also for their great sense of humour). I would also like to thank Cecil Korzenowski for being the best friend I know.

This thesis is dedicated to my parents, Anne and Carl Noga for their love and support throughout my career as a student and to Faith Jacyk who shared so much of her life with me.

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Introduction

It has been demonstrated that the spinal cord contains a network of cells capable of generating locomotion when appropriately activated (Grillner and Zangger, 1979). Two brainstem systems have been described which are capable of activating this network. Both systems are fundamentally different in their internal organization and their descending trajectories. One is thought to initiate locomotion by a fast conducting, relatively direct pathway, whereas, the other is thought to initiate locomotion via a slower conducting, polysynaptic pathway. A controversy has arisen concerning the contribution and independence of the two systems to the production of locomotion. Central to the resolution of this controversy is a better understanding of their internal organization, interaction and brainstem origin. The proposed research is designed to answer many of these questions in order to better understand the brainstem-spinal cord pathways by which locomotion may be initiated.

The MLR-MRF-VLF System

Stimulation of the classical mesencephalic locomotor region or MLR (Shik et al., 1967), of the midbrain (stereotaxic coordinates: P2, L4, H-1; Berman, 1968) produces locomotion in decerebrate cats placed on a treadmill. Descending fibers from this site terminate in the gigantocellular (FTG) and magnocellular (FTM) tegmental fields of the medial pontomedullary reticular formation (MRF) and do not project directly to the spinal cord (Steeves and Jordan, 1984). As summarized by Jordan (1986), reticulospinal cells in the MRF then produce locomotion by a pathway travelling in the ventral lateral funiculus (VLF) of the spinal cord. This projection is a fast conducting and direct pathway to the spinal cord.

The evidence in favor of this pathway comes from a number of observations. Anatomical studies in the cat have demonstrated that the MLR projects to the FTG and FTM of the reticular formation and doeso not project directly to the spinal cord (Steeves and Jordan, 1984). This has also been confirmed in the rat (Garcia-Rill et al., 1986). Garcia-Rill et al. (1983b) have also demonstrated a small projection to the MRF from an area situated slightly medial

to the MLR which is also capable of producing locomotion when electrically stimulated. Moreover, pronounced locomotor deficits have been described following lesions in the FTG and FTM (Zemlan et al., 1983).

The reticular formation sites which receive projections from the MLR have been previously shown to project to the cervical and lumbar enlargements in the spinal cord through the VLF (Kuypers and Maiskey, 1977; Martin et al., 1978; Peterson et al., 1975). This supports the observation that the VLF is the only essential quadrant of the spinal cord that is required for MLR-evoked locomotion (Steeves and Jordan, 1980) or for locomotion in otherwise intact animals, including man (Afelt, 1974; Afelt et al, 1975; Eidelberg, 1980, 1981; Eidelberg et al., 1981a,b; Sholomenko and Steeves, 1987). Electrical stimulation of the VLF in the decerebrate cat (Yamaguchi, 1986, 1987) or the intermediate portions of the lateral funiculus of the spinal cord in the decerebrate chick (Jacobson and Hollyday, 1982) or the spinal stingray (Williams et al., 1984) also can initiate locomotor movements.

Electrophysiological evidence also points to the MRF as the mediator of MLR-evoked locomotion. For example, Mori et al. (1978a, 1980b) demonstrated that stimulation of the ventral aspect of the MRF, in sites later confirmed to receive projections from the MLR (Steeves and Jordan, 1984), could produce hindlimb or quadrupedal locomotion or facilitate locomotion produced by MLR stimulation. The capacity of the MRF to produce locomotion when electrically stimulated has subsequently been confirmed in a number of different species (Garcia-Rill and Skinner, 1987a; Livingston, 1986; Ross and Sinnamon, 1984; Steeves et al., 1987). In addition, stimulation of the MRF has been shown to be capable of resetting the locomotor rhythm (Drew and Rossignol, 1984).

In an early series of papers, Orlovsky demonstrated that stimulation of the MLR could orthodromically activate reticulospinal neurons bilaterally (1970a) and increase the activity of pathways within the ipsilateral and contralateral VLF (1969). Furthermore, he demonstrated that reticulospinal neurons became rhythmically active during locomotion produced by stimulation of the MLR (Orlovsky, 1970b). These initial studies have recently been confirmed and extended. Both MLR cells (Garcia-Rill et al., 1983a) and reticulospinal cells (Drew et al., 1986; Perret, 1976; Shimamura et al., 1982; Shimamura and Kogure,

1983) have been shown to be rhythmically active during spontaneous locomotion (chronic intact or thalamic cats). Furthermore, these studies demonstrate that the activity of many of these cells can been correlated to the excitation of specific muscle groups in one or more limbs (Drew et al., 1986; Garcia-Rill et al., 1983a; Shimamura et al., 1982). Shimamura et al. (1984a) have also shown the presence of descending volleys in the VLF during spontaneous forelimb locomotion in thalamic cats which can be correlated to the activity of forelimb flexors or extensors. Garcia-Rill and Skinner (1987b) have demonstrated that stimulation of the MLR may activate reticulospinal cells located ipsilaterally and/or contralaterally to the side of stimulation. They have also demonstrated that the reticulospinal cells may descend unilaterally or bilaterally in the ipsilateral or contralateral VLF. Cells in the MRF also show increased metabolic activity during MLR-evoked fictive locomotion (Kettler and Jordan, 1984). While these studies indicate that cells in the MRF may be involved in the mediation of MLR-evoked locomotion, they provide only indirect evidence. The direct evidence implicating the involvement of cells in the MRF in the mediation of locomotion includes the observation that cooling restricted areas in the MRF to block synaptic transmission can reversibly abolish both spontaneous and MLR-evoked locomotion (Shefchyk et al., 1984). In addition, injections of substance P or cholinergic agonists into the MRF can elicit locomotion and injections of cholinergic antagonists into the MRF abolish MLR-evoked locomotion (Garcia-Rill and Skinner, 1987a). While this and another study (Garcia-Rill et al., 1987) indicate the importance of acetylcholine and substance P in MLR or MRF-evoked locomotion, the involvement of other putative neurotransmitters in the locomotion produced by activation of the MLR is indicated by a number of recent findings. Cholinergic activation is not required for initiation of locomotion because intravenous injection of cholinergic antagonists do not block the inititation of locomotion from the classical MLR (Noga et al., 1987a; Noga et al., unpublished). Furthermore, few ACh (Lee et al., 1986; Rye et al., 1987) or substance P-containing cells (Leger et al., 1983) are found within the region of the classical MLR.

While the evidence summarized above suggests that the MLR mediates its effects via the reticulospinal pathways in the VLF of the spinal cord, it has been suggested that other spinal cord pathways may mediate some of the locomotion produced by stimulation of the MLR (Yamaguchi, 1986). It was claimed that

forelimb locomotor movements could be evoked by MLR stimulation if bilateral lesions spare either the dorsolateral funiculus (DLF) or the VLF on one side with transection of both the DLF or the VLF on the opposite side. On the basis of these results it was concluded that the descending pathways for the activation of the spinal locomotor network of the single forelimb are located ipsilaterally in the DLF as well as in the VLF. Thus, it was suggested that the activity of a polysynaptic propriospinal chain (see next section) was important in the initiation of forelimb locomotion by stimulation of the MLR. While there is no doubt that stimulation of the DLF of the spinal cord may initiate locomotion in a number of different species (Jacobson and Hollyday, 1982; Kazennikov et al., 1983b, 1985; Lennard and Stein, 1977; Williams et al., 1984; Yamaguchi, 1987) the relationship of this area to the MLR is unresolved (see next section for further discussion). It is difficult to accept the conclusions made by Yamaguchi (1986) on the basis of the presented evidence. Examination of the lesions depicted indicate that the VLF was not entirely transected. In fact, these experiments indicate that following the destruction of the DLF and VLF on the opposite side, any further destruction of the DLF on the ipsilateral side (in the presence of the incomplete transection of the VLF) is without any additional effect on MLR-evoked locomotion.

The PLS-DLF-Propriospinal System

Electrical stimulation of another region known as the pontomedullary locomotor strip (PLS) has also been shown to produce locomotion in decerebrate cats (Kazennikov et al., 1979, 1983a; Mori et al., 1977, 1978a,b; Selionov and Shik, 1981, 1984; Shik and Yagodnitsyn, 1977, 1978) and swimming in turtles (Kazennikov et al., 1980b; Selionov and Shik, 1982). The type of locomotion produced by the PLS is similar to MLR locomotion but is often accompanied by spastic hindlimbs (Mori et al., 1977). The PLS is a very small, discrete area 0.5-1.0 mm in diameter (Mori et al., 1977, 1978b; Shik and Yagodnitsyn, 1978) and extends throughout the lateral tegmentum of the pons and medulla medial and ventral to the spinal nucleus of the fifth nerve (Mori et al., 1977, 1978a; Shik and Yagodnitsyn, 1977, 1978). However, in some experiments the PLS may be dorsolateral or lateral to the spinal nucleus (Shik and Yagodnitsyn, 1977). The PLS extends to the C1 spinal cord level to the border of gray matter and dorsolateral funiculus (DLF) where it produces, when stimulated, ipsilateral

hindlimb (Shik and Yagodnitsyn, 1977; Mori et al., 1978b) or quadrupedal locomotion (Budakova and Shik, 1980).

While the evidence summarized above indicates that the MLR projects to cells in the MRF which then project through the spinal cord via the VLF, others (Mori et al., 1977, 1978b; Shik and Yagodnitsyn, 1978) have claimed that the MLR projects via the PLS. The evidence, based primarily on electrophysiological techniques, is inconclusive. For example, while stimulation of the PLS produces field potentials in the MLR, stimulation of the region between the PLS and midline, where locomotion cannot be produced, also gives rise to field potentials in the MLR (Mori et al., 1977). Although subthreshold stimulation of the MLR and the PLS will generate locomotion when delivered together (Mori et al., 1977), and stimulation of the PLS and the region surrounding it facilitates MLR-evoked locomotion (Mori et al., 1978a), it is not necessarily true that this facilitation is due to a projection of the MLR via the lateral tegmentum. The best evidence to implicate the MLR as the origin or rostral pole of the PLS comes from the results presented by Mori et al. (1977). They mapped the brainstem areas caudal to the MLR that could produce locomotion when electrically stimulated and concluded that the MLR was continuous with the PLS. However, a site medial (P2, L2.7, H-0.5) to the classical MLR, which includes the mesencephalic nucleus of the fifth nerve and the periaqueductal grey, has also been shown to produce locomotion when electrically stimulated (Garcia-Rill et al., 1983b; Shefchyk et al., 1984). This site, referred to as the "medial MLR" (Shefchyk et al., 1984) may produce locomotion by a pathway which travels through the PLS. The evidence for this is twofold. Firstly, descending projections of the medial MLR region terminate within the lateral tegmentum in the region of the PLS (Garcia-Rill et al., 1983b; Skinner et al., 1984). Secondly, cooling of the PLS reversibly abolishes locomotion produced by stimulation of the medial MLR but does not affect the locomotion produced by stimulation of the classical MLR (Shefchyk et al., 1984). Thus, it is possible that Mori et al. (1977) could have been stimulating fibers projecting from the medial MLR sites to the PLS region. This may also explain the results obtained by Shik and Yagodnitsyn (1978), who showed that stimulation of regions somewhat caudal to the MLR (P4, L4) could activate cells within the PLS.

On the basis of electrophysiological recording techniques, it has been suggested that the lateral tegmental locomotor region is a polysynaptic pathway

with the cells of origin located either within the PLS (Shik and Yagodnitsyn, 1978; Selionov and Shik, 1984) or located medial and ventral to the PLS (Selionov and Shik, 1984). While it is thought that activity may propagate through the cells in the strip (Shik and Yagodnitsyn, 1978) the fact that continuity of the PLS is not essential for PLS-evoked locomotion (stimulation at more rostral levels) has led to the suggestion that the cells located outside the strip may also mediate the effects of PLS stimulation (Budakova and Shik, 1980). These cells, which are located in the adjacent lateral reticular formation (Selionov and Shik, 1981, 1982, 1984; Kazennikov et al., 1980b), have been termed the pontomedullary locomotor column or PLC (Selionov and Shik, 1984). Activity is said to propagate polysynaptically along the column through their axonal collaterals located within the locomotor strip (Kazennikov et al., 1980b; Selionov and Shik, 1984). The cells are thought to project for only short distances (4-9 mm) in the rostro-caudal direction (Selionov and Shik, 1984). It is assumed that when repetitive stimulation achieves a threshold for locomotion the propagation occurs without decrement and as a result, spinal stepping generators are activated (Selionov and Shik, 1984). Cells of the PLC show monosynaptic and polysynaptic activation to stimulation of the ipsilateral (Kazennikov et al., 1980b; Selionov and Shik, 1982, 1984) and contralateral (Kazennikov et al., 1980b; Selionov and Shik, 1984) locomotor strip and have been shown to have both descending and ascending projections (Selionov and Shik, 1982, 1984). While locomotion may be produced by stimulation of the PLS, it is thought that stimulation of the PLC is ineffective for producing locomotion because the cells are too diffusely distributed (Selionov and Shik, 1984). Others (Garcia-Rill et al., 1983b) have claimed that the PLS corresponds to Probst's tract, a fiber pathway descending from the mesencephalic nucleus of the trigeminal nerve (Corbin, 1942). However, the only direct evidence suggesting that cell bodies in the PLS region are actually involved in the production of locomotion comes from the observation that cooling to abolish synaptic transmission of a restricted portion of the PLS reversibly abolishes locomotion elicited by stimulation of the "medial MLR" (Shefchyk et al., 1984).

The spinal pathway of the PLS is not known with certainty although two possible routes have been described on the basis of electrophysiological experiments. One possible route is via the MRF of the medulla since the caudal PLS has been shown to activate cells located in the this region (Mori et al., 1986; Selionov and Shik, 1981, 1984). These cells are located in the same area that have

been suggested to be involved in the mediation of MLR-evoked locomotion (Shefchyk et al., 1984; Steeves and Jordan, 1984) and may thus project through the VLF of the spinal cord. However, this has not been proven. If this is the case, then an alternate explanation for the observation that continuity of the PLS is not essential for PLS-evoked locomotion (Budakova and Shik, 1980) may be that the signal activates the spinal centers for locomotion via the projection to the MRF.

The PLS is also thought to continue through the spinal cord in the DLF (Kazennikov et al., 1980a, 1983a,b). It has been suggested that the PLS may activate the pattern generator for locomotion in the cat spinal cord via a column of propriospinal neurons located primarily within the dorsal horn of the spinal cord (Kazennikov et al., 1979, 1983a,b; Shik, 1983). Activity is thought to propagate polysynaptically along this column of propriospinal cells, referred to as the "spinal stepping column" (Kazennikov et al., 1985). It has been suggested that the axons of the propriospinal cells in the upper cervical segments (Kazennikov et al., 1983a) are located in the DLF of the spinal cord at a spot that corresponds to the "stepping point" described by Sherrington (1910). Evidence for this spinal organization is provided by the following observations:

1) The PLS has been traced as a continuous strip to the spinal cord DLF (Shik and Yagodnitsyn, 1977, 1978).

2) Electrical stimulation of the DLF, unilaterally, throughout the spinal cord (C1-L1) can induce stepping in spinal or decerebrate animals (Jacobson and Hollyday, 1982; Kazennikov et al., 1983b, 1985; Lennard and Stein, 1977; Williams et al., 1984; Yamaguchi, 1986).

3) The upper cervical propriospinal cells respond to stimulation of a number of different locomotor points including the bulbar locomotor strip (Kazennikov et al., 1979, 1983a) and MLR (Kazennikov et al., 1983a, 1985). Responses are primarily polysynaptic (some monosynaptic from the PLS and possibly from the MLR), and repetitive stimuli are needed to generate responses in some cells (Kazennikov et al., 1979, 1983a). These cells project primarily for distances of 3-5 mm but do not project to low cervical or upper lumbar segments directly (Kazennikov et al., 1979).

4) Propriospinal neurons of the low thoracic and upper lumbar region (T12-L1) respond synaptically to stimulation of the stepping strip in the DLF (Kazennikov et al., 1985).

The evidence which supports the hypothesis that the DLF-propriospinal pathway mediates the production of locomotion by PLS stimulation is that destruction of the gray matter at C2-3 for a distance of 4-6 mm is required to make induction of walking by the PLS impossible, despite preservation of the lateral and ventral funiculi (Kazennikov et al., 1980a). As the propriospinal cells typically project caudally to similar grey matter levels for 3-5 mm (Kazennikov et al., 1979) they are unable to bypass the lesion (Kazennikov et al., 1980a). However, the results of this experiment are unclear. For example, it has been demonstrated that stimulation of the caudal PLS activates cells located in the MRF (Mori et al., 1986; Selionov and Shik, 1981, 1984) in areas implicated in the mediation of MLR-evoked locomotion (see above). If the MRF cells activated by PLS stimulation are related to the production of locomotion, why does stimulation of the PLS following the spinal lesion fail to produce locomotion? In addition, if the MLR produces locomotion by a pathway which projects via the PLS (Mori et al., 1977, 1978b; Shik and Yagodnitsyn, 1978), and PLS-evoked locomotion is abolished by lesions of the dorsal cord (Kazennikov et al., 1980a), why does locomotion produced by stimulation of the MLR only require the integrity of the VLF of the spinal cord at the cervical spinal level (Steeves and Jordan, 1980)? While a number of explanations are possible, the results of the dorsal cord lesion experiment must be interpreted with caution since the capacity for PLS- evoked locomotion following the extended spinal grey lesion may simply reflect a generalized deterioration in the health of the experimental animal and not necessarily represent an interruption of the descending pathway. While the available data concerning the descending spinal pathway(s) of the PLS are suggestive, the pathway(s) mediating the effect of PLS stimulation remain(s) unclear.

In the lower segments, the spinal organization of the propriospinal pathways is very complex. Stimulation of the stepping strip at these levels does not activate the hindlimb spinal centres for locomotion directly (Kazennikov et al., 1983b, 1985). Rather, it is thought that the activation is mediated by propriospinal cells which project caudally to the lumbar segments via the ventral funiculi

(Kazennikov et al., 1983b, 1985). Furthermore, the propriospinal neurons must be activated over a considerable length of the spinal cord (minimum of 120 mm) in order to activate the spinal centres for locomotion (Kazennikov et al., 1983b). Propriospinal neuron activation is achieved by both ascending and descending projections of the stepping strip in the DLF (Kazennikov et al., 1983b, 1985).

The brainstem and spinal organization for the PLS described above is in sharp contrast to the relatively direct pathway (MRF- VLF) thought to mediate MLR-evoked locomotion (see above). The fact that the ventral half of the spinal cord contains the pathways required for the initiation of locomotion in all mammalian species studied (Eidelberg, 1981) suggests that the PLS-DLF system may be involved in functions other than the initiation of locomotion from higher brain (motor) centers. An alternate interpretation of the PLS-DLF in the production of locomotion may be that it is part of a sensory pathway which has access to the locomotor systems. It has been known since the time of Sherrington that sensory pathway activation may induce locomotion (Sherrington, 1910). For example, stimulation of the flexion reflex afferents induces locomotor-like activity in spinal animals (Jankowska et al., 1967b), and dorsal root or dorsal column stimulation can induce locomotion in mesencephalic or spinal animals (Budakova, 1972; Grillner and Zangger, 1979). In addition, mechanical stimulation of the dorsal root entry zone in the upper cervical cord (Huang et al. 1970) and stimulation of the pinna in mesencephalic cats (Aoki and Mori, 1981) are also sufficient in producing locomotion. While DLF stimulation can produce locomotion in spinal and decerebrate animals (Jacobson and Hollyday, 1982; Kazennikov et al., 1983b, 1985; Lennard and Stein, 1977; Williams et al., 1984; Yamaguchi, 1986, 1987), recent evidence suggests that this pathway may be involved in sensory activation of locomotion. Shimamura et al. (1984b) found that bilateral lesions of the DLF could abolish locomotion produced by stimulation of joint afferents in the thalamic cat. Although the neural elements comprising the PLS have not been defined, the effective stimulus sites are always in close proximity to the trigeminal system (Garcia-Rill et al., 1983b; Shik and Yagodnitsyn, 1977, 1978). While there is evidence available which shows that peripheral stimuli within the trigeminal receptive field can induce locomotion (Aoki and Mori, 1981), no studies have been carried out to distinguish between PLS structures and the brainstem trigeminal system. Taken together, the PLS would therefore be a likely candidate for mediating locomotion produced by

stimulation of the trigeminal receptive field. In this case, the PLS could be considered to be analogous to the flexion reflex afferent system for the limbs.

Activation of the Spinal Centers for Locomotion by the MLR

During locomotion, limb alpha motoneurons (flexor and extensor) undergo periodic depolarization and hyperpolarization, termed locomotor drive potentials (LDPs), during the step cycle (Edgerton et al., 1976; Jordan, 1983; Jordan et al., 1981; Perret and Cabelguen, 1980; Perret, 1983). Motoneuron membrane conductance measurements (Jordan, 1981; Jordan et al., 1981; Shefchyk and Jordan, 1985b) and intravenous injections of strychnine (Pratt and Jordan, 1987) during fictive locomotion produced by stimulation of the MLR has demonstrated that these membrane oscillations are due to alternating excitatory and inhibitory synaptic input during the appropriate phase of the step cycle. Rhythmic inhibition of motoneurons during fictive locomotion has also been verified using intracellular current or chloride injection to reverse the hyperpolarized phase of the LDP (Edgerton et al., 1976; Perret, 1983; Orsal et al., 1986). The relative importance of the excitatory input to the generation of the rhythmic activity of motoneurons is emphasized by the observation that the hyperpolarized phase of the motoneuron can be removed by intravenous administration of strychnine (Pratt and Jordan, 1987), without abolishing the rhythmic firing of the motoneuron. During the removal of the rhythmic inhibitory input by strychnine administration, large excitatory postsynaptic potentials (EPSPs) are also revealed in the motoneuron during MLR-evoked locomotion (Pratt and Jordan, 1987). It is reasonable to assume that the spinal interneurons involved in the production of motoneuron LDPs are also activated by MLR stimulation. A system of spinal neurons has been described in cats which may be part of the spinal centres for locomotion (Jankowska et al., 1967a,b), and evidence has been provided which shows that the pathways which activate these neurons may be facilitated during MLR-evoked locomotion (Grillner and Shik, 1973). Unidentified rhythmically active neurons in the cat spinal cord have been described before during MLR-evoked locomotion (Baev et al., 1979; Orlovsky and Feldman, 1972). While some of these cells could conceivably be part of the locomotor generator or part of the output stage of the generator, they could be ascending tract cells or cells driven by other rhythmically active descending pathways such as the vestibulospinal or rubrospinal tracts (Orlovsky, 1972) since

they were not shown to be synaptically driven by MLR stimulation and their trajectory is unknown. Direct evidence for the activation of identified last order spinal interneurons in the 4th lumbar segment by MLR stimulation has recently been obtained (Shefchyk et al., 1987). Furthermore, unidentified last-order interneurons which may participate in the production of locomotion have also been localized in the 5th, 6th, and 7th lumbar segments (Noga et al., 1987b).

A recent paper has shown that stimulation of the MLR (in a site which is maximally effective for the initiation of locomotion) produces both short latency excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs) in alpha motoneurons (Shefchyk and Jordan, 1985a). Since the amplitude of these PSPs increase when the motoneuron LDP (and locomotion) appears and since they are strongly modulated during the locomotor step cycle it has been suggested that they are linked to the process of locomotion (Shefchyk and Jordan, 1985a). Evidence in favor of this hypothesis comes from the demonstration that the pathway involved in the production of these PSPs converge on interneurons mediating the effects of flexion reflex afferents (Shefchyk and Jordan, 1985a). Interneurons receiving FRA input have previously been implicated in the production of locomotion (Jankowska et al., 1967a,b). If the MLR-evoked PSPs are linked to the process of locomotion, then they may reflect processes in the spinal cord which are responsible for the production of the LDPs and locomotion (i.e., output of last order excitatory and inhibitory interneurons of the CPG). The latencies of EPSPs suggest that they are transmitted from the MLR through a few synaptic delays (the IPSPs may arrive after an additional synaptic delay). Thus they probably reflect passage through the MLR-MRF-VLF pathway to the spinal cord with an additional 1 or 2 synaptic delays within the cord. In order to establish their potential role in the genesis of locomotion it is crucial that the PSPs be demonstrated to be inexorably linked to the initiation of locomotion. While this is difficult without actually recording from identified interneurons and then showing that they are synaptically activated by the MLR and produce PSPs of the appropriate latencies in motoneurons, further evidence may be obtained by procedures which can perturb the process of MLR- evoked locomotion (reversible cooling of the descending pathways, for example). If the PSPs are related to the process of locomotion one would expect that they share a common pathway in the spinal cord and covary with the amplitude of the LDP as locomotion is abolished.

Anatomical tracing studies have shown that the MLR projects bilaterally to the MRF (Garcia-Rill et al., 1983b; Steeves and Jordan, 1984). Unilateral stimulation of the MLR has also been shown to activate cells in the MRF bilaterally (Kettler and Jordan, 1984) as well as reticulospinal cells which descend in the VLF of the spinal cord bilaterally (Garcia-Rill and Skinner, 1987b; Orlovsky, 1970a). Evidence for a crossed spinal influence in the production of locomotion is also available. For example, while acute loss of locomotor function occurs on the same side as a unilateral hemisections (Brown-Sequard syndrome), recovery of locomotor function occurs in the chronic situation in otherwise intact animals (Eidelberg, 1981; Eidelberg et al., 1986; Kamishima, 1978; Sholomenko and Steeves, 1987). Partial locomotor recovery has also been reported following chronic staggered bilateral hemisections (Jane et al., 1964; Kato et al., 1984). While acute hemisections of the spinal cord in cats results in the loss of MLR-evoked locomotion on the side of the lesion (Steeves and Jordan, 1980); and stimulation of the lateral funiculus of the cervical spinal cord produces monopodal locomotion (on the side of stimulation), rhythmic excitation of the contralateral forelimb extensors has also been observed (Yamaguchi, 1986, 1987). In birds, stimulation of the MRF may produce bilateral hindlimb locomotion after acute spinal hemisections (Sholomenko and Steeves, 1987). Unilateral stimulation of the spinal cord may also induce stepping or swimming of the contralateral limbs in a number of other animals (Jacobson and Hollyday, 1982; Lennard and Stein, 1977; Williams et al., 1984). If unilateral stimulation of the MLR produces a bilaterally descending signal and an intraspinal crossover, it is reasonable to expect that this may be detectable in the MLR- evoked PSPs during cooling to abolish locomotion. This would provide further evidence for the hypothesis that these short latency PSPs are related to the production of LDPs during MLR-evoked locomotion.

Research Outline

Experiments were designed to investigate key questions regarding the organization of the pathways descending from the brainstem that can produce locomotion when stimulated. The specific objectives were:

I) to confirm the presence of neuron somata in the MRF and PLS which can produce locomotion when stimulated and to map their distribution in the

brainstem. To accomplish this a technique for the selective activation of cell bodies by the localized injection of small quantities of putative neurotransmitters (Goodchild et al., 1982) was adapted. It was assumed that selective activation of cell bodies in the MRF and PLS by a variety of neuroactive substances would be effective in the initiation of locomotion in the precollicular, postmamillary decerebrate cat, indicating that these sites may serve as relays for descending systems capable of initiating locomotion;

II) to establish a relationship between the brainstem trigeminal system and the pontomedullary locomotor strip by determining whether stimulation of the trigeminal receptive field could produce locomotion after injection of chemical agents into the PLS. As the PLS is anatomically coextensive with the brainstem trigeminal sensory nuclei it was hypothesized that chemical activation of the trigeminal sensory nuclei and subsequent stimulation of the trigeminal peripheral receptive field would be capable of producing and facilitating drug-induced treadmill locomotion, respectively;

III) to determine the descending trajectories of the MLR and PLS. It was hypothesized that the classical mesencephalic locomotor region produces locomotion by a pathway which descends in the ventral funiculus of the spinal cord and does not require the lateral tegmentum of the brainstem (PLS) nor a pathway descending in the dorsal half of the spinal cord. Furthermore it was hypothesized that the descending pathway of the PLS may converge, in part, on the medial reticular formation and produce locomotion by a pathway which descends in the ventral funiculus of the spinal cord. Alternatively, the PLS may produce locomotion by a pathway which requires the continuity of the dorsal funiculus and is not dependant on the integrity of connections to the MRF or pathways within the ventral funiculus. These hypotheses were tested by examining the effect of reversible (cooling) and irreversible lesions of the brainstem and/or the spinal cord on locomotion produced by stimulation of either the MLR or the PLS.

IV) to determine the relationship between the short latency postsynaptic potentials and locomotor drive potentials observed in spinal motoneurons during MLR-evoked locomotion. To examine this relationship a detailed characterization of the PSPs during fictive locomotion was necessary. It was

hypothesized that the transition between depolarization and hyperpolarization of the LDP should always be accompanied by similar rapid amplitude changes in the MLR-evoked PSPs if they are related to the production of locomotion. Changes in overall amplitude of the LDPs should also be correlated to similar changes in the amplitude of the MLR-evoked PSPs. As it has been suggested that MLR-evoked PSPs are produced by the activity of spinal neurons (Shefchyk and Jordan, 1985a), it was hypothesized that stimulation of the MLR should activate spinal neurons at latencies appropriate for the production of MLR-evoked PSPs. It was hypothesized that if the short- latency PSPs are related to MLR-evoked locomotion, a gradual decline in peak amplitude and eventual removal of both the short- latency MLR-evoked PSPs and LDPs should occur concurrently to the loss of MLR-evoked locomotion. Furthermore, it was hypothesized that this would occur during reversible cooling of the MRF or the VLF of the spinal cord. This positive correlation would suggest a common pathway for the production of the short latency PSPs, LDPs, and locomotion and would stress the importance of the MLR- MRF-VLF pathway in the initiation of locomotion. In contrast, cooling of the dorsal half of the spinal cord should have no effect unless the signal responsible for the production of PSPs, LDPs and the initiation of locomotion produced by MLR stimulation travels through the dorsal fasciculus or propriospinal system. Our current understanding of the descending pathway from the MLR suggests that the signal for the initiation of locomotion from unilateral stimulation of the MLR descends bilaterally in the VLF and that a crossover of the signal involved in the production of locomotion may occur at the spinal level (see above). Therefore, cooling of the VLF will be done ipsilateral and contralateral to the sites of MLR stimulation and of intracellular recording to distinguish the relative contribution of a bilaterally descending signal and a spinal crossover to MLR-evoked short-latency PSPs, LDPs, and hindlimb locomotion.

Materials and Methods

The experiments were carried out on cats using two types of preparations. Treadmill locomotor preparations were used to determine a) the location of cell bodies within the brainstem capable of producing locomotion (Series I) and the relationship between the PLS and the trigeminal sensory nuclei (Series II), and b) the descending trajectories of the brainstem sites that can produce locomotion when electrically stimulated (Series III). "Fictive" locomotion experiments were used to characterize the MLR-short latency PSPs and to establish the link between MLR- evoked short latency PSPs, LDPs, and locomotion (Series IV).

Treadmill Locomotion Preparations:

The experiments were carried out on 46 (32 + 14) adult cats weighing 2.0 to 4.0 kg. Animals were initially anesthetized with a mixture of nitrous oxide and halothane. The trachea was intubated, the left common carotid was cannulated, and blood pressure was monitored with a pressure transducer. Each animal was given 4 mg of dexamethasone (Hexadrol phosphate, Organon) intravenously to reduce tissue swelling. The head of each animal was fixed in a stereotaxic headframe with all four limbs free to step on a treadmill belt. The hindquarters were suspended by a sling under the abdomen or by pins attached to the iliac crests. The animals were decerebrated with a precollicular-postmamillary transection of the brainstem and the anesthesia was subsequently terminated. All wound margins were anesthetized with topically applied xylocaine ointment or by small injections of xylocaine hydrochloride. Following a recovery period of approximately 1 - 1.5 hours, the cerebellum and brainstem were exposed by an extended craniotomy.

Series I and II

32 cats were used in order to demonstrate that cells within the projection sites of either the classical (Steeves and Jordan, 1984) or medial (Garcia-Rill et al., 1983b) MLR are capable of producing locomotion and to examine the relationship between the PLS and the trigeminal sensory nuclei. An assembly consisting of an insulated monopolar stimulating electrode (tip diameter 0.1 mm, 0.25 mm exposed tip length) glued alongside a 30 gauge cannula was used for

electrical stimulation and drug injection. After exposure of the cerebellum and brainstem, the junction of the superior and inferior colliculi (AP0), the obex (P14) and the midline (L0) were visualized (coordinates from Berman, 1968). The electrode-cannula assembly was lowered through the cerebellum and stereotaxically positioned in the brainstem. A region extending from P3-P14 and L0-6 was stimulated at a variety of depths (square wave pulse, 0.5 msec duration, 20-30 Hz, 10-275 uA) to induce locomotion on the treadmill. The treadmill belt was maintained at a constant speed of 0.4 m/sec. A "locomotor movement" was considered to be any limb movement which shows a rhythmical alternation between flexion and extension resulting in a characteristic swing and stance phase of the limb on the treadmill. Any limb which illustrates this type of movement is considered to be in the process of locomotion regardless of the presence of locomotor activity in any other limb. Locomotion was scored as quadrupedal, bipedal, monopedal, and so forth on the basis of visual observations using this criterion. Locomotion was monitored in all four limbs by using electromyographic (EMG) electrodes placed into the following muscles: biceps brachii (BB); triceps brachii (TB); tibialis anterior (TA); and, lateral gastrocnemius (LG). The EMGs were amplified and recorded on an 8- track FM analog magnetic tape recorder (bandpass: 10 Hz to 2.25 KHz) for later analysis.

Drugs were infused in those sites where electrical stimulation could produce locomotion consistently. The various drugs dissolved in 0.9% saline and maintained at pH 7.2-7.5 included gamma amino butyric acid (GABA) (0.005-0.01 M), muscimol (MUS) (0.005-0.01 M), picrotoxin (PIC) (0.005-0.01 M), D-L-Homocysteic acid (DL-HCA) (0.1 M), L-glutamic acid (GA) (0.005M- 0.1 M), glutamic acid diethyl ester (GDEE) (0.005-0.1 M), and substance P (SP) (0.74 mM). Concentration ranges were chosen on the basis of previous successful experiments using drug injections for the initiation of locomotion (Garcia-Rill et al., 1985). The excitatory compounds DL-HCA and GA were infused on the assumption that they would produce effects similar to those seen with electrical stimulation, since iontophoresis of these compounds is known to excite cells in both the MRF and PLS regions (Greene and Carpenter, 1985; Henry et al., 1977; Tebecis, 1973). SP has been shown to excite cells within the region of the PLS (Henry et al., 1977) and to induce locomotion after injection into either the MRF or MLR (Garcia-Rill and Skinner, 1987a). PIC was used because it has been shown to be effective in inducing locomotion after infusion into the MLR (Garcia-

Rill et al., 1985). GABA and MUS were used to reverse the actions of PIC while GDEE was used to antagonize the action of GA. The electrode and cannula assembly was removed and rinsed thoroughly with saline between applications of drugs. Drugs were usually infused at a rate of 1 ul/min for 1 to 5 minutes. Flow was controlled by a Model 341 Syringe Pump (Sage Instruments) with a Hamilton 10 or 50 ul syringe attached to the cannula by PE 60 polyethylene tubing. Control injections of 0.9% NaCl at similar infusion parameters were also given in some sites.

In some experiments drugs were infused into sites 1.5 mm lateral or medial to sites producing locomotion with electrical stimulation in order to evaluate the extent of drug diffusion to surrounding tissue. An assessment of drug spread could only be made if locomotion could be produced by a subsequent injection of the same drug into the adjacent "locomotor" region.

The relationship of the PLS to the trigeminal sensory nuclei was examined in 5 experiments. Attempts were made to induce treadmill locomotion before and after drug infusion by stimulation of various parts of the face, neck and chest regions by gentle or brisk rubbing, pinching, or with a calibrated mechanical stimulus (Rowan aesthesiometer RA-100).

The locations of effective stimulus and/or infusion sites were determined at the end of each experiment. In most cases sites were marked electrolytically (2-5 mA for 3 sec) or with a dye injection of 1% Procion Rubine or 10% Fast Green at volumes equal to the drug injection. The locations of effective sites within the brainstem were also estimated stereotaxically after removal of the cerebellum by repositioning the electrodes and noting the depth of the electrode with respect to the floor of the IVth ventricle. At the end of most experiments the brainstem was removed and placed in Lillies' neutral buffered formalin overnight. Frozen sections (30 um) were cut and stained according to the method of Kluver and Barrera (1953).

Ten second periods of full wave rectified and low pass filtered EMG signals were analyzed with a Hewlett Packard 9836 computer at the end of each experiment. The EMG linear envelope signals from all muscles were digitized at 100 Hz and the data stored on floppy disk. The signals were then plotted for visual

inspection. All calculated means (thresholds for electrically induced locomotion, drug infusion volumes, etc.) for series I and II, include standard deviations of the mean.

Series III

Fourteen cats were used in this experimental series. In order to establish the descending trajectories of the MLR and PLS, the ability of electrical stimulation of these sites to produce locomotion was assessed before and after various lesions (reversible or irreversible) of the brainstem or spinal cord. Stimulating electrodes were stereotaxically positioned in the MLR and PLS (and sometimes in the MRF) regions of the brainstem following the decerebration and recovery period. Control bouts of locomotion of 10 to 30 seconds following electrical stimulation of the MLR and PLS were obtained using the stimulation parameters listed in Series I (above). All stimulation parameters were noted as well as the electrical threshold for the initiation of locomotion (determined by slowly increasing the strength of stimulation until locomotion ensues). Locomotion was monitored in all four limbs using electromyographic (EMG) electrodes placed bilaterally in the LG, TA, TB and BB muscles. The EMG signals were amplified and recorded using an 8-track FM analog magnetic tape recorder (bandpass: 10Hz to 2.25 KHz) for later analysis and the channels calibrated with a premeasured pulse.

Reversible lesions of the brainstem were made by cooling a localized area with a coaxial 18 gauge stainless steel probe (Shefchyk et al., 1984) through the inner shaft of which cooled alcohol was circulated. Cooling probe temperatures were monitored by a tissue implantable thermocouple microprobe (Type IT-23, Bailey Instruments) glued to the outside surface of the probe tip. To provide information concerning the extent of cooling, tissue temperatures around the tip of the cooling probe were determined in separate experiments using a thermocouple attached to a hypodermic needle placed at various distances from the probe. Control isotherms produced during cooling of the MRF are illustrated in Fig. 1. Probe tip temperatures were kept at that which will effectively block synaptic (18-20 degrees C) but not axonal (4-10 degrees C) transmission (Brooks, 1983). Probe tip temperatures of 13-15 degrees were sufficient to block synaptic transmission in the brainstem for distances of 1-2 mm from the probe. The

temperature of the region was allowed to return to normal control by stopping the flow of cold alcohol.

In some experiments the cervical spinal cord was exposed by removal of the C2 or C3 vertebral lamina. Reversible lesions of various areas of the cervical spinal cord were obtained by cooling (intradurally) the surface of the spinal cord with a specially designed cooling probe through which cooled alcohol was circulated. Probe tip and tissue temperatures were monitored with a thermocouple as described above. Probe tip temperatures were kept at that which would effectively block axonal (4-10 degrees C) transmission (Brooks, 1983), in the immediately adjacent spinal cord quadrant (see discussion of isotherms produced during spinal cord cooling, below).

Irreversible brainstem and spinal cord (C2-C3) lesions were made using surgical methods. To prevent movement during this procedure, a temporary paralysis was obtained by intravenous injection (0.5-1.0 ml of 20mg/ml) of succinylcholine chloride (Quelicin Chloride) and the animals artificially respirated. The spinal cord was cooled prior to the lesion by application of frozen artificial cerebrospinal fluid to the dorsal and lateral surfaces of the cord. Once neuromuscular transmission was restored (usually 10-20 minutes) the artificial ventilation was discontinued. Intravenous injection of diluted norepinephrine was given only in those cases where the blood pressure dropped below 60 mm Hg.

The location of the brainstem stimulus sites were marked electrolytically (2-5 mA for 3-5 sec) at the end of each experiment. The brainstems were then removed and placed in Lillies' neutral buffered formalin overnight. Frozen sections (30 um) were cut and stained according to the method of Kluver and Barrera (1953).

10 to 20 second periods of full wave rectified and low pass filtered EMG signals were analyzed with a Masscomp MC563 computer at the end of each experiment. The EMG linear envelope signals from all muscles were digitized at 200 Hz using a 1MHz 16 channel analogue to digital (A/D) converter and stored on computer hard disk. The signals were then plotted for visual inspection.

Fictive Locomotion Experiments

The experiments were carried out on 31 adult cats weighing 2.0 to 3.8 kg. The data was obtained from precollicular postmamillary decerebrate cats, similar to a preparation described previously (Jordan et al., 1979). The animals were anesthetized, cannulated, and intubated as described for the treadmill locomotion experiments (see above). In some experiments a constant infusion of 5% glucose - sodium bicarbonate was used to replace fluid loss and help maintain a normal pH balance in the animal. Branches of the sciatic nerves were dissected free bilaterally and mounted (after decerebration) on bipolar stimulating/recording electrodes submerged in a mineral oil bath contained in a specially designed plastic horizontal tray. The nerve branches included anterior biceps (AB), semimembranosus (SM), posterior biceps (PB), semitendinosus (ST), medial gastrocnemius (MG), lateral gastrocnemius-soleus (LG), flexor digitorum longus (FDL), tibialis anterior (TA), and common peroneal (CP). In addition, branches of the femoral nerve were unilaterally dissected free, cut, and nerve cuff electrodes were placed around them for stimulating and recording. These nerve branches included vastus (Vast) (lateralis, intermedialis, and medialis), and sartorius (Sart) (lateralis and medialis). The contralateral femoral nerve was cut because it has been observed that symmetric tonic afferent input to the spinal cord enhances the efficacy of MLR stimulation in producing fictive locomotion. Following a T12 (or T13) and a L4-L7 laminectomy each animal was suspended in a stereotaxic frame with its limbs hanging vertically. A back pool (over the L4 to L7 laminectomy area) was formed, filled with mineral oil, and the temperature maintained at 37 degrees Celsius by a feedback controlled heating lamp. Following the decerebration, termination of the anaesthesia, and a recovery period of about 1-1.5 hours, the animals were paralyzed with gallamine triethiodide (1-2 ml, 8 mg/ml, i.v., Flaxedil, Rhone-Poulenc). The animals were artificially ventilated and the end tidal carbon dioxide maintained between 2.5 and 5.0%. Periodic injections of Flaxedil were used throughout the experiment to keep the animals in a state of flaccid paralysis. In some experiments, an extended craniotomy was performed to expose the cerebellum and brainstem.

Locomotion was initiated by electrical stimulation (0.5-1.0 ms square wave pulses, 10-20 Hz., 30-220 uA) of the MLR as described by Jordan et al. (1979). The rhythmic activities of a L7 ventral root filament and/or one or more nerves

(electroneurogram or ENG) (unilaterally or bilaterally) were used as monitors for locomotion. Since no actual movement occurs, locomotion elicited in this manner is known as **"fictive"** (or fictitious) **locomotion.**

Intracellular recordings from alpha motoneurons in the L6 and L7 spinal cord segments were obtained using single-barreled glass microelectrodes filled with 2 to 3 M potassium citrate (1- 10 Mohms resistance, 1-2 um tip diameter). Cells were identified as motoneurons using antidromic activation from the cut ventral roots or peripheral nerves. In addition, the cells were usually characterized by their pattern of synaptic input from the peripheral nerves. In cases where antidromic or synaptic characterization was not definitive, the motoneurons were classified as extensor- or flexor-coupled by comparing their activity during the fictive step cycle to that of the identified ventral root or the peripheral nerves. Activity of unidentified spinal neurons in the L6 and L7 spinal segments were also recorded from (intracellularly and extracellularly) using potassium citrate microelectrodes. These cells were distinguished from motoneurons on the basis of their high rate of discharge, their repetative response to seperate stimulation of a number of peripheral nerves, their very fast spike rise time and short spike duration, their low capacitance, and their inability to be activated antidromically with peripheral nerve stimulation (Hunt and Kuno, 1959). The cells were classified as extensor- or flexor coupled in a manner similar to that described for motoneurons. Their responses to peripheral nerve stimulation were not sytematically investigated. During each experiment, high gain AC-coupled and low gain DC-coupled intracellular signals were captured along with the cord dorsum potential (negative indicated by upward deflection on cord dorsum traces), ventral root filament activity, ENG activity, and a timing pulse, on an 8-track FM magnetic tape recorder (bandwidth 0-2.25 kHz) or collected directly through 1 MHz 16 channel analogue to digital (A/D) converter and stored on computer (Masscomp MC563) disk. Tape recorded data was subsequently transferred to computer. Capture rates (A/D digitization) were usually at 5-10 kHz for the DC trace, 5 kHz for the high gain AC trace, 10 kHz for the timing pulse, 3.3 kHz for the cord dorsum, 5 KHz for raw ENGs, and 200 Hz for the full wave rectified and low pass filtered ENGs. While the DC trace and ENG activity was collected continuously throughout each locomotion trial, the AC and cord dorsum signals were time-locked to each MLR stimulus and collected for a 30-50 msec period (triggered by each timing pulse). The cord dorsum electrode was

placed ipsilateral and immediately above the intracellular recording electrode site.

Series IV

In order to demonstrate that the MLR stimulus-locked short latency PSPs observed in motoneurons are related to the production of both motoneuron LDPs and locomotion, the activity of single motoneurons recorded intracellularly and groups of motoneurons recorded extracellularly (ENGs) were examined during fictive locomotion produced by stimulation of the classical MLR. The onset of each fictive step cycle was determined (start of the rising phase of the LDP or in some cases as the beginning of activity in the ventral root or ENG). The step cycle was then normalized and divided into equal intervals or bins. The motoneuron membrane potential (Em) levels occurring during the same bin in each normalized step cycle were then averaged together. The averages obtained over 50-100 bins were then displayed with respect to the normalized step cycle. The MLR-triggered AC and cord dorsum recording electrode traces occurring during the same bins of each normalized step cycle were also averaged together. Typically, for these averages, the normalized step cycle was divided into 5-10 bins. The shape of PSPs from a wide variety of motoneurons during MLR locomotion were compared to each other and to LDPs in order to determine their characteristics. A comparison between the short latency PSPs and LDPs observed from the motoneuron was made before, during, and after local cooling of the MRF or spinal cord. Measurements of the MLR stimulus-locked PSPs included latency for arrival of the descending volley at the cord dorsum recording electrode (descending volley latency), latency between arrival of the descending volley to the onset of the PSP (segmental latency), and peak amplitude modulation with respect to step cycle and membrane potential (Em) during locomotion. Measurements of the averaged LDP included amplitude and duration (proportion of step cycle that the LDP occupied) during the normalized step cycle. The presence of locomotion was monitored by unilateral or bilateral ENGs.

The activity of unidentified spinal neurons was also examined during fictive locomotion produced by stimulation of the MLR. The presence of short-latency synaptic activation by stimulation of the MLR was determined by examination of individual or averaged MLR stimulus-triggered responses.

Latencies of activation by stimulation of the MLR were also determined. The location of some cells was estimated by stereotaxic measurements of electrode tip position: spinal segment, position of electrode entry point into the spinal cord (mm from midline), angle of electrode relative to the vertical, and depth within the spinal cord.

Reversible lesions of the brainstem were made by cooling a localized area of the MRF as described above (control isotherms are illustrated in Fig. 1). Cooling to block synaptic transmission was done in regions previously shown to receive projections from the classical MLR (Steeves and Jordan, 1984) and which could either produce or facilitate MLR-evoked locomotion when electrically stimulated. Cooling of the spinal cord was done at the ventral lateral funiculus (unilaterally) or dorsal half of the spinal cord (bilaterally) at the thoracic level (T12 or 13). Reversible lesions were made by cooling the surface of the spinal cord (extradurally) with a specially designed cooling probe as described above. Control isotherm mapping during cooling of the VLF (Fig. 2) indicates that probe tip temperatures approaching 0 to -1 degrees C completely block fiber transmission in the VLF unilaterally and synaptic transmission in the spinal grey bilaterally. Cooling of the VLF (to these same probe tip temperatures) was shown to block the transmission of a signal through the ventral quadrant of the spinal cord (produced by stimulation of the VLF with an insulated monopolar stimulating electrode and recorded from the ventrolateral surface of the spinal cord) (Fig. 2B). Cooling of the VLF to these probe temperatures reduced but did not abolish the transmission of a signal through the dorsal quadrant of the spinal cord (produced by stimulation of the dorsolateral funiculus and recorded from the dorsal surface of the spinal cord) (Fig. 2C). DLF surface temperatures were always 10-12 degrees warmer than cooling probe temperatures (measured in three additional cooling trials). Fig. 2D and E show the changes in probe tip and ventral horn temperature (ipsilateral and contralateral to the cooling probe) during a VLF cooling trial. Plateau temperatures occur approximately 2 minutes into the cooling trials. These control trials indicate that fiber transmission may be blocked in specific areas adjacent to the cooling probe with probe tip temperatures of approximately 0 to -1 degree. Further cooling may result in block of fiber transmission in the ipsilateral DLF and contralateral VLF. In a few cases, cooling of the spinal cord was done intradurally. The probe tip temperatures sufficient for complete blockage of fiber transmission in the adjacent spinal quadrant were

approximately 4-8 degrees C. Examination of Fig. 2A indicates that fiber transmission for a distance of 1-1.5 mm from the cooling probe may be blocked during cooling of the VLF to probe temperatures of 0 to -1 degree. Similarly, blockage of fiber transmission within the dorsal half of the spinal cord would occur during cooling of the dorsal aspect of the spinal cord to similar temperatures (during placement of the cooling probe over the dorsal surface of the cord, the spinal cord 'flattens' and accommodates itself to the shape of the probe). In addition, intraspinal temperatures will be lowered sufficiently to block synaptic transmission throughout the cord region adjacent to the cooling probe. However, dorsal cord cooling will not affect fiber transmission through the ventral funiculi, as this region is too distant from the cooling probe and is adjacent to the the body of the animal which provides a large heat 'sink'.

Cooling of the VLF was done ipsilateral and contralateral to the sites of MLR stimulation and of intracellular recording to distinguish the relative contribution of a bilaterally descending signal in the VLF (Garcia-Rill et al., 1987b; Orlovsky, 1969, 1970a,b; Steeves and Jordan, 1980) to hindlimb locomotion, LDPs and PSPs produced by stimulation of the MLR unilaterally. The role of synaptic transmission through the grey matter (propriospinal neurons) to the production of locomotion, LDPs and PSPs during stimulation of the MLR was tested with a dorsal cord cooling control trial.

Results

Series I and II

Electrically Induced Locomotion

Treadmill locomotion could be produced by electrical stimulation (25-225 uA) of the medial pontomedullary reticular formation and the pontomedullary locomotor strip (Figs. 3,4). The region of the MRF which was capable of electrically induced locomotion extended from P4 to P14 and L0 to L2 and included the FTG, FTM, inferior central raphe nucleus (CI) and postpyramidal nucleus of the raphe (PPR) (Fig. 4). This region is more extensive than the P3-P10 MRF regions previously shown to be capable of producing locomotion when electrically stimulated (Garcia-Rill and Skinner, 1987a; Mori et al., 1978a, 1980b). The effective PLS sites included the lateral tegmental field (FTL) medial and ventral to the spinal nucleus of the fifth nerve as previously described (Mori et al., 1977; Shik and Yagodnitsyn, 1977) but also included the spinal nucleus of the fifth nerve (5SM, 5SP). These effects were obtained in trials throughout the longitudinal extent of the brainstem from P3 to P13.

The brainstem sites where treadmill locomotion was produced by electrical stimulation are illustrated in Fig. 3. The MRF and PLS are separated by areas that were relatively ineffective for producing locomotion. Stimulation of the region bounded by L3.5 to 5.5 (PLS) produced locomotion in 62% (n=184) of the trials, while stimulation of the region bounded by L0 to 1.5 (MRF) was effective in 49% of trials (n=103). In contrast, stimulation of the area between these two regions (L2.0-3.0) evoked locomotion in only 17% of trials (n=41).

The locomotion produced by stimulation of the MRF and PLS was often preceded by increased extensor tonus of the limbs, whereas stimulation of areas 1 to 2 mm dorsal to the effective MRF sites often decreased extensor tonus as described by Mori et al. (1982). Extensor tonus was not evaluated systematically in the present study, however. A significantly higher ($p<0.001$, Students' t-test) threshold for electrically induced locomotion was observed for the MRF (mean=145.0 +/- 53.7 uA; n=26) as compared to the PLS (95.1 +/- 43.7 uA; n=51).

Stimulation of the MRF or PLS produced quadrupedal locomotion in 13.5% of the trials. However, other stepping patterns during locomotion were also observed (Fig. 3A), including: bilateral forelimb (47.4%); ipsilateral (with reference to site of stimulation) forelimb (14.6%); contralateral forelimb (5.9%); bilateral hindlimb (7.0%); ipsilateral hindlimb (1.8%); contralateral hindlimb (0.6%); ipsilateral fore- and hind-limb (4.7%); and bilateral forelimb and ipsilateral hindlimb (2.3%). The remaining 2.2% displayed various combinations of the above categories. A somatotopic organization was not apparent when data from all animals was grouped together: stimulation of a similar site in different animals could produce different stepping patterns during locomotion. However, within the same animal slightly different stepping patterns could be seen with stimulation of adjacent areas within the brainstem.

Drug-Induced Locomotion

Drug infusions were made in sites that could reproducibly elicit locomotion with electrical stimulation. Control saline injections in the MRF and PLS at all rates used for test drug infusions did not produce any observable changes in EMG activity or influence the threshold for electrically induced locomotion.

Extent of Drug Diffusion

To estimate the extent of drug diffusion with injections into the brainstem, procion rubine (1%) and fast green (10%) were infused in volumes similar to the drug infusion trials. Infusions of 3 ul and 5 ul of procion rubine produced detectable spread (total diameter) of 0.5-1 and 1-2 mm, respectively. Infusions of 1 ul and 3 ul of fast green produced spread of 1 and 2 mm (total diameter), respectively. With both dyes an additional 0.5 mm vertical spread (along the cannula tract) was also observed. Additional experiments were conducted to determine whether the diffusion pattern of PIC was comparable to that seen after injections of similar quantities of procion rubine or fast green. A site where electrical stimulation did not produce locomotion, 1.5 mm medial or lateral to a PLS site (see Fig. 8: P7.1 and P12.1), was infused with 3 ul (15 nmol, 0.005 M) PIC. No locomotion was observed after 30 minutes of the PIC injection. In

addition, no change was observed in the threshold for electrically induced locomotion produced by stimulation of the PLS site. Subsequent infusion into the PLS site, however, lowered the threshold for electrically induced locomotion in one case and produced locomotion by itself in the other (Fig. 9). This indicates that for injections of 3 ul PIC, drug diffusion was on the order of $\leq$1.5 mm (total diameter) and thus comparable to the diffusion pattern observed with infusions of 3 ul procion rubine or 1 ul fast green.

MRF Drug Infusions

Infusions of various drugs into the MRF could influence the locomotor capability of the animal by either initiating stepping or by decreasing the threshold for electrically induced locomotion. Glutamic acid was the only compound tested that produced locomotion after infusion into the MRF. Fig. 5A-B illustrates the quadrupedal locomotion produced by electrical stimulation or by injection of 500 nmol GA (5 ul, 0.1 M) into a midline site (PPR and FTM) 2 mm rostral to the obex and 3.8 mm ventral to the floor of the 4th ventricle (Fig. 5E) in one animal. Within 17 minutes of drug infusion the threshold for electrically induced locomotion decreased from 100 uA to 20 uA and muscle tonus increased in forelimb extensors (as determined by passive resistance to limb flexion and EMG activity). Within 30 minutes of drug infusion spontaneous quadrupedal locomotion (Fig. 5B) began in an episodic fashion (1-3 minutes duration) separated by periods of quiescence (0.5-2 minutes). During quiescent periods locomotion could be reestablished by swinging the forelimbs backward and releasing them (Fig. 5C). Although the forelimb and hindlimb flexors (BB,TA) were phasically active during electrical and drug-induced locomotion, the activity of forelimb and hindlimb extensors (TB,LG) was different following GA: the right and left TB became phasically active whereas the phasic activity of the right and left LG ceased. Although efficient uptake systems for GA have been described (cf. Mayer and Westbrook, 1987), episodes of drug-induced locomotion continued to occur for 100 minutes after the infusion, possibly due to the large quantity of GA injected. At this time, infusion of the quisqualate receptor antagonist GDEE (Mayer and Westbrook, 1987) (500 nmol in 5 ul, 0.1 M) into this site decreased the vigour and duration of the GA-induced locomotion within 3 minutes and locomotion completely ceased after 12 minutes. Forelimb swinging was completely ineffective in initiating locomotion during this period (Fig. 5D).

However, the effects of the antagonist were short-lasting, and locomotion began again within 15 minutes of the infusion of GDEE.

In a different animal, infusion of 500 nmol GA (5ul, 0.1M) into the MRF (P9, L0, H-8.3) produced episodic forelimb locomotion after 8 minutes for a period lasting 20 minutes. Muscles demonstrating phasic activity during the control remained phasic following GA. During the period in which episodic GA-induced locomotion was evident, stimuli previously unable to evoke stepping (forelimb swinging and rubbing of the maxillary region of the face) were found to induce locomotion.

Infusions of smaller amounts of GA (40-80 nmol in 8ul, 0.005-0.01 M) into the MRF of 4 other animals were ineffective in producing locomotion in 6 trials. However, a 40-66% decrease in the threshold for electrically induced locomotion in sites 0.5-1 mm lateral to the midline (P7.7 and P10.0, Fig. 8) was observed in two of these trials.

Infusions of DL-HCA in two of four trials (200 nmol in 2 ul, 0.1 M) (2 animals) and PIC in three of six trials (15 nmol in 3 ul, 0.005 M) (6 animals) also reduced the threshold for electrically induced locomotion by 20-27% and 53-61%, respectively. Extensor muscle tonus increased following drug infusion but no stepping movements were produced. After 15-25 minutes electrical thresholds began to return to pre- infusion values. No effect was observed in the remaining trials even when 300-800 nmol DL-HCA or 25-50 nmol PIC was infused.

Injection sites within the MRF are illustrated in Figs. 7 and 8. Effective sites were found from P6 to P12, L0 to L1 1/2 and within the CI, PPR, FTG, FTM in the ventral half of the brainstem.

PLS Drug Infusions

A variety of compounds were capable of either producing locomotion or decreasing the threshold for electrically induced locomotion after infusion into the PLS. Locomotion was produced by infusions of GA, DL-HCA, PIC, and SP into the PLS. Increased extensor muscle tonus was seen (visual observations) prior to the onset of drug-induced locomotion.

Fig. 6A illustrates forelimb locomotion typically produced by electrical stimulation (150 uA) of the PLS. Fig. 6B illustrates the locomotion produced by infusion of 100 nmol GA (in 1 ul, 0.1 M) into the ventromedial aspect of 5 SP at P7 (Fig. 6G) in a different animal. After infusion of 300 nmol into this site, periodic episodes of spontaneous locomotion lasted for 15- 20 minutes. Infusions of 40-80 nmol GA (in 8 ul of 0.005-0.01M) produced 35-67% decreases in the threshold for electrically induced walking (Fig. 8: P7.7). In one case the infusion of 40 nmol was sufficient to maintain locomotion for up to 8 seconds after the termination of electrical stimulation. In contrast, locomotion stopped upon termination of the electrical stimulus in the control situation. The location of this latter infusion site was within 5SM (Fig. 8: P6.0). In 6 other cases, GA (50-80 nmol) was ineffective other than occasionally increasing the discharge of motor units. In an additional case, infusion of 300 nmol GA produced convulsive movements but did not induce locomotion.

Infusion of 700 nmol DL-HCA (in 7 ul, 0.1 M) produced locomotion within 4 minutes of injection into a site 4 mm lateral to the midline (Fig. 6C), but no accurate depth measurement was obtained for this trial. Injections of smaller quantities 300 nmol in 3 ul, 0.1 M) in two other trials were ineffective.

Injections of PIC (0.005-0.01 M) into the PLS produced threshold decreases and eventually spontaneous locomotion in 11 trials (8 cats). Locomotion could be produced after injection of 10-50 nmol (mean = 30.0 +/- 13.8 nmol). The mean infusion volume was 5.5 +/- 2.6 ul. At high concentrations (0.01M) a smaller infusion volume was required to produce locomotion, and in one case locomotion was induced before 1ul (10 nmol) had been infused. The threshold for electrically induced locomotion decreased by 24-40% of the original values in three other trials after injection of 15-30 nmol (mean = 21.7 +/- 7.6 nmol) PIC, but locomotion was not produced by the drug infusion. PIC was without effect in 17 other trials at similar quantities (31.7 +/- 11.0 nmol, range 25-60 nmol). Infusions of PIC produced locomotion within 8.4 +/- 7.1 minutes (range 2-20 minutes).

Fig. 6D illustrates the spontaneous forelimb locomotion produced after infusion of 25 nmol PIC (in 5 ul, 0.005 M) into the PLS at P6 (Fig. 6G). During

the infusion a decrease (from 150 to 50 uA) in the threshold for electrical activation of stepping occurred prior to the drug-induced locomotion. The stepping sequences lasted from 10 seconds to 1.5 minutes and were separated by quiescent periods lasting 1 to 2 minutes. This periodic drug-induced locomotion occurred for about 20 minutes. A second infusion into this site produced an even greater EMG output, but stepping ceased when the animal began to display a tegmental response (Ingram et al., 1932). In other PLS sites, PIC-induced locomotion lasted for periods of up to 60 minutes with injections of 15 nmol (in 3 ul, 0.005 M) PIC and up to 90 minutes with injections of 30 nmol (in 6 ul, 0.005 M). Stepping episodes gradually ceased or were replaced by convulsive movements.

PIC-induced locomotion and electrical stimulation control trials showed the same stepping pattern in 8/11 trials. However, in one experiment electrically induced quadrupedal locomotion was replaced by ipsilateral fore- and hind-limb locomotion and in two other experiments bilateral forelimb and contralateral hindlimb locomotion was replaced by forelimb locomotion when PIC was infused. No consistent differences in step cycle rate and the EMG amplitudes during stepping were observed between electrical and PIC-induced locomotion.

The presence or absence of rhythmic EMG activity was assessed from recordings of locomotion produced by electrical stimulation or PIC injection into the PLS. In 65% of the EMG records, rhythmic activity remained during PIC-induced locomotion. In contrast, loss of activity was observed in 16% of the recorded muscles, and recruitment of phasic activity occurred in 19% of the recorded muscles during PIC-induced locomotion. We found no relationship between injection site and the pattern of loss or gain of phasic muscle activity during drug-induced locomotion.

PIC-induced locomotion was antagonized by infusions of either GABA and MUS. Injections of MUS completely blocked PIC-induced locomotion whereas the effects of GABA were transient. A decrease in the frequency and vigour of PIC-induced locomotion was usually apparent within 5 minutes of infusion of an equivalent (nmol) dose of GABA. As illustrated in Fig. 6E, locomotion induced by forelimb swinging was reduced to a short stepping sequence (2-3 steps) after infusion of GABA. Within 10 to 15 minutes, however,

the frequency, length, and intensity of the locomotor episodes gradually approached their original levels.

Injection of 2 nmol SP (in 3 ul, 0.74 mM) into the PLS (Fig. 8) produced locomotion (Fig. 6F,G) similar to that produced by electrical stimulation of the same site in the two animals tested. Periodic episodes of walking were observed shortly after drug infusion (within 1-2 minutes) and were maintained for periods of up to 30 minutes. During periods with no spontaneous locomotion, the threshold current for producing locomotion decreased from 60 uA (pre-drug value) to 20 uA. Unlike the pre- drug trials, locomotion was also maintained for 5-10 seconds after termination of the electrical stimulus.

Injection sites into the PLS are shown in Figs 7 and 8. Injections producing locomotion or decreases in the threshold for electrically induced locomotion were found from P5 to P13, L3 to L5 and within or just ventral and medial to 5SM, 5SP in the adjacent FTL. Most ineffective sites were located ventrally in the FTL.

Trigeminal Receptive Field-Induced Locomotion

Following PIC infusion into the area of the PLS, mechanical stimuli applied to the receptive field of the trigeminal nerve could induce locomotion. Stimulation of the trigeminal field was attempted prior to drug administration. These stimuli never produced locomotion by themselves. Fig. 9 illustrates examples of these effects found after a 15 nmol (in 3 ul, 0.005 M) injection of PIC in the site illustrated in Fig. 9N. Stimulation of the trigeminal receptive field ipsilateral to the injection site was more effective for initiating locomotion than stimulation of the contralateral side. Stimuli of greater intensity also produced more vigorous locomotion than did gentle stimuli. Ipsilateral mandibular stimulation (D) immediately and invariably induced locomotion whereas a 2 and 5 second latency period was observed for ipsilateral maxillary (E) and pinna (F) stimulation, respectively. Ipsilateral corneal stimulation (G) produced locomotion within 2 seconds in 1 out of 3 trials but locomotion was not maintained for the duration of the stimulus. Similarly, jaw opening (H) produced 4 to 5 steps in 2 of 6 trials. A longer latency (7 seconds) and weaker stepping response was produced with stimulation of the contralateral maxillary region (I). Stimulus sites which failed to produce any locomotion included: contralateral pinna (J), contralateral

cornea (K), nape of neck (L), chest (M) and perianal regions. Injection of 15 nmol PIC (in 3 ul, 0.005M) into a site (which could not produce locomotion when electrically stimulated) 1.5 mm lateral to the PLS (Fig. 9N) was without effect. Infusion sites from 4 other experiments which showed similar locomotion production by trigeminal field stimulation are illustrated by an asterisk in Fig. 8 including a GA infusion site in the MRF. In one experiment (Fig. 8: P9.2, left side) a 14 gm stimulus applied to the ipsilateral pinna induced locomotion but was ineffective when applied to the contralateral pinna.

Series III

As described in the "Introduction" two differing views have emerged concerning the descending trajectories of the MLR and PLS. On the one hand, evidence has been presented which shows that the classical MLR projects through the VLF of the spinal cord via a relay in the MRF (Shefchyk et al., 1984; Steeves and Jordan, 1983). On the other hand, it has been claimed that the MLR relays via the lateral tegmentum (PLS) which then projects through the spinal cord DLF as a polysynaptic propriospinal pathway with the cells of origin located primarily in the dorsal horn (Mori et al., 1977; Kazennikov et al., 1980a,b, 1983a, 1985; Shik, 1983). While the data presented in Series I and II demonstrate that the proposed relay sites (MRF and PLS) contain cell bodies capable of generating locomotion when chemically activated, the anatomical (and functional) relationship between these two areas remain unclear. Experiments using subtotal spinal cord or brainstem lesions were therefore conducted to determine the anatomical trajectories of the MLR and PLS.

The responses to stimulation of either the MLR or the PLS before and after various lesions to either the brainstem or spinal cord were examined in 14 cats. Only those lesions which did not totally abolish the ability of the animal to produce locomotion with brainstem stimulation are reported since it is difficult to determine with certainty whether loss of the capacity to produce locomotion following an irreversible lesion and a recovery period of 1 to 2 hrs is due to the lesion itself or to a general deterioration in the state of health of the animal. This selection of data is designed to remove the possibility of the spontaneous loss in the capacity of a brainstem site to produce locomotion which normally occurs during the course of locomotion experiments. The loss in the capacity of a site to

produce locomotion when stimulated during a reversible lesion (cooling) is acceptable, however.

Spinal Cord Lesions

A number of different spinal cord lesions (C2-C3) were made to determine the spinal trajectories of the MLR and PLS. Two basic results were obtained from acute spinal cord lesions experiments: 1) the MLR does not require the continuity of the dorsal half of the spinal cord; 2) the PLS may also produce locomotion in the absence of the dorsal half of the spinal cord but also shows loss of locomotion with these spinal lesions more often than that seen with MLR stimulation. These results are presented in Figures 10-12.

Figure 10 shows the effect of an extended (8 mm) dorsal hemisection at the C2-C3 level on MLR-evoked locomotion. Following this extensive lesion stimulation of the MLR (either side) could still produce quadrupedal locomotion that was essentially the same as that seen in the control situation. No essential change in the threshold for the production of locomotion by electrical stimulation of the MLR was observed. The effect of this lesion on PLS-evoked locomotion is illustrated in Fig. 11. In this experiment, stimulation of the left or right PLS was capable of producing bouts of hindlimb or forelimb locomotion, respectively. No consistent differences in the threshold for the production of this locomotion was observed. However, in both cases, the locomotion observed following the lesion was bipedal compared to the quadrupedal control locomotion. Interestingly, stimulation of the right PLS following the dorsal hemisection was also capable of producing right hindlimb locomotion but it was not observed simultaneously with the bilateral forelimb locomotion. Some rhythmic activity was also observed in the left triceps brachii muscle with stimulation of the left PLS following the spinal lesion but no locomotor movements were observed in the left forelimb.

Figure 12 summarizes the effect of various spinal cord lesions on locomotion produced by stimulation of either the MLR or PLS. In general, stimulation of the MLR could produce locomotion similar to that seen in the control situation following the disruption of the dorsolateral funiculus, dorsal horns, or the dorsal aspect of the spinal cord by either simple transections or extended lesions (5/7 experiments and 9 tested MLR sites) (A,B,D,F,I). No

consistent differences in the thresholds for the production of locomotion by electrical stimulation of the MLR were observed in these experiments (cf. Fig. 10). Changes in MLR-evoked locomotion following lesions to the dorsal spinal cord were observed only in 3 sites (2/7 experiments) (G,H). In one additional experiment, transection of the VLF ipsilateral to the MLR stimulation site, resulted in the loss of ipsilateral hindlimb locomotion and disrupted flexion-extension alternation in the ipsilateral forelimb and contralateral hindlimb (C).

Forelimb or hindlimb locomotion could also be produced with stimulation of the PLS following hemisection of extended portions of the dorsal aspect of the spinal cord (Fig. 12H,I,J). These results show that PLS stimulation may produce locomotion by pathways travelling in the ventral half of the spinal cord at the C2-C3 level. This was supported by the observation that following a unilateral transection of the VLF (C), stimulation of the ipsilateral PLS (in multiple locations throughout the brainstem) was incapable of producing locomotion (even though stimulation of the ipsilateral MLR could still produce contralateral forelimb locomotion and some ipsilateral forelimb and contralateral hindlimb locomotion). Furthermore, in one additional experiment, intradural cooling of the spinal cord at C2 region (over a length of 1 cm) to temperatures sufficient for the block of fiber transmission in the unilateral VLF (probe temperature of 10 degrees C) reversibly abolished locomotion produced by stimulation of the ipsilateral PLS. On the other hand, cooling of the spinal cord (intradurally) to temperatures which block fiber transmission within the DLF and synaptic transmission throughout the C2 spinal cord level (probe temperatures of -4 degrees C) only decreased the vigour of PLS- evoked locomotion (EMG amplitude and limb displacement). The contribution of the dorsal aspect of the spinal cord to the production of PLS locomotion is more difficult to assess. While lesions of the dorsal cord may have no noticeable effect on PLS locomotion in some cases (Fig. 12A,F,G) the observation that lesions of the dorsal spinal cord affected PLS locomotion (6/9 experiments) more often than MLR locomotion (see above) suggests that the DLF or dorsal horn may mediate the production of locomotion by PLS stimulation in some cases (Fig. 12B,D,E).

Brainstem Lesions

The results of the spinal cord lesion experiments demonstrate that the PLS can produce locomotion (forelimb or hindlimb) via pathways descending in the ventral half of the spinal cord. One candidate for the mediation of this locomotion is the reticulospinal system originating in the MRF. Cells in the MRF have been shown to receive projections from the MLR (Garcia-Rill and Skinner, 1987b; Orlovsky, 1970a; Steeves and Jordan, 1984), abolish MLR-evoked locomotion when cooled (Shefchyk et al., 1984) and produce locomotion when chemically activated (see "MRF Drug Infusions", above). In addition, electrophysiological studies have demonstrated that the caudal PLS may project to the MRF (Selionov and Shik, 1981, 1984).

A total of 5 reversible MRF cooling trials were obtained during PLS-evoked locomotion in one animal. Figure 13 demonstrates that cooling of the MRF to temperatures sufficient for the block of synaptic transmission (probe temperature of 14.7 degrees C) reversibly abolished PLS-evoked quadrupedal locomotion. Cooling of this same site also reversibly abolished MLR locomotion at temperatures similar to that described previously (Shefchyk et al., 1984). The PLS site (D) was located 3-3.5 mm lateral to the midline, in a region slightly more medial and ventral to ones which could produce locomotion with injection of PIC or SP (Fig. 8) and may therefore comprise part of the medially directed projection of the PLS on its course to the reticular formation (Selionov and Shik, 1981, 1984).

The spinal cord lesion and brainstem cooling experiments indicate that PLS stimulation activates reticulospinal pathways projecting through the ventral aspect of the spinal cord, but do not prove that the PLS may produce locomotion via a projection that is independent of the medial reticular formation (Kazennikov et al., 1979, 1983a,b; Shik, 1983). Furthermore the results do not demonstrate that the MLR can produce locomotion independently of the PLS. While evidence has been presented which demonstrates that cooling of the PLS to temperatures which block synaptic transmission do not abolish MLR-evoked locomotion (Shefchyk et al., 1984), it is possible that locomotion produced by MLR stimulation may have occurred via a fiber projection which bypassed the cooled PLS area. These questions may be addressed by experiments which isolate the lateral tegmentum from both the MLR and more medial brainstem areas (MRF).

The effects of isolation of the lateral tegmentum of the brainstem on locomotion produced by stimulation of the MLR and the PLS was assessed in two experiments. The results from one of these experiments is illustrated in Fig. 14. Unilateral separation of the lateral tegmentum at the level of the pons and medulla (P 4-12) did not abolish the capacity of the MLR to produce quadrupedal locomotion when electrically stimulated. Furthermore, stimulation of PLS within the lateral tegmental area (P 9.0) was still capable of producing locomotion in the absence of direct connections to the MRF region implicated in the production of locomotion. While increases in the electrical threshold for the initiation of locomotion were observed for both the MLR and PLS sites they were not unreasonable considering the extensive brainstem lesion performed (the change in MLR threshold is not outside the normal variation seen during prolonged experimental procedures). In the second experiment, surgical isolation of the lateral tegmentum also did not abolish the ability of the ipsilateral MLR to produce quadrupedal locomotion when stimulated, although the electrical threshold for the initiation of the hindlimbs increased from 115-170 uA. Stimulation of the PLS within the isolated tegmental region was ineffective in initiating locomotion following the lesion. However, the lesion also destroyed the main blood supply to the lateral tegmental area and any loss of locomotor capability could have been due to the death of this region and not to the interruption of any descending pathways from the PLS.

Series IV

Short-latency MLR-evoked PSPs

As described in the "Introduction", it has been suggested that the short-latency PSPs produced in spinal motoneurons by stimulation of the MLR are involved in the production of motoneuron LDPs during fictive locomotion (Shefchyk and Jordan, 1985a). Furthermore, it has been suggested that the MLR-evoked PSPs are produced by a pathway with a minimum of two synaptic relays: one in the MRF and one on interneurons in the spinal cord (Shefchyk and Jordan, 1985a). The results of the spinal cord and brainstem lesion experiments described in the previous "Results" section are consistent with this concept and rule out the possibility that a polysynaptic propriospinal pathway is involved in the mediation of MLR-evoked locomotion (cf. Shik, 1983). However, to establish the

role of the short latency MLR-evoked PSPs in the generation of LDPs and locomotion, further characterization is necessary.

Shapes

The shapes of short-latency PSPs produced by stimulation of the classical MLR were examined in 69 motoneurons (16 SMAB, 12 LGs-MG, 1 Vast, 6 PBST, 5 FDL, 19 unidentified extensor motoneurons, 7 TA, and 3 unidentified flexor motoneurons). Table 1 summarizes the distribution of PSP types (observed within a 30 msec triggered period) with respect to motoneuron type and side of MLR stimulation during locomotion. The short-latency stimulus- locked PSPs were classified into 4 groups: excitatory only (E) (single or multiple) (Fig. 16F, 18C, 19A,E); excitatory followed by similarly sized inhibitory PSP and possibly by a longer latency excitatory PSP [EI(E)] (Fig. 17D, 18H, 20G); small excitatory-large inhibitory PSP followed possibly by another excitatory PSP [eI(E)] (Fig. 20B, 23C); and inhibitory possibly followed by an excitatory PSP [I(E)]. Table I and II indicate that the E and EI(E) PSPs are the 2 most common types of short- latency PSPs produced by stimulation of the MLR, comprising 33.3 and 42.7% of the observed types of PSPs, respectively. In contrast, the eI(E) and I(E) types were observed much less frequently (14.7 and 9.3%, respectively). While a total of 69 cells were sampled, the responses to stimulation of more than one MLR site were recorded in some cells (see below). These cells are indicated in Table I (common superscript indicate same cell).

In flexor motoneurons (TA and unidentified) the short- latency MLR-evoked PSPs are predominantly excitatory in nature (63.6%) although longer latency inhibitory PSPs [EI(E),I(E)] were also found in 36.4% of the flexor motoneurons (see Fig. 18H). While the majority of short-latency MLR-evoked PSPs in extensor motoneurons (bifunctional motoneurons groups were considered as extensors when their activity patterns were predominantly or entirely extensor-related) are excitatory and possibly followed by similarly sized inhibitory or excitatory PSPs [E=28.1%; EI(E)=45.3%], some also show short-latency PSPs which are predominantly inhibitory in nature [eI(E)=17.2%; I(E)=9.4%]. A discussion of these latter types is given below.

Table II also groups the percentages of PSP type seen in flexor and extensor motoneurons with stimulation of either the ipsilateral (i) or contralateral (c) MLR. In extensor (and bifunctional extensor-related) motoneurons, stimulation of the iMLR produced a slightly lower percentage of E or EI(E) PSP types and a higher percentage of eI or I(E) PSP types than that seen with cMLR stimulation. A comparison is slightly more difficult to make for the flexor motoneuron group as the total number sampled for each side is low.

Latency

While determination of the total latency for the production of MLR-evoked PSPs is relatively simple, the determination of the descending volley and segmental latency is much more difficult. An estimate may be obtained, however, by examination of the spinal potentials produced following MLR stimulation with cord dorsum recording electrodes. It must be stressed at this point that the information content of the cord dorsum potential recording is not known with certainty. While an initial descending volley is clearly seen in many cases, at other times the volley is not well differentiated from the rising phase of the first negative wave. While blocking of locomotion by cooling of the spinal cord always affects the size of the first negative wave of the cord dorsum recording, the initial volley is also affected in some cases. Thus, as the first volley may be related to the production of locomotion, latency measurements were made from its onset.

Figure 15E,F illustrates an example of the measuring technique used to determine the descending volley latency and the segmental latency for the first EPSP in a TA motoneuron. The descending volley latency was determined by measuring the time between stimulus artifact (line 1) and the arrival of the first descending volley at the cord dorsum recording electrode (line 2). The segmental latency was determined by measuring the time between the arrival of the descending volley at the cord dorsum recording electrode (line 2) and the onset of the first EPSP (line 3).

Table III summarizes the MLR-evoked PSP latency measurements obtained from all motoneurons. The mean latency from stimulus artifact to cord dorsum (CD) produced by stimulation of 39 different MLR sites (both iMLR and cMLR) was 4.0 +/- 0.6 msec (range 2.9 - 5.2 msec). Assuming a 1 msec delay for

activation of reticulospinal cells in the brainstem medial reticular formation (Orlovsky, 1969) and a distance of 32 +/- 2 cm (measured in 7 cats) from the MLR to the lumbar cord dorsum, an average conduction velocity of 103.3 m/sec is obtained for the fastest signals descending to the lumbar cord dorsum recording electrode by reticulospinal cells activated by the MLR (assuming a maximum caudal distance of the reticulospinal cells of approximately 1 cm from the MLR). The latency for arrival of the descending volley at the cord dorsum recording electrode was not significantly different (parametric unpaired T-test) for stimulation of the iMLR (4.1 +/- 0.6 msec) compared with stimulation of the cMLR (3.9 +/- 0.6 msec) when data from all cats was grouped together. When the latencies for arrival of the descending volley at the cord dorsum recording electrode for iMLR or cMLR stimulation were compared in the same animal (n=3), no essential differences were noted: the latency for iMLR and cMLR was equal in 1 experiment, the cMLR latency was shorter in 1 experiment, and the iMLR latency was shorter in 1 experiment. In any particular experiment, differing latencies were also observed for stimulation of different MLR sites on the same side of the brainstem. Measured from 11 experiments (and from 27 different unilateral MLR sites) the latencies for the arrival of the descending volley at the cord dorsum recording electrode could vary by as much as 1.3 msec. While the latency for arrival at the cord dorsum of the descending volley produced by stimulation of any particular MLR site (for unperturbed locomotion trials) usually remained constant during intracellular recording from the same cell, this latency could change slightly over prolonged periods of stimulation of the same MLR site throughout an experiment. For this reason, the latency measurements from each MLR site tabulated for Table III were obtained from the earliest (and most consistent) unperturbed locomotion trials during each experiment.

As seen in Table III, the mean segmental latency to the first EPSP for all motoneurons (stimulation of either iMLR or cMLR) was 2.3 +/- 0.7 msec (range: 0.9-3.0 msec). This indicates that one (Shefchyk and Jordan, 1984) or more spinal interneurons may be involved in the relay of this excitatory input to spinal motoneurons during MLR-evoked locomotion. The segmental latency was significantly ($p<0.04$) longer (0.4 msec) for activation of extensor motoneurons during stimulation of the cMLR as opposed to that seen with iMLR stimulation. This significance was maintained when the latencies of cMLR and iMLR stimulation for all motoneurons (extensors plus flexors) were compared. No

significance difference in the excitation of flexor motoneurons was seen when the segmental latencies obtained with stimulation of the iMLR and cMLR were compared. Similarly, the segmental latency for activation of flexor motoneurons compared to extensor motoneurons (produced by stimulation of the iMLR or cMLR) was not significantly different ($p<0.1$). However, the flexor motoneuron sample size was small.

The total latency to the first MLR-evoked EPSP for all motoneurons averaged 6.5 +/- 0.8 msec (range 4.1 - 8.6 msec). Unlike that seen with segmental latency measurements, no significant difference in the total latency was found between iMLR or cMLR stimulation. This may have been due to the added variability for the arrival of the descending volley to the cord dorsum recording electrode. Similarly, no significant difference was found between activation of flexor or extensor motoneurons (for stimulation of either iMLR or cMLR) although a longer latency for activation of flexor motoneurons approached the $p<0.08$ level of confidence.

Short-latency MLR stimulus-locked IPSPs were characteristically observed in many motoneurons. As the IPSP follows MLR-evoked EPSPs in most cases, it is difficult to establish the onset of the IPSP with any certainty. Thus, latency measurements were made to the onset of membrane hyperpolarization following each MLR stimulus: hyperpolarization onset was defined as the point of intersection between the Em baseline and the slope of the IPSP. (It is recognized that this method overestimates the onset of the MLR-evoked IPSP). The mean latency from cord dorsum to the first measurable hyperpolarization for all motoneurons (stimulation of either iMLR or cMLR) was 4.4 +/- 1.4 msec (range 2.2 - 8.7 msec) (see Table III). The mean latency from stimulus artifact to the onset of the first measurable hyperpolarization for all motoneurons (stimulation of either iMLR or cMLR) was 8.5 +/- 1.3 msec (range 6.5 - 12.5 msec). The segmental and total latency for the onset of the hyperpolarization (in all motoneurons grouped together) was significantly longer ($p<0.01$) than that seen for the EPSP (for iMLR and cMLR compared separately or together). These results indicate that additional spinal interneurons (interposed between the MLR and spinal motoneuron) may be required to produce the MLR stimulus-locked IPSPs than are required for the production of stimulus-locked EPSPs. A significantly ($p<0.01$) longer segmental (1.2 msec) and total latency (0.9 msec) for

the onset of the first MLR-evoked hyperpolarization was also observed for stimulation of the cMLR when compared to that seen with iMLR stimulation.

PSP Characteristics During Locomotion

PSP and LDP Amplitude Modulation in the E or EI(E) Type

Figures 15 and 16 show typical examples of PSPs [E, EI(E)] produced by stimulation of the MLR. As the motoneuron goes through its characteristic depolarization and hyperpolarization (LDP) during locomotion produced by stimulation of the MLR (Fig. 15B-C, 16B-C), the PSPs change in amplitude. As shown in Fig. 15D and 16E the short-latency EPSPs are largest during the depolarized phase of the LDP and occur with nearly every stimulus. The slightly longer latency IPSPs occur primarily during the hyperpolarized phase of the LDP. Averages of these responses over a number of step cycles are shown in Fig. 15F-G and 16D,F,H. Comparison of the averaged PSP amplitude measurements (Fig. 15G, 16F,H) to the LDP averages (Fig. 15C, 16C) illustrates the strong correlation between the MLR-evoked EPSPs and IPSPs to the depolarized and hyperpolarized phase of the LDP, respectively. In these examples, the mean amplitudes of the EPSPs were observed to vary throughout the step cycle between 1-5 mV (Fig. 15G) and 0.3-0.9 mV (Fig. 16F). In addition, the amplitude of the EPSPs were found to sometimes vary in size with respect to their occurrence during the depolarized phase of the LDP: individual EPSPs could be of slightly different size although they were evoked at similar Em levels during the LDP (Fig. 15D, 16E). Despite these minor changes in amplitude throughout the LDP, the overall amplitudes of the EPSPs were positively correlated (larger) to membrane depolarization (Fig. 15H, 16G). The mean IPSP amplitudes were found to vary from 0 to -1.2 mV during the step cycle, being largest during the hyperpolarized phase of the LDP (Fig. 16H). The IPSPs also showed a positive correlation (larger) to membrane hyperpolarization (Fig. 16H). These results indicate that the amplitude of the stimulus-locked EPSPs and IPSPs are not simply determined by changes in the Em (Eccles, 1968) during the LDP.

In many cells the EPSPs were significantly reduced in size but not abolished during the hyperpolarized phase of the LDP (Fig. 15F,G; 16D,F). In other cells, the EPSPs were only present in the depolarized phase of the LDP

(Fig. 17C). The same feature applies for MLR-evoked IPSPs: in some cells, IPSPs were only apparent during the hyperpolarized phase of the LDP (Fig. 17H), while in others, they were present during the depolarized phase of the LDP but at significantly reduced amplitude to that seen during the hyperpolarized phase (Fig. 16D).

The peak (mean) EPSP amplitude in the E subtype during MLR- evoked locomotion ranged from 0.5-7.2 mV. In all cases (25/25), the EPSP was significantly modulated during the step cycle being maximal during the depolarized phase of the LDP and thus positively correlated with Em depolarization. Similar results were seen in 27/28 cells of the EI(E) type. In one cell, the EPSP was not significantly modulated throughout the step cycle and not significantly correlated to Em depolarization or hyperpolarization. The peak (mean) EPSP amplitude in the EI(E) subtype ranged from 0.5-4.0 mV. In two measured cases, long latency EPSPs (visible within the 30 msec triggered window) were found to be significantly modulated during the step cycle and positively correlated to Em depolarization. The peak amplitude of these longer latency EPSPs ranged from 3.2 and 3.5 mV. During the step cycle, peak (mean) IPSP amplitudes were found to vary between -0.5 and -4.2 mV, being largest during the hyperpolarized phase of the LDP and thus positively correlated to Em hyperpolarization (21/23). In the remaining two cells, no significant step cycle modulation or Em dependence of the IPSP was observed.

The transition between depolarization and hyperpolarization of the LDP is accompanied by rapid amplitude changes in the EPSPs and IPSPs (at the same part of the normalized step cycle), respectively. These similar cycle-related amplitude transitions for MLR-evoked LDPs and PSPs indicate that the PSPs are strongly related to the duration of each phase of the LDP. This feature is apparent for a TA motoneuron in Figure 17 for locomotion produced by stimulation of the MLR site indicated in E. Higher strength stimulation (F-I) of the MLR produced a slightly shorter step cycle duration, a prolonged depolarized phase of the LDP, and a prolongation of the occurrence of the short-latency EPSP (H) compared to that seen with lower strengths of stimulation (A-D).

A relationship between the amplitude of the LDPs and EPSPs during stimulation of the same MLR site but at differing strengths of stimulation was also

apparent in Fig 17. Higher strengths of stimulation produced larger amplitude LDPs and larger amplitude EPSPs than that seen with lower stimulation strengths (compare panel B with G and panel D with I). Increasing the strength of MLR stimulation (same site) during locomotion increased the size of the LDP and short-latency EPSPs in 3/3 cells examined [with E or EI(E) PSPs]. In two cells with the eI(E) type, increased strength of stimulation of the same MLR site also increased the size of the short-latency EPSP (see below). In 2 additional cells, stimulation of the MLR at strengths subthreshold for locomotion did not produce LDPs nor any large short-latency EPSPs. However, increasing the MLR stimulation to strengths suprathreshold for the production of locomotion resulted in the appearance of LDPs and large, well modulated EPSPs.

Effects of Different MLR Stimulation Sites

While the PSP modulation paralleled the LDP duration during the normalized step cycle, a link between the amplitude of the EPSPs and the LDPs was not as apparent during stimulation of different MLR sites. Fig. 18 shows locomotion produced by stimulation of a more caudal MLR site (E) for the same cell that is illustrated in Fig. 17. A shorter step cycle length was observed for the trial of locomotion illustrated in Fig. 18. While the averaged LDP amplitude was smaller for stimulation of this caudal MLR site (Fig. 18), the EPSPs were larger in amplitude than those seen with stimulation of the rostrally located MLR site (compare Fig. 17C,H with 18C). However, a longer latency IPSP (in the hyperpolarized phase of the LDP) (Fig. 18C: arrow) was also present during stimulation of the caudal MLR site. The fact that a different descending volley and segmental latency was observed (an overall 1 msec shorter latency), indicates that different brainstem and spinal interneurons may have been activated during stimulation of this more caudal MLR site. Thus, comparisons of PSP and LDP amplitude changes with variation in stimulation strength are valid only with stimulation of the same MLR site.

Figures 17 and 18 thus illustrate a general finding: stimulation of different MLR sites may produce PSPs of varying shape and/or latency in the same motoneuron. Of the 8 tested motoneurons, differences in latency were observed in 7 of them and differences in the shape of the PSPs (segregated on the basis of PSP type) were observed in 4. Two additional examples of this site dependency are

shown in Figs. 19 and 20. In Fig. 19, slight differences in the descending volley and segmental latencies between the two sites are seen resulting in a 0.3 msec longer total latency (MLR stimulus artifact to onset of the first EPSP) for the contralateral MLR EPSP. While contralateral MLR stimulation produces single EPSPs in this TA motoneuron (A), stimulation of the ipsilateral MLR results in multiple EPSPs (D). In Fig. 20, stimulation of the ipsilateral (E) or contralateral (J) MLR produced e(I) and EI PSPs, respectively. While LDPs were observed in this SM motoneuron during stimulation of the ipsilateral MLR, the amplitude of the IPSPs were largest during the depolarized phase of the LDP (possibly determined by Em) and the amplitude of the EPSPs were too small and variable to show any significant modulation during the step cycle (see next section below). In contrast, stimulation of the contralateral MLR (J) produced well modulated EPSPs and IPSPs which were largest during the depolarized and hyperpolarized phase of the LDP, respectively.

eI(E) and I(E) PSPs - A Special Case?

The fact that stimulus-locked PSPs of the eI(E) type may be observed in the same cell during locomotion produced by stimulation of one MLR site and not necessarily observed by stimulation of another site (Fig. 20) leads one to conclude that this PSP type is not necessarily due to poor health of the motoneuron. In fact, many of the cells illustrating this PSP type were found to have good resting Em potentials. The observation that the modulation of the PSPs may not reflect modulated excitatory or inhibitory drive but rather postsynaptic membrane properties (Em) (Fig. 20D) necessitates a more detailed examination of this PSP type if one is to determine whether these PSPs are related to locomotion, or not.

As seen from Table II, the eI(E) and I(E) type make up a small percentage of the total stimulus-locked PSP types observed in motoneurons with stimulation of the MLR (14.7 and 9.3%, respectively). The averaged peak amplitudes of the first excitatory PSP in the eI(E) type are generally very small (0.2- 0.6 mV). However, this small averaged amplitude measurement may not just reflect the production of a small EPSP with every stimulus delivered to the MLR. As seen in Fig. 20A (*), large EPSPs were seen following some MLR stimuli but not in others. A lowered response index (measure of the one-to-one correspondence of stimulus to response), indicating a failure to produce a

response with every stimulus or a greater variability than that seen with the E or EI(E) type, may result in a small and highly variable averaged EPSP. As a result, the early (averaged) EPSP was not significantly modulated in amplitude during the normalized step cycle in 90.9% of the cases (10/11). A positive correlation between the amplitude of the first EPSP on the strength of MLR stimulation was also observed for two cells. In contrast, the IPSP of this type showed significant modulation throughout the normalized step cycle in 81.8% of the cases (9/11). However, in 6 of the 9 significantly modulated IPSPs, the IPSPs were larger during the depolarized phase of the LDP and appeared to be simply determined by Em during the LDP. These IPSPs were generally very large: averaged peak amplitudes ranged from -2 to -12.8 mV. In 3 of the 9 cases, significantly modulated IPSPs were larger during the hyperpolarized phase of the LDP and thus possibly related to the production of the hyperpolarized phase of the LDP. These IPSPs were small: -1.0 to -1.6 mV (averaged peak amplitude). It is unclear from these observations whether or not locomotor-related inhibitory input is present (but masked by another non-locomotor related inhibitory input) in those cases where the amplitude of the IPSPs appear to simply follow changes in the Em (Eccles, 1957). A long-latency EPSP was observed in the 30 msec AC trace window in two cases. In both cases, this EPSP (peak amplitude: 0.8-1.8 mV) was significantly modulated during the normalized step cycle and largest during the depolarized phase of the LDP.

Fig. 21 illustrates two additional features concerning the short-latency eI(E) PSP type. Panel A demonstrates that in many instances, spike initiation would occur on the MLR-evoked short- latency EPSP (see section on MLR-evoked spiking, below). The substantially large EPSPs, which were generating the action potentials, were not included in the PSP averages illustrated in C. Coupled with this selective removal of large EPSPs and a low response index, the averaged EPSPs showed small amplitudes and very small modulation (with high variability) during the step cycle (D). This figure also shows that long latency EPSPs outside of the 30 msec AC trace window were capable of producing locomotor-related spiking in this cell. Of the 98 action potentials observed in this SM motoneuron during this trial of locomotion, 7 were produced by the short-latency EPSP and 29 were generated by the long-latency EPSP. Panel C (bottom trace) also shows (at least in this case) that the short-latency EPSP was not a due to an extracellular field potential.

The short-latency IPSPs in the I(E) type were significantly modulated during the normalized step cycle in only 42.9% of the cases (3/7 different MLR trials in 6 different cells). Of these, 2 of the 3 were largest during the depolarized phase of the LDP and may have simply been determined by changes in Em). These IPSPs were very large (-4.0 mV averaged peak amplitudes). Only 1 of the 3 modulated IPSPs were largest during the hyperpolarized phase of the LDP (-1.0 mV averaged peak amplitude) indicating that it may be related to the production of locomotion. During locomotion trials in 4 of the 6 cells (which showed either IPSPs which were largest during the depolarized phase of the LDP or non-significantly modulated IPSPs during the step cycle) locomotion was not evident in recordings from ENGs on the ipsilateral side. Although the cells possessed LDPs, the normal excitatory and inhibitory drives upon motoneurons (Jordan, 1983) present during well-formed locomotion may not have been optimal or at least well synchronized to the delivery of the MLR stimulus. In one cell, a long latency EPSP of 0.6 mV (peak amplitude) was observed within the 30 msec triggered AC trace window. It was significantly modulated during the step cycle and largest during the depolarized phase of of the LDP. In this cell, spontaneous (non-stimulus triggered) firing and LDP amplitude modulation was very pronounced.

MLR-evoked Spiking and the Issue of Reciprocity

During fictive locomotion produced by stimulation of the MLR, two basic firing patterns (intracellular recording of single motoneurons or extracellular recording of ENGs) were observed. This is best illustrated in Fig. 22 for intracellular and extracellular recordings obtained from different experiments. Comparison of the position of the action potentials to the delivery of each MLR stimulus for the SMAB motoneuron plotted in A shows that the motoneuron spiking does not (for the most part) occur at any fixed latency past the MLR stimulus. Thus, the firing is not dependent upon any particular EPSP produced by the MLR stimulus and may, therefore, be termed stimulus-independent firing. This type of firing is consistent with the idea that with this form of MLR-evoked locomotion, stimulation of the MLR is insufficient in synchronizing the firing of the spinal interneurons interposed between the MLR and the spinal motoneuron. Even though stimulus-locked EPSPs may be observed in the motoneurons they may not be large enough in amplitude to elicit action potentials. In contrast, the

spiking produced in the TA motoneuron illustrated in panel B occurs on the rising phase of an EPSP at a fixed latency from the MLR stimulus. Thus, the firing is timed to the MLR stimulus and may be termed stimulus-locked or stimulus-dependent firing. This type of MLR-evoked locomotion is consistent with the idea that the MLR stimulus is sufficiently strong to result in the synchronous firing of the spinal interneurons interposed between the MLR and the spinal motoneurons. In this case, the interneurons produce fixed-latency EPSPs of sufficient amplitude to result in the production of action potentials in the spinal motoneurons. Examples of stimulus-independent and -dependent spiking (and an intermediate stage between) recorded from a large number of motoneurons (ENGs) can be seen in Fig. 22C-E. The synchronization of the motoneuronal pool to the MLR stimulus is indicated by the appearance of bursts of activity in D and E. Just as increasing the strength of stimulation to a particular MLR site will result in larger EPSPs and LDPs in a particular motoneuron (see above), increasing the strength of stimulation to the MLR may also result in greater stimulus-dependent spiking.

The fact that stimulation of the MLR may produce stimulus- dependent firing which is modulated (phasic) during locomotion may provide clues concerning the organization of the cells within the spinal cord which may be related to the generation of locomotion. This is illustrated in Fig. 23 for recordings from two unidentified extensor motoneurons and various ipsilateral agonist and antagonist ENGs. It is clear in these examples that stimulus-locked spiking (ENGs) and PSPs (intracellular records) are well developed and that this activity is highly modulated during the step cycle. For the extensor motoneuron shown in A-C, stimulus-locked EIE PSPs are present during the hyperpolarized phase of the LDP (C). Taking into consideration the time that would be required for conduction of the large unit action potential (bottom trace) to reach the TA ENG recording electrode, the activity in this single unit in an antagonist ENG may be strongly correlated to production of either the stimulus-locked EPSP or IPSP. Evidence to suggest that activation of the TA single unit is related to the appearance of the IPSP, rather than the EPSP, comes from the observation that other TA units (B) show activity when only IPSPs are present in the extensor motoneuron. In addition, in other step cycles (not illustrated) activity in the large TA unit was always associated with the presence of an IPSP and independent of the presence of any EPSP in the extensor motoneuron.

Figure 23D-F illustrates the high correlation of stimulus- locked PSPs and spiking in a group of ipsilateral agonists during locomotion produced by stimulation of the MLR. Examination of the latencies of onset for each of the events shown in F and taking into consideration the time required for conduction of the action potentials to the SM ENG and the even more distally located MG ENG, indicate that these extensors are probably activated at a similar latency during this trial of locomotion. The similar activation of this group of agonists further relates the stimulus-locked EPSPs to the process of locomotion.

The behaviour of MLR-evoked PSPs during locomotion presented in the above results sections indicate that they are highly related to the production of LDPs and locomotion. Indeed, the reciprocal organization between intracellularly recorded MLR- evoked IPSPs during the hyperpolarized phase and the spiking of antagonist motor units and the common excitation of agonist motor units (Fig. 23) suggests that the MLR-evoked PSPs are somehow related to the activity of the spinal neurons generating the locomotion. While the demonstration of a reciprocal organization between antagonists during the locomotor step cycle does not necessitate a causal relationship between the two events this interpretation is greatly strengthened in light of the evidence presented in Fig. 24. In this example of MLR-evoked fictive locomotion, the hyperpolarized phase of the LDP appears to spontaneously 'drop out' during the locomotor step cycle. This phenomenon occurred following an extensive condition-test stimulation paradigm between the MLR and the medial reticular formation. This 'drop out' does not simply reflect a prolonged depolarized phase of the LDP. Rather, examination of the spiking pattern of the MG motoneuron indicates that the cell still receives a rhythmic excitatory drive which is separated by a period of reduced or abolished spiking. Examination of the bottom trace reveals that in the reduction or absence of rhythmic inhibition to the MG motoneuron, rhythmic excitatory drive to the TA ENG (antagonist at the same joint) is likewise reduced or abolished. This strongly suggests that the the MLR-evoked IPSP observed during the hyperpolarized phase of the LDP in the motoneuron in Fig. 23 and the spiking in the antagonist ENG are causally related and strengthens the idea that the MLR-evoked PSPs are not unrelated phenomena which are merely coincidental with the generation of LDPs and locomotion.

Spinal Neurons and Their Activation from the MLR

The segmental latency results presented in Table III indicate that the excitation and inhibition of spinal motoneurons from stimulation of the MLR is accomplished by the activation of at least 1 (and possibly more) interposed spinal interneurons. This conclusion is supported by recordings made from spinal neurons located in the same lumbar spinal segments from which motoneuron recordings were obtained (cf. Baev et al., 1979; Feldman and Orlovsky, 1975; McCrea et al., 1980; Orlovsky and Feldman, 1972; Pratt and Jordan, 1987). Recent work using the transneuronal labelling technique (Jankowska, 1985) has demonstrated that many of the last-order spinal interneurons which are active during the process of locomotion project to spinal motoneurons located in the same lumbar segments (Noga et al., 1987b).

The activity of 23 spinal neurons in the L6-L7 spinal segments was examined during locomotion produced by stimulation of the MLR. Many of these cells were synaptically activated or inhibited by stimulation of various peripheral nerves (sural, saphenous, tibial, vastus, etc.) although no systematic attempt was made to identify these cells on the basis of their activation properties from peripheral stimulation. MLR-stimulus-evoked responses were measured in 13 cells. Average latencies for activation of these cells by stimulation of the MLR ranged from 5.5 to 8.1 msec. These latencies are appropriate for the role of these spinal neurons in mediating MLR-evoked PSPs in motoneurons found in the same lumbar segments. The responses for some cells were highly variable (one cell was not synaptically driven by MLR stimulation at 1 Hz although it was driven at 20 Hz stimulation). The location within the spinal cord for some of the spinal neurons is shown in Fig. 27D.

All recorded cells were rhythmically active during locomotion produced by stimulation of the MLR. An example of the activity of a spinal neuron from the rostral L7 spinal segment is illustrated in Fig. 25. This cell was rhythmically active and out of phase with the activity of the ipsilateral TA ENG. As seen in the raw record and the averaged record in B and C, respectively, this cell was activated from the MLR after a 6.6 msec latency. The time course of excitation in this extensor-related cell was nearly identical to that seen for excitation of an LG motoneuron in the same experiment (6.7 msec), indicating that the two cells may

have been receiving excitation from common interneurons (segmental latency for activation of either cell was calculated as approximately 1.8 msec). With termination of the MLR stimulus, this extensor-related neuron (like one other recorded from in this experiment) showed an after-discharge which was inhibited by stimulation of the ipsilateral sural nerve (not illustrated). This indicates that this cell may have been related to the inhibition of flexor activity during locomotion produced by stimulation of the MLR.

Further evidence which relates the activity of these spinal neurons to the process of locomotion is shown in Fig 26. As illustrated in A, stimulation of the MLR produced rhythmic activity in a flexor-related interneuron. Stimulation of the MLR also produced a short-latency response (mean latency of 8.1 msec). In addition, this cell responded to stimulation of the ipsilateral FDL, sural (Fig. 26A3) and posterior tibial nerves and to stimulation of the contralateral sural and superficial peroneal nerves (not illustrated). Long-lasting discharges to stimulation of some of these nerves was also observed. An interesting feature of the cell illustrated in panel B was that it showed long bursts of activity (separated by relatively less active periods) when a train of 4 stimuli were delivered to the MLR (this was in addition to the rhythmicity which could be induced with tonic 20 Hz stimulation of the MLR). When this stimulation paradigm (train of 4 stimuli to the MLR) was given in a repetitive fashion (every second), locomotor-like activity was observed. C illustrates that tonic stimulation of the MLR (20 Hz, 200 uA, 1 msec duration) can produce tonic activation of a flexor-related spinal neuron during the interburst period of the step cycle (total latency for the stimulus-locked spike was 6.7 +/- 1.3 msec). Thus the presence of small stimulus-locked EPSPs during the hyperpolarized phase of the LDP (cf Fig. 15) in many motoneurons may be the result of the maintained (but reduced) activity of some spinal neurons during their interburst period. This is consistent with the idea that the large EPSPs present during the depolarized phase of the LDP are produced by the same spinal interneurons that produce the smaller EPSPs during the hyperpolarized phase of the LDP.

Brainstem and Spinal Cord Cooling During Fictive Locomotion

The descending pathways mediating the production of short- latency MLR-evoked PSPs and the relationship of these PSPs to the production of LDPs

and locomotion were investigated by reversible cooling of selective quadrants of the spinal cord or specific regions of the brainstem. As shown in Fig. 1, temperatures required to block synaptic transmission could be achieved by cooling specific regions of the brainstem. Likewise, temperatures required to block nerve fiber transmission in the ventrolateral funiculus (VLF) of the spinal cord could be achieved by cooling of the VLF (Fig. 2). These temperatures were also shown to be cool enough to block synaptic transmission, bilaterally, and reduce (but not completely block) fiber transmission through the ipsilateral dorsolateral funiculus at the level of cooling. Temperatures sufficient to block fiber transmission in the dorsal half of the spinal cord (and synaptic transmission, throughout) could also be attained by dorsal cord cooling.

Cooling of the Brainstem During MLR-Evoked Locomotion

Cooling of the MRF was performed in areas that either facilitated MLR-evoked locomotion (same experiment) during concurrent stimulation or in areas that could produce locomotion when stimulated (cf. Fig. 4), that have been shown to receive projections from the MLR (Steeves and Jordan, 1984) and that abolish MLR-evoked treadmill locomotion when cooled (Shefchyk et al., 1984). Cooling was performed during intracellular recording from 4 motoneurons (3 experiments). A total of 6 cooling trials were obtained. Cooling of the MRF to probe temperatures of 9-14 degrees C could either abolish (2 trials from different cells) or significantly reduce (remaining trials) MLR-evoked fictive locomotion as measured by LDPs and ENG recording. These effects were reversed upon rewarm of the MRF. In 3 cells exhibiting MLR- evoked EPSPs without the presence of spiking, the EPSP was decreased in amplitude concurrent to the reduction in LDP amplitude (5 trials) and were lost in the same trial in which locomotion was abolished (1 trial). In one cell MLR-evoked PSP data was not obtainable as the motoneuron spiked throughout the cooling trial.

Reversible loss of MLR-evoked locomotion with cooling of the MRF for a SMAB motoneuron is illustrated in Fig. 27. Locomotion was abolished (C) at cooling probe temperatures of 11 degrees C. At an intermittent probe temperature (B) (13 degrees C), a temporary decrease in the step cycle length and concurrent reduction in the number of motoneuronal spikes/burst (decrease from 25.4 +/- 6.6 to 7.4 +/- 1.0) was observed. ENG amplitudes were also reduced

during this trial of locomotion. Upon MRF rewarm, the number of spikes/burst approached control levels (D). It should be noted that the step cycle length increased in the other five MRF cooling trials.

Concurrent decrease in the amplitude of the LDP and MLR- evoked PSPs in an FDL motoneuron during cooling of the MRF is illustrated in Fig. 28. A decreased ENG amplitude and an increased step cycle length was also observed during cooling of the MRF (B). These changes were reversed upon rewarm of the MRF. As shown in D-F, the amplitude of the MLR-evoked EPSP was reversibly decreased during cooling and rewarming of the MRF. Only small amplitude EPSPs and IPSPs remained following cooling. At probe temperatures of 12.5 degrees, the PSP amplitudes were so decreased that modulation during the step cycle were insignificant. With rewarming of the MRF, the locomotion was more vigourous (larger amplitudes of the LDP and ENGs). Concurrently, larger amplitude EPSPs than that seen in the precool control trial were observed. Throughout the cooling trial, no change in the descending volley latency was found.

The hyperpolarized phase of the LDP was sometimes observed to 'drop out' during cooling of the MRF. This is illustrated in Fig. 29 for an LG motoneuron. Concurrent to the loss of the hyperpolarized phase of the LDP, the rhythmic excitatory output of the antagonist motoneuron pool across the same joint (TA ENG) was abolished. Rhythmic excitation of the LG motoneuron during this prolonged depolarized phase of the LDP was still present since the cell still showed small Em oscillations. In addition, the VR filament (extensor agonist) was rhythmically active. It should be noted during this loss of rhythmic inhibition, the interburst period of the VR filament was reduced in duration compared to that observed during the presence of the hyperpolarized phase of the LDP. This reciprocal organization of excitation and inhibition between antagonists at the same joint was similar to that seen following an extensive stimulation paradigm of the MLR and the MRF in a different experiment (Fig. 24).

As the cells in the MRF region capable of generating locomotion when chemically stimulated are widely distributed (Fig. 7), complete abolition of MLR-evoked locomotion will depend upon the placement (and subsequent area of synaptic block) of the cooling probe within the MRF region. The state of vascular

perfusion surrounding the cooling probe may also determine the temperatures required for abolition of MLR-evoked locomotion during cooling of the MRF. This would be expected to fluctuate with time after placement of the cooling probe into the brainstem. This was observed in multiple cooling trials of the same MRF site in one experiment: probe tip temperatures sufficient to abolish MLR-evoked locomotion increased with prolonged placement of the cooling probe in the same site.

Cooling of the Spinal Cord During MLR-Evoked Locomotion

Reversible cooling of selected regions of the spinal cord (T12 region) during MLR stimulation was found to be an effective method by which one could investigate the location of descending pathways required to produce locomotion. In general, cooling of the VLF was found to be an effective method to block locomotion as monitored by extracellular and intracellular recordings. In contrast, cooling of the dorsal half of the cord (blocking synaptic transmission throughout the cooled T12 spinal cord segment) never abolished locomotion and only rarely affected the amplitude of the ENGs or LDPs.

ENG Recordings

An example of the effect of cooling of the VLF on MLR-evoked locomotion is illustrated in Fig. 30. Locomotion was monitored bilaterally with ENG electrodes. Cooling of the right VLF abolished locomotion on the right side of the animal and reduced the amplitude of the ENG activity on the left side. In this case, the rhythmic activity of the L MG was also lost. In 25 extradural VLF cooling trials in which ENG activity was monitored, MLR- evoked locomotion (as monitored by the amplitude of the ENG recordings) began to decrease at temperatures around 12-5 degrees and was abolished at temperatures of 4.3 +/- 3.2 (range 10-0 degrees). (Temperatures required for the removal of ENG phasic activity during intradural VLF cooling were slightly higher: 7.2 +/- 1.6 (range 9-6 degrees). In many cases, extensor activity was abolished slightly sooner than flexor activity on the same side.

The effects of cooling of the VLF on ENG activity during MLR-evoked locomotion suggest that the signal for the production of locomotion descends bilaterally in the VLF of the spinal cord. Regardless of the side of stimulation or the side of VLF cooling, locomotion as monitored by the ENG recording electrodes was always abolished on the side of cooling. However, in some cases, locomotion observed with ENG recording electrodes was also abolished on the contralateral side during unilateral cooling of the VLF. However, it is not clear from ENG recordings alone whether or not the reduced or abolished activity in the hindlimb nerves contralateral to the cooling site is the result of the removal of a crossed descending signal on the cooled side. It should be noted that in some cases the loss or reduction of contralateral locomotion with unilateral VLF cooling may depend upon the functional integrity of the pathways involved in the production of locomotion on that side. For example, in one experiment cooling of the left VLF abolished right-sided (and left-sided) locomotion produced by stimulation of the left MLR. However, stimulation of the right MLR was only capable of producing left-sided locomotion implying that transmission in the right side of the spinal cord was insufficient for the production of locomotion on that side. The observation that locomotion on both sides was abolished simultaneously during cooling of the left VLF indicates that the generation of the locomotion was related to a common signal descending in the left side of the spinal cord and does not support the idea that the loss of locomotion was the result of the block of fiber transmission in the contralateral side of the spinal cord. The idea that the loss of locomotion was a result of the block of synaptic transmission at the cooling site is not supported by the observations that cooling of the dorsal part of the spinal cord to temperatures that block synaptic transmission throughout the spinal cord cooling area never abolished MLR-evoked locomotion (see below).

Intracellular Recordings

A total of 40 (32 VLF and 8 dorsal cord) reversible spinal cord cooling trials during MLR-evoked locomotion were obtained during intracellular recording from 21 lumbar motoneurons. From these cooling trials, an analysis of the relationship between the LDP and the MLR-evoked PSPs was completed. During cooling of the VLF, the latency for arrival of the descending volley at the cord dorsum recording electrode was usually increased by 0.3 msec but rarely more (range 0-0.5 msec). On some occasions, no change was observed. During

cooling of the VLF, the loss of locomotion as monitored with ENG recordings was accompanied by a reduction in the size of the LDP. Concurrent to the reduction in size of the LDP, the amplitude of the MLR-evoked EPSPs and the amount of amplitude modulation seen during the normalized step cycle were decreased. The complete abolition of the LDP was dependent upon the side of the spinal cord which was cooled and evidence for a crossed spinal pathway capable of generating LDPs (and PSPs) was obtained. In most cases, the complete loss of the LDP was associated with the complete loss of MLR-evoked EPSPs although residual IPSPs were sometimes seen. In cases where LDPs were not abolished, modulated MLR-evoked PSPs remained. Cooling of the dorsal part of the spinal cord did not abolish MLR-evoked locomotion, LDPs or MLR-evoked PSPs.

Of the 32 VLF cooling trials completed, LDPs were completely abolished in 22 of the attempts. Probe temperatures required for the complete block of LDPs with extradural cooling of the VLF was 2.8 +/- 3.2 degrees (range 9 to -2 degrees). Probe temperatures required for complete block of the LDP with intradural cooling of the VLF was 7.0 +/- 1.4 degrees (range 8 to 6 degrees). In 19 of the trials in which LDPs were abolished, residual PSPs were found. In cases where EPSPs remained (9), the amplitude of the EPSPs were reduced (compared to the peak size in the control depolarized phase of the LDP) 47-96% and in all cases were equal or smaller in amplitude than that seen during the hyperpolarized phase of the control LDP. In 14 cooling trials, IPSPs were not abolished completely with the loss of LDPs. In 10 of these trials the residual IPSP was reduced in size (38-86%) from that seen in the control LDP (hyperpolarized phase). However, in 3 trials (1 cell) the residual IPSPs were as large as that seen during the control locomotion trials. In the 22 trials in which LDPs were abolished, MLR-evoked EPSPs were completely abolished in 8 of the cases and IPSPs in two of the cases. Thus with the loss of the LDP during cooling of the VLF, the greatest effect on MLR-evoked PSPs seems to be the removal or reduction in their amplitude. While IPSPs may be completely abolished with cooling of the VLF in some cases, they are more often than not, left as residual PSPs with the loss of MLR-evoked LDPs. While a reduction in IPSP amplitude is often seen with cooling of the VLF, some residual IPSPs are as large as that seen in the control locomotion trials.

Complete removal of the LDP and short-latency MLR-evoked EPSPs with cooling of the VLF of the spinal cord is shown in Figs. 31 and 32. In Fig. 31, cooling of the left (ipsilateral) VLF to probe temperatures of 1.7 degrees abolished left MLR- evoked locomotion (LDPs and ENG rhythmic activity). In addition, the modulated, MLR-evoked EPSPs seen during control trials of locomotion (middle and lower panels in A) were completely lost with cooling of the VLF (lower panel in B). This effect was reversed with rewarming of the VLF (C). It should also be pointed out that with lower MLR stimulation strengths in the rewarm locomotion trial, smaller amplitude LDPs and short-latency EPSPs (with a smaller amplitude of modulation) were observed. In this example, cooling of the VLF simultaneously blocked locomotion and MLR-evoked EPSPs.

Figure 32 also demonstrates the loss (B) of the MLR-evoked LDP and short-latency EPSP (A) in a left LG motoneuron, with cooling of the left VLF. In this cooling trial, the LDP was abolished at probe temperatures of 2 degrees. The ipsilateral TA ENG showed some phasic modulation (with reduced interburst inhibition) to temperatures of 0 degrees. At 0 to -1 degrees (B), only tonic excitation of the TA ENG remained. No LDPs were observed in the LG motoneuron. At this temperature, the phasically modulated MLR-evoked EPSP (which was largest during the depolarized phase of the LDP) was absent. However, a longer- latency IPSP remained. This IPSP was of similar segmental latency as that seen in the control hyperpolarized phase (latency determined from the control traces which showed the smallest EPSP: time of onset determined as the point of intersection of the slope of the IPSP with the baseline Em). However, because of the presence of a shorter latency EPSP in the control it was difficult to determine the latency of onset of the IPSP to an accuracy of less than 1 synaptic delay. It is thus impossible to determine whether the residual IPSP was the result of activation of the same interneurons producing the IPSP in the control situation or the result of the activation by a newly revealed contralateral pathway. The amplitude of the residual IPSP in Fig. 32 is plotted in A (bottom panel) which also shows the amplitude of the MLR-evoked control IPSP with respect to Em. It can be seen that the amplitude of this IPSP approximates the IPSP amplitude seen during the hyperpolarized phase of the LDP (most negative Em levels) and at Em potentials that are even slightly more depolarized. This IPSP is also larger in size than that seen at similar Em levels in the control locomotion trial. [It is interesting that in this same cell, cooling of the contralateral VLF was also conducted with

similar results: locomotion (ENG modulation and LDPs) was abolished, the short-latency MLR-evoked EPSP was greatly reduced (but not abolished) and an IPSP with similar amplitudes as that seen during the hyperpolarized phase of the LDP, left residual. In addition, cooling of the dorsal cord was found to have no significant effect on the MLR-evoked locomotion or PSPs over that seen in the control trials.]

While cooling of the ipsilateral VLF blocked the MLR-evoked short-latency EPSP in the LG motoneuron, it did not abolish the tonic excitation of the L TA (as seen by the maintained MLR- evoked excitation in the ENG recording). In fact, aside from the slight change in descending volley latency (0.3 msec longer) caused by cooling of the spinal cord, no change was noticed in the latency for MLR-evoked excitation of the TA ENG (10.5 msec in control) after cooling of the ipsilateral VLF (determined by spike triggered averaging of the raw TA signal).

Another interesting feature linking the excitation of extensors and the inhibition of flexors during MLR-evoked locomotion is revealed in Fig. 32. During rewarm of the ipsilateral VLF and the reestablishment of MLR-evoked locomotion, the depolarized phase of the LDP occasionally failed to occur (Fig 32C). Concurrent to the loss of excitation to the LG motoneuron, a reduced inhibitory input to the ipsilateral TA during the step cycle was seen. This was evidenced as a reduced interburst period (maintained low level excitation) between the higher amplitude excitatory bursts. Upon complete rewarm of the ipsilateral VLF, normal locomotion (ENG modulation and LDPs) was restored. In addition, appropriately modulated MLR-evoked PSPs were seen. This reciprocity is similar to that seen during MRF cooling (Fig. 29) except that the depolarized phase rather than the hyperpolarized phase of the LDP and the inhibition rather than the excitation of the antagonist failed to occur.

Cooling of the VLF during MLR-evoked locomotion also revealed a composite nature to the IPSP observed in the MLR- evoked eI PSP type. This was observed in 2 of the 3 motoneurons with this PSP type that were tested. As seen in Fig. 33A2, stimulation of the MLR produced eI PSPs in this SM motoneuron. The IPSPs were largest during the depolarized phase of the LDP (3) and thus positively correlated to membrane depolarization (4). Cooling of the VLF decreased the amplitude of the LDPs, EPSPs, and IPSPs (B). No significant

modulation or Em dependence of the MLR-evoked IPSP was seen. However, upon rewarm (C) of the VLF the IPSPs were found to be significantly modulated during the step cycle, being largest during the hyperpolarized phase (3). In addition, the IPSPs were positively correlated with membrane hyperpolarization (4). This complete reversal in the behaviour of the IPSPs during MLR-evoked locomotion and following VLF cooling indicates that in the control situation there are either two different (except for latency) components to the IPSP or that there may be a failure to inhibit the interneurons responsible for the production of the IPSPs during the depolarizing phase of the LDP. No causal connection to either suggestion is possible from these experiments.

While cooling of the spinal cord to temperatures sufficient for the block of fiber transmission in the ipsilateral VLF always blocked the rhythmic activity of the ENGs on the side of cooling it did not always block the LDPs in the intracellularly recorded motoneurons. Of the total 32 VLF cooling trials, 10 trials failed to completely abolish the LDPs (all trials at equivalent probe temperatures). Of the 12 trials during which locomotion was produced by stimulation of the contralateral MLR (to the side of motoneuron recording) cooling of the ipsilateral VLF failed to abolish LDPs in 6 of the trials (50%). Residual LDPs were left less frequently with other combinations of MLR stimulation and VLF cooling: 40% (2/5) with ipsilateral MLR and contralateral VLF; 16.7% (2/12) with ipsilateral MLR and VLF; and 0% (0/3) with contralateral MLR and VLF.

In all cases in which LDPs were not abolished with cooling of the VLF, MLR-evoked PSPs were present. Concurrent to decreases in LDP amplitude of 50-83% were significant reductions in PSP amplitudes. As a result of significant decreases in the amplitudes of the MLR-evoked PSPs (42-71%), the amplitude modulations of the PSPs were not always significantly modulated during the normalized step cycle.

Evidence for the suggestion that pathways descending from the opposite side of the spinal cord may contribute to the production of LDPs and MLR-evoked PSPs is presented in Figs. 34 and 35. Fig. 34 illustrates the responses of a left TA motoneuron to stimulation of the right MLR before and after cooling of the left VLF. Cooling of the VLF (1 to 0 degrees) reduced but did not abolish the

LDP in this motoneuron (A). In addition, a longer latency (1 msec) MLR-evoked EPSP was revealed (C). Examination of the cord dorsum recordings in B shows the portion of the potential that was lost during cooling of the VLF. No change in the descending volley latency can be detected.

Further evidence that a crossed spinal pathway may contribute to the production of locomotion, LDPs and MLR-evoked PSPs in motoneurons is illustrated in Fig. 35. In this unidentified motoneuron (which received IPSPs from stimulation of the nerve to Vast), stimulation of the left MLR produced EIE PSPs (A). Cooling of the right VLF decreased the amplitude of ENG modulation and LDPs on the left side but did not completely abolish locomotion (B). In addition, a longer-latency EPSP was abolished with cooling of the contralateral VLF. Upon rewarm of the cord (C), the longer latency EPSP and LDP was reestablished. As illustrated in D, no effect was observed on LDPs or MLR-evoked PSPs with cooling of the dorsal part of the spinal cord to temperatures of 0 to -2 degrees.

A positive correlation between LDP amplitude and the amount of MLR-evoked PSP modulation was observed during VLF cooling trials to block locomotion (Fig. 36). PSP modulation was determined in those motoneurons which showed simple E or distinct EI PSP types. The degree of modulation was determined by the difference between the maximum and minimum PSP amplitude. In cases of EI PSPs, the amplitude modulations of the excitatory and inhibitory components were added together to obtain an estimate of MLR-excitatory and inhibitory input (synaptic drive) onto the motoneuron during the LDP. The results plotted in Fig 36, indicate that PSP modulation increases with larger LDPs.

Discussion

The MLR-Reticulospinal Pathway

Cellular Activation of Locomotion and the MRF

The results of this study demonstrate that cell bodies in the pontine and medullary medial reticular formation are capable of producing treadmill locomotion. The location of the cell bodies in the lower brainstem which are capable of initiating locomotion correspond to the MLR fiber terminations reported previously (Steeves and Jordan, 1984) and to the site where cooling to block synapse transmission reversibly abolished locomotion produced by electrical stimulation of the classical MLR (Shefchyk et al., 1984). The present study extends the MRF region in the cat previously shown to be capable of generating locomotion when electrically stimulated (Garcia-Rill and Skinner, 1987a; Mori et al., 1978a, 1980b) and shows that an area from the midline to L2.0 can be stimulated electrically to induce locomotion. This confirms the previous finding by Mori et al. (1978a) that midline stimulation is effective for production of locomotion, but is in contrast to the report by Garcia-Rill and Skinner (1987a) who found that midline stimulation did not produce locomotion. The results of the present study are consistent with the concept that midline stimulation can produce locomotion by activating descending MLR fibers which have been observed to cross through and terminate within the raphe nuclei in the pons and medulla (Steeves and Jordan, 1984). The fact that the electrical threshold for the initiation of locomotion by stimulation of the MRF is higher than that observed for the MLR (Mori et al., 1980b) or PLS (this study) suggests that a larger volume of tissue must be stimulated to produce locomotion, possibly due to a more diffuse distribution of neurons within the reticular formation. Slight differences in the effective MRF sites and in the relative electrical thresholds for MRF-evoked locomotion (compared to the MLR) reported in this study and by Garcia-Rill and Skinner (1987a) may simply reflect differences in the type of stimulating electrode used and the consequent volume of tissue activated with each pulse.

The variability in stepping patterns observed with MRF stimulation suggests that there is a somatotopic organization for the initiation of locomotion within the reticular formation. Although no somatotopic organization was

apparent when data between animals was grouped together, electrical stimulation of adjacent areas in individual animals could produce different stepping patterns during locomotion. A somatotopic organization for individual muscles may also be present. The fact that some drug-induced locomotion trials showed the loss of phasic activity in specific muscle groups implies that the cell bodies which activate those muscle groups are outside of the infusion area and that their activity in the electrically induced locomotor trials were the result of activation of passing fibers. Similarly, recruitment of phasic EMG responses implies spread of the drug to cell bodies outside of the electrical stimulation site. Similar site-specific effects were observed by Ross and Sinnamon (1984) for electrical stimulation of the medulla in rats and by Garcia-Rill and Skinner (1987a) in cats. A locomotor somatotopic organization within the MRF is supported by observations that some reticulospinal neurons only project to cervical spinal cord levels (Peterson et al., 1975) and that the firing patterns of reticulospinal cells may be linked to the activity of individual muscles from particular limbs during locomotion (Drew and Rossignol, 1984). Other factors, such as the excitability levels of the brainstem and spinal cord, may affect the ability of the brainstem to produce locomotion when stimulated (Mori et al., 1977, 1982). How this affects the recruitment of individual limbs or muscles during controlled locomotion is unclear.

Stimulation of the MRF by either electrical or chemical means produced postural changes prior to the onset of locomotion. Our data indicate that the postural changes occur in those monitored extensor muscles that are rhythmically active during electrically or drug-induced locomotion. For example, after infusion of GA into the MRF an increase in EMG activity prior to stepping was found in the forelimb extensors which were rhythmically active during locomotion. In contrast, the hindlimb extensors were neither tonically nor phasically active after infusion of GA. Electrical stimulation of this area produced postural and locomotor activity in the hindlimb extensors, possibly by activation of the fibers of cells located more rostrally in the brainstem. This has been suggested to explain the effects of ventral tegmental field stimulation described by Mori and co-workers (1978a, 1980b), which produces increased hindlimb extensor activity and locomotion when electrically stimulated. Postural adjustments prior to the onset of locomotion have previously been reported during stimulation of the MLR (Garcia-Rill et al., 1985; Mori et al., 1980a) and its reticular formation projection sites (Mori et al., 1978a, 1980b, 1982). Thus, while the MLR relays its descending

command signal for locomotion, it may also influence parallel postural systems also located in the MRF. The degree of functional dependence or independence of these systems remains to be determined.

Although the present study was not intended to be an exhaustive neuropharmacological examination of the transmitters involved in the descending locomotor pathways, it does contribute to our understanding of them. It demonstrates that injection of GA into the MRF was effective in the production of locomotion. Infusion of either DL-HCA or PIC only produced decreases in the electrical threshold for the initiation of locomotion from these same sites. From these results we suggest that reticulospinal cells related to the production of locomotion possess receptors for excitatory amino acids and may only be partially under the control of a GABAergic inhibitory input. (Alternatively, receptors for excitatory amino acids may be located on the presynaptic terminals of pathways terminating on reticulospinal cells). Similar results have been reported in previous studies on cats (Garcia-Rill and Skinner, 1987a) and stingrays (Livingston, 1986). This is in agreement with iontophoretic studies on medial reticulospinal neurons (Greene and Carpenter, 1985; Tebecis, 1973). These results are different from those obtained with drug infusions into the MLR of cats, where PIC is effective in producing locomotion but GA is not (Garcia-Rill et al., 1985). However, infusions of GA into the MLR of intact rats has recently been reported to substantially increase the locomotor activity in the freely-moving animal (Brudzynski et al., 1986).

The identity of the neurotransmitter(s) released by the MLR at the MRF relay is still unknown. Garcia-Rill and Skinner (1987a; Garcia-Rill et al., 1987) suggest that a cholinergic and/or SP input from the MLR to the reticular formation may act to trigger locomotor events. However, recent studies using both the fictive (Noga et al., 1987a) and treadmill locomotor preparations (Noga et al., unpublished) demonstrate that no significant alteration of the electrical threshold for MLR-evoked locomotion is seen following the intravenous administration of the acetylcholine antagonists mecamylamine and atropine. The fact that few cells within the classical MLR contain SP (Leger et al., 1983) is also in contrast to this proposal. Thus, fibers from the MLR locomotor region contain other neurotransmitters which are involved in the transmission of the signal for initiation of locomotion through relays with the brainstem. The release of an

excitatory amino acid at the MRF relay site may be inferred from the present results, but more evidence is needed to confirm this.

MLR Descending Pathway

The results of the present study demonstrate that extended spinal dorsal hemisections or isolation of the lateral tegmentum caudal to the MLR does not abolish MLR-evoked locomotion. The results also demonstrate that cooling of the MRF can abolish MLR- evoked locomotion and thus confirm the results obtained by Shefchyk et al. (1984). Furthermore, lesions of the VLF produce deficits in locomotion produced by stimulation of the MLR. The importance of the VLF for the mediation of MLR-evoked locomotion has been reported previously (Steeves and Jordan, 1980). The results thus provide evidence for the hypothesis that the classical MLR produces locomotion by activation of reticulospinal cells in the MRF which descend in the VLF of the spinal cord (Steeves and Jordan, 1980, 1984). In addition, these findings support the idea proposed by Orlovsky (1970a) that the mediating link for the initiation of locomotion is the reticulospinal system. The reticulospinal system also has been shown to be important in the production of locomotion in many animals (including man) subject to incomplete spinal cord lesions (Afelt, 1974; Afelt et al., 1975; Eidelberg, 1980, 1981; Eidelberg et al., 1981a,b; Livingston, 1986; Sholomenko and Steeves, 1987). In addition, lesions in the MRF areas in otherwise intact animals produce profound deficits in posture and locomotion (Zemlan et al., 1983).

The results of the present study demonstrate that forelimb locomotion can be produced in the absence of the DLF or dorsal half of the spinal cord (extended lesion) with stimulation of the MLR. Furthermore, lesions which disturb the VLF of the spinal cord can abolish ipsilateral hindlimb locomotion and reduce the quality of forelimb locomotion ipsilateral to the lesion. This is in contrast to the conclusions reached by Yamaguchi (1986), who stated that pathways within the DLF may mediate some of the locomotion produced by stimulation of the MLR. However, as indicated in the "Introduction", the results were based on experiments in which the VLF was not completely transected. In addition, these results were consistent with the suggestions that destruction of the DLF, in the presence of incomplete transection of the VLF, is without any effect on MLR-evoked forelimb locomotion on the same side as the lesion or that there is a

crossed component in the spinal cord for pathways descending from the MLR. The results obtained in the present study support the suggestion that stimulation of the MLR produces quadrupedal locomotion by pathways which descend in the VLF (Steeves and Jordan, 1980) and that there is a crossed spinal component for pathways descending from the MLR (see later).

The results also demonstrate that the MLR does not require a pathway projecting through the lateral tegmentum of the brainstem or the dorsal half of the spinal cord as has been previously suggested (Mori et al., 1977, 1978b; Kazennikov et al., 1980a, 1983a; Shik, 1983; Shik and Yagodnitsyn, 1978). The fact that no consistent changes in the electrical threshold for MLR-evoked locomotion were observed following the spinal or brainstem lesions suggest that little (if any) of the signal producing locomotion is transmitted through lateral brainstem pathways with stimulation of the MLR. As summarized in the "Introduction", the evidence suggesting that the MLR projects to the spinal cord via the PLS-DLF pathway is based primarily on electrophysiological data which is only suggestive and not conclusive. While a region capable of producing locomotion appears to continue caudally into the lateral tegmentum from the MLR (Mori et al., 1977), it would appear that this continuity does not reflect the major descending projection from the MLR. Evidence has been provided that a region medial to the MLR (mMLR) also capable of producing locomotion when stimulated projects through the lateral tegmentum (Garcia-Rill et al., 1983b; Shefchyk et al., 1984). Thus, the results reported by Mori et al. (1977) may be due to stimulation of fibers projecting from the mMLR sites to the PLS region which would overlap the medially directed projections (to the MRF) of the classical MLR region (Steeves and Jordan, 1984). This explanation could also account for the results obtained by Shik and Yagodnitsyn (1978) who demonstrated that stimulation of areas caudal to the MLR could activate cells within the PLS. Alternatively, some cells in the classical MLR region may project to the lateral tegmental region in a function not necessary for the production of MLR-evoked locomotion. Recently, Skinner et al. (1984) have shown that some cells in the region of the classical MLR may be labelled by injections of tracer substances into the region of the PLS. The demonstrated convergence between the MLR and PLS (stimulation of the PLS facilitates locomotion produced by stimulation of the MLR) has also been used as evidence for a projection from the MLR to the PLS (Mori et al., 1978a). However, cooling of the MRF can abolish locomotion produced by stimulation of the PLS

(or MLR). Thus the convergence may simply reflect the fact that locomotion produced by stimulation of the PLS may be mediated by a projection to the MRF as demonstrated electrophysiologically (Selionov and Shik, 1981) (see "PLS Descending Pathways" below, for further discussion). The fact that the MLR and PLS converge onto propriospinal cells in the C2-3 spinal segments has also been used as evidence for the suggestion that the MLR projects via the PLS (Kazennikov et al, 1983a). The fact that removal of these cells by extended dorsal hemisections does not abolish MLR-evoked locomotion (nor affect the electrical threshold) demonstrates that their function is not related to the production of locomotion by MLR stimulation. What their function is remains unclear (see "PLS Descending Pathways" below, for further discussion). Shik (1983) has also suggested that the field potentials recorded in the MLR from stimulation of the PLS (Mori et al., 1977) may represent ascending fibers from the PLS and that the MLR may, therefore, be part of an ascending system: stimulation of the MLR would evoke locomotion due to antidromic excitation of the PLS. This hypothesis is false in light of the present results.

The Pontomedullary Locomotor Strip

Cellular Activation of Locomotion and the Trigeminal System

The results from the present experiments support the idea that the area classically defined as the PLS (Mori et al., 1977; Shik and Yagodnitsyn, 1977) contains cell bodies that can produce locomotion with the appropriate chemical stimulus. The effective drug-induced locomotor sites are also located within the spinal nucleus of the trigeminal nerve. This is in disagreement with the proposal that the PLS contains only axons and that its cells of origin, the "pontomedullary locomotor column", are located even more ventral and medial to the PLS (Selionov and Shik, 1984). While we were able to produce locomotion by electrical stimulation within the "pontomedullary locomotor column", the lack of drug-induced locomotion in this region suggests that we were activating fibers of passage and not cell bodies involved in the generation of locomotion. With regard to the cells of origin of the PLS, we propose that they are a collection of cells located in the spinal nucleus of the trigeminal nerve and in the immediately adjacent lateral reticular formation (LRF). Both anatomical and physiological evidence is available to support the idea that the LRF contains cells that are

related to the trigeminal system. The LRF is contiguous dorsolaterally with the trigeminal spinal nucleus and no obvious borders between them exist (Torvik, 1956). In addition, cells within and adjacent to the trigeminal nuclear complex (within the LRF) are activated by noxious and/or non-noxious stimulation of the trigeminal receptive field (Azerad et al., 1982; Nord and Kyler, 1968; Nord and Ross, 1973; Segundo et al., 1967) in a manner dependent upon stimulus intensity (Biedenbach, 1977; Burton, 1968). This activation is both monosynaptic and polysynaptic (Nord and Kyler, 1968; Nord and Ross, 1973). This has led to the suggestion that the LRF cells are a ventromedial extension of (at least) the trigeminal subnucleus caudalis and that this system has a integral role in the mediation of facial pain at the bulbar level (Nord and Kyler, 1968; Nord and Ross, 1973).

The fact that PIC injection into the PLS region could make previously ineffective trigeminal field stimulation initiate treadmill locomotion indicates that the PLS region is closely related to the physiology of the spinal nucleus of the trigeminal nerve. Thus it may be hypothesized that the PLS region contains a brainstem system which provides a substrate for sensory-motor reflex initiation of locomotion. Stimulation of the pinna, which is innervated in part by the trigeminal nerve, has been shown to sometimes elicit quadrupedal treadmill locomotion in the acute precollicular-postmamillary decerebrate cat (Aoki and Mori, 1981) and a characteristic "reflex posture" (Sherrington, 1906) resembling a part of locomotor movements. Since the area of the LRF may be involved in the integration of facial pain (see above), activity in this system may thus result in locomotor behaviour as part of a generalized response to the appropriate stimulus applied in the facial or trigeminal peripheral field. In this context, it is suggested that this facial afferent initiation of locomotion is similar to initiation of locomotion by segmental afferents. For example, stepping movements may be elicited in cats by nociceptive stimuli applied to localized areas of the skin (Sherrington, 1910) including the perineal region (Afelt, 1970). Furthermore, stepping movements may also be initiated by dorsal root stimulation in mesencephalic (Budakova, 1972), chronic spinal, and in acute spinal cats pretreated with nialamide and DOPA (Grillner and Zangger, 1979). After injection of DOPA into acute spinal cats stimulation of flexion reflex afferents (FRAs) also induces locomotor-like activity in spinal animals (Jankowska et al., 1967b). Thus facial and segmental afferent systems can produce locomotion when

appropriately stimulated and, in this context, the trigeminal and FRA system for the limbs may be considered analogous.

In decerebrate cats the adequate stimulus for eliciting locomotion applied to the pinna is pressure rather than touch (Aoki and Mori, 1981). However, in cats which have received a PIC injection into the PLS, gentle stroking of the trigeminal field may also evoke locomotion. This implies that the PLS region is normally under GABAergic inhibitory control. Removal of the GABAergic inhibition with an injection of PIC would produce a situation in which the afferent input would have a greater influence on the output activity of the cells in this region. This supposition is supported by the observation that pre- and postsynaptic inhibition in the trigeminal sensory nuclei is depressed by PIC (Ishimine et al., 1980; Nakamura and Wu, 1970). One may expect, therefore, that the production of locomotion by stimulation of various trigeminal receptive fields may be related to the somatotopic organization of the injected area in the brainstem. Although the somatotopic organization of the trigeminal area of the brainstem is complex (Azerad et al., 1982; Nord and Kyler, 1968; Yokota and Nishikawa, 1980), a differential effectiveness in producing locomotion by stimulation of the trigeminal receptive field was observed after injection of PIC into the PLS. The response characteristics described for the experiment illustrated in Fig. 9 would correspond with an injection site predominantly located in the ipsilateral mandibular projection area of the LRF with diffusion spread partially including the maxillary and ophthalmic zones. Locomotion produced by contralateral trigeminal field stimuli may be due to a contralateral trigeminal nuclear projection (Lovick and Wolstencroft, 1983; Nord and Kyler, 1968).

The concept that the PLS and the trigeminal system are synonymous or closely related is strengthened by a number of other observations. Both the PLS (Shik and Yagodnitsyn, 1977) and the trigeminal sensory nuclei (Olszewski, 1950) have similar rostro-caudal distributions. In addition, the PLS originates from the medial MLR (Garcia-Rill et al., 1983b) which includes the mesencephalic nucleus of the fifth nerve. Thus locomotion produced by opening of the jaw after the injection of PIC into the PLS observed in this study may be the result of increased activity in the medullary projection of the mesencephalic nucleus (Corbin, 1942; Walberg et al., 1984). The fact that SP injection into the PLS produces locomotion also supports the idea of an close relationship between the PLS and trigeminal

system. SP- containing trigeminal afferents have been shown to project to the spinal nucleus of the trigeminal nerve and the adjacent LRF (Cuello et al., 1978) where they may mediate the transmission of nociceptive and non-nociceptive information (Henry et al., 1977; Salt et al., 1983). In addition, a calcium-dependent release of SP in the trigeminal nuclei has been described (Jessell and Iversen, 1977).

PLS Descending Pathways

The results of the present study demonstrate that the PLS can produce locomotion by activation of cells in the MRF and through a pathway in the ventral half of the spinal cord. It is suggested that the PLS converges onto cells in the MRF which mediate locomotion produced by stimulation of the MLR. These conclusions are based on the observations that a) cooling of the MRF (in an area which also blocks locomotion produced by stimulation of the MLR) can abolish the locomotion produced by stimulation of the PLS; b) stimulation of the PLS may produce forelimb or hindlimb locomotion following extended spinal cord dorsal hemisections, and c) cooling of the ipsilateral VLF can reversibly abolish PLS-evoked locomotion. The PLS may thus be considered as an auxiliary route for the initiation of locomotion. In this way, sensory activation of locomotion is achieved via the connection between the PLS and the reticulospinal system. This is supported by the observation that trigeminal field stimulation produced locomotion when GA was injected into the MRF. Thus the activation of cells in the MRF by stimulation of the PLS (Mori et al., 1986; Selionov and Shik, 1981, 1984) or trigeminal afferents (Lamarche et al., 1960) may be mediated by similar pathways. The medial projections are thought to be primarily di- or polysynaptic (Mori et al., 1986; Selionov and Shik, 1981) although some monosynaptic responses may also occur (Mori et al., 1986). This is consistent with anatomical studies which demonstrate the presence of trigeminoreticular projections (Nauta and Kuypers, 1958; Stewart and King, 1963). The fact that only electrical stimulation of areas medial and ventral to the PLS was effective in producing locomotion suggests that this area consists of fibers projecting from the PLS to the MRF. Alternatively, cells in this intermediate area, involved in mediating the activation of reticulospinal cells by stimulation of the PLS, are not responsive to the drugs that were tested.

The observations that locomotion may be produced by a pathway which projects to the MRF and through the VLF is in contrast to the hypothesis that locomotion produced by stimulation of the PLS is mediated by a polysynaptic pathway which descends in the lateral tegmentum of the brainstem and continues in the spinal cord in the DLF (Kazennikov et al., 1979, 1980a, 1983a,b, 1985; Selionov and Shik, 1981, 1982, 1984; Shik, 1983). The hypothesis was based primarily on the report that destruction of the grey matter at C2-C3 (cells of origin of the pathway) for distances of 4-6 mm was required to abolish PLS-evoked locomotion if the lateral and ventral funiculi remained intact, whereas local (rostrocaudal length of 1 to 1.5 mm) lesions of the grey matter had no effect (Kazennikov et al., 1980a). It was thought that activity in the propriospinal pathway could bypass the local lesions of the grey matter since the characteristic length of axons in the polysynaptic pathway is 3-5 mm at this level (Kazennikov et al., 1979). While it may be true that stimulation of the PLS may project via this pathway (see below), it is certainly not the only route of activation since stimulation of the PLS may produce locomotion following lesions which destroy the spinal grey or the dorsal half of the spinal cord for up to 8.5 mm. The reasons for the loss of locomotor capability of the animals in the study by Kazennikov et al. (1979) are not clear. One possibility is that the thresholds for activation of the PLS-MRF pathways were outside of the tested range of stimulation. Alternatively, the loss of locomotor capability may have resulted from damage to the MRF-VLF pathway when the spinal cord was lesioned.

The results of the present study also indicate that PLS stimulation may produce locomotion by a pathway which descends in the lateral tegmentum of the brainstem and through the dorsal half of the spinal cord. This conclusion is based on the following observations: a) stimulation of the PLS may produce locomotion following isolation of the lateral tegmentum of the brainstem from the MRF and the MLR; b) loss of PLS-evoked locomotion may occur following transections of the DLF or extended dorsal hemisections of the spinal cord (even when MLR stimulation can still produce locomotion). In this way sensory activation of locomotion may also be achieved via a pathway descending from the PLS through the lateral tegmentum of the brainstem and dorsal aspect of the spinal cord. Similar results have been reported for the Atlantic stingray by Livingston (1986), who showed that swimming may be obtained by stimulation of lateral tegmental areas following lesions which isolate it from the MRF (Livingston, 1986). The

evidence linking the PLS to the trigeminal system (see above) suggests that the pathway descending in the lateral tegmentum and dorsal aspect of the spinal cord may be synonymous to the intranuclear trigemino- spinal pathways described previously (Matsushita et al., 1981, 1982). Whether the propriospinal system proposed to mediate the effects of PLS stimulation (Kazennikov et al., 1979, 1980a, 1983a,b, 1985; Selionov and Shik, 1981, 1982, 1984; Shik, 1983), is equivalent to the pathway revealed in this study remains to be shown. If it is so, one would expect that the propriospinal pathways which are activated by stimulation of the PLS (Kazennikov et al., 1979, 1983a,b; Shik, 1983), should be responsive to stimulation of the trigeminal peripheral field independently of any possible activation via the MRF (this study; Lovick and Wolstencroft, 1983).

Brainstem and Spinal Pathways for the Initiation of Locomotion

The pathways which are capable of producing locomotion in the decerebrate animal are summarized in Fig. 37. Briefly, the "classical MLR" relays its signal for the production of locomotion in the medial pontomedullary reticular formation (FTG, FTM). Reticulospinal cells (which may be somatotopically organized) then activate spinal centers for the generation of locomotion (at cervical and lumbar levels) via a pathway descending bilaterally in the VLF. This pathway is essential for the initiation of locomotion in intact animals since locomotion may still occur after transection of all areas of the spinal cord except the VLF (Eidelberg, 1981). The controlling elements activating this locomotor system and the transmitters released at the reticulospinal or spinal interneuronal levels are not fully understood. However, this study suggests that these reticulospinal cells may be partially under GABAergic control and that amino acid receptors may participate in the initiation of locomotion. Furthermore, the MRF may be activated by peripheral trigeminal stimuli. The PLS consists of trigeminal cells located in the lateral tegmentum and extends the length of the brainstem trigeminal spinal nucleus, receiving inputs from the peripheral field of the trigeminal nerve (5), contralateral trigeminal sensory nuclei, and from the area of the mesencephalic nucleus or "medial MLR" (mMLR) (Garcia-Rill et al., 1983b). The mMLR has also been shown to have a small projection to the MRF but its contribution to the initiation of locomotion during stimulation of the mMLR remains to be elucidated. Results from this study suggest that the PLS system is under GABAergic control and may be activated by amino acids and SP

to produce locomotion. Sensory activation of locomotion by stimulation of the trigeminal peripheral field may be mediated by two different pathways. The PLS may activate reticulospinal cells in the MRF (involved in mediating MLR-evoked locomotion) which descend via the VLF of the spinal cord. Alternatively, the PLS may project to the spinal cord via the DLF and possibly via polysynaptic propriospinal pathways already described (Kazennikov et al., 1980a, 1983a,b, 1985; Selionov and Shik, 1981, 1982, 1984; Shik, 1983).

MLR-evoked PSPs

Relationship to the Production of Locomotion

The results of the present study give further evidence to support the concept that the MLR-evoked PSPs are related to the process of locomotion. The evidence is based on observations of the characteristics of the PSPs during unperturbed fictive locomotion and during local cooling of the brainstem and spinal cord to abolish locomotion. The results confirm and extend those obtained by Shefchyk and Jordan (1985a).

During locomotion produced by stimulation of either the ipsilateral or contralateral MLR, LDPs and excitatory and inhibitory PSPs are observed in lumbar motoneurons. In the majority of cases, the MLR-evoked PSPs are either excitatory (E) or excitatory followed by an inhibitory PSP (EI). These PSPs are modulated during the step cycle, with the short-latency EPSPs being largest during the depolarized phase of the LDP and the IPSPs being largest during the hyperpolarized phase of the LDP. The amplitude of the LDPs and PSPs are positively correlated to each other and any increase in the strength of MLR stimulation results in both larger PSPs and LDPs. In addition, the greatest amplitude transitions of the MLR-evoked PSPs and LDPs occur during the same period of the step cycle. This indicates that the occurrence of MLR-evoked EPSPs and IPSPs are highly correlated to the duration of the depolarized and hyperpolarized phase of the LDP, respectively. Furthermore, Shefchyk and Jordan (1985a) have demonstrated that the site for the optimum production of MLR-evoked PSPs corresponds to the area of lowest electrical threshold for the initiation of locomotion and that the PSPs frequently increase in amplitude at the same time that fictive locomotion commences. Taken together, these results are

consistent with the suggestion that the excitability (and thus the output) of the spinal interneuron pool producing the MLR- evoked PSPs (see below) is modulated during the step cycle. (Increased excitability in this context means that each interneuron is closer to the threshold for activation and hence generates a larger response and/or that more interneurons in the pool are recruited with a pulsed synaptic excitatory input.) The excitability of the interneurons producing EPSPs in motoneurons is greatest during the depolarized phase of the LDP and they are thus easily activated (synchronized in firing) by stimulation of the MLR. Conversely, interneurons producing IPSPs in motoneurons with stimulation of the MLR are most excitable during the hyperpolarized phase of the LDP. In this context, the MLR-evoked EPSPs and IPSPs during the depolarized and hyperpolarized phase of the LDP, respectively, may simply reflect the predominant interneuronal synaptic imput occurring on motoneurons during each phase of the LDP. Considering that the depolarized and hyperpolarized phases of the motoneuron LDP are due to excitatory and inhibitory synaptic input, respectively (Jordan, 1981; Jordan et al., 1981; Perret, 1983; Pratt and Jordan, 1987; Orsal et al., 1986; Shefchyk and Jordan, 1985b), it seems reasonable to suggest that the interneurons producing the PSPs are linked to the process of LDP formation. While the results from the present experiments provide support for the suggestion that presynaptic mechanisms (changes in the excitability of spinal interneurons) are responsible for the amplitude modulation of the MLR-evoked PSPs, a contribution of postsynaptic factors cannot be ruled out until experiments designed to address these issues are conducted.

The fact that stimulation of the MLR may also produce phasic, stimulus-dependent firing in motoneurons also supports the concept that the MLR-evoked PSPs are related to the production of locomotion. Synchronization of activity to stimulation of the MLR has been reported previously for reticulospinal cells in the MRF (0rlovsky, 1970b), for fibers in the VLF of the spinal cord (Orlovsky, 1969), and for individual hindlimb motor units recorded by electromyography (Severin et al., 1967). Although this synchronization may be considered to be artifactual to controlled locomotion experiments in which stimuli are delivered to the MLR, there is evidence to support the suggestion that this phenomenon reflects the natural activation of motoneurons during spontaneous locomotion. For example, spike triggered averaging techniques have shown that during spontaneous locomotion in thalamic cats, responses in muscles are highly

correlated to the discharge of individual cells in the MLR (Garcia-Rill et al., 1983a) and MRF (Drew et al., 1986; Shimamura et al., 1982). Results from the present experiments using intracellular recording techniques demonstrate that stimulus-dependent firing of motoneurons was only observed during the depolarized phase of the LDP and occurred during the rising phase of the MLR-evoked EPSPs. Furthermore, the stimulus- dependent activity of antagonist motoneuron pools (ENG recordings) was shown to occur during the hyperpolarized phase of the intracellularly recorded motoneuron and was highly related to the occurrence of MLR-evoked IPSPs. The fact that spontaneously occurring spikes in antagonist ENGs were also associated with the IPSPs during the hyperpolarized phase of the LDP further strengthens the concept that the PSPs produced by stimulation of the MLR reflects a reciprocally organized network of spinal interneurons involved in the production of motoneuron excitation and inhibition during locomotion. This reciprocity is also observed in the phasing between flexors and extensors during the step cycle. For example, loss of the hyperpolarized phase (inhibition) of the LDP in extensor motoneurons (Figs. 24 and 29) was always associated with the loss of excitation to antagonist motoneuron pools (ENGs). Similarly, loss of the depolarized phase of the LDP in extensor motoneurons was associated with the loss of inhibition in antagonist motoneuron pool (Fig. 32). Quantitative analysis of the activity of hindlimb motoneurons during fictive locomotion indicates that the excitatory input for flexor LDPs covaries with the inhibitory input for extensor LDPs (Kriellaars et al., 1987) and further strengthens the reciprocal organization revealed by the present study.

A reciprocal organization of interneurons mediating excitatory and inhibitory action to alpha motoneurons of flexors and extensors has been previously described in spinal cats treated with L-dopa (Jankowska et al., 1967a,b). Evidence has been provided which shows that this organization may be involved in the production of locomotion produced by stimulation of the MLR. For example, the pathways which activate these neurons may be facilitated during MLR-evoked locomotion (Grillner and Shik, 1973). In addition, the interneurons involved in the production of MLR-evoked PSPs are activated by flexion reflex afferents (Shefchyk and Jordan, 1985a) which also mediate the effects described by Jankowska et al. (1967a,b). Whether these cells are the same ones involved in the production of MLR- evoked PSPs is unclear, but the demonstration of a

reciprocal excitation and inhibition to flexors and extensors (this study, Kriellaars et al., 1987) during MLR-evoked locomotion provides evidence in favor of this view.

Data collected during reversible cooling of the brainstem or spinal cord also support the idea that the MLR-evoked PSPs are related to the production of locomotion during MLR stimulation. Loss of locomotion was always associated with the reduction or loss of MLR-evoked LDPs and PSPs. Complete loss of LDPs was associated with either a significant reduction in the size or a complete loss of MLR-evoked PSPs. In cases where LDPs were not abolished, residual MLR-evoked PSPs remained. Furthermore, a positive correlation between LDP amplitude and the amount of MLR- evoked PSP modulation during cooling of the spinal cord was observed. From these results it is suggested that there is a common pathway for the production of PSPs, LDPs and locomotion (see below for further discussion).

While the data summarized above provides evidence that the interneurons that produce MLR-evoked PSPs are involved in the production of motoneuron activity during locomotion, it is not certain whether these cells are involved in locomotor rhythm generation. While the properties of these interneurons have not been investigated, it is of interest that stimulation of the MLR could produce bursts of activity in one spinal neuron tested (Fig. 26). In addition, stimulation of the MLR produced a response in this neuron that was appropriate for it to be intercalated in the pathway between the MLR and the motoneuron (see below for further discussion). Bursting pacemaker neurons have been described previously in spinal cord cultures (Legendre et al., 1985), indicating that cells endowed with properties of this type may be present in the intact spinal cord.

The hypothesis concerning the relationship of the MLR-evoked PSPs to the production of LDPs and locomotion rests on the assumption that the PSP size reflects the excitatory or inhibitory input to the motoneuron during locomotion. It is thus important to account for the fact that some cells exhibit LDPs but do not show well modulated EPSPs or IPSPs, i.e., the eI(E) or I(E) PSP type. As demonstrated in the Results section, cells which exhibited this MLR-evoked PSP type were not common. It was also shown that the averaging technique may sometimes mask the presence of large MLR-evoked EPSPs. This indicates that

the absence of any EPSP may simply reflect the possibility that the cells providing the excitatory input during the step cycle cannot be synchronously activated by stimulation of the MLR. In this context, a number of explanations may be forwarded. This inability may simply reflect a poor response index (low probability of a stimulus producing a response) in the pathway from the MLR to the motoneurons due to conduction failure of one of the interposed cells or the result of small amounts of neurotransmitter release. Alternatively, the interneurons may not be in an excitability state that is favourable for a synchronous activation by MLR stimulation. For example, the interneurons may be so asynchronously active that they are somewhat refractory to pulsed synaptic input. Furthermore, the MLR-evoked EPSPs may have been outside of the 30 msec window used in the analysis of the motoneuron response to stimulation of the MLR. In this case, a slower conducting pathway or one which has a greater number of interposed interneurons between the MLR and the motoneurons may have been activated. In cases where stimulation of the MLR produced large IPSPs, it is possible that the primary imput for the production of the LDPs in those cells, may have been inhibitory (phasic inhibition only). Evidence was also provided indicating that the IPSP was actually composed of two components, only one of which was related to the process of locomotion (larger during the hyperpolarized phase of the LDP or positively correlated to membrane hyperpolarization). In those cases, the presence of a larger IPSP in the depolarized phase of the LDP IPSP does not necessarily mean that stimulation of the MLR was not providing an appropriately modulated inhibitory input.

Latencies of Response and Mediation by Spinal Interneurons

The results of the present experiments demonstrate that stimulation of the MLR produces short-latency PSPs in lumbar motoneurons and thus confirm the results obtained by Shefchyk and Jordan (1985a). The mean total latency from the MLR stimulus artifact to the first EPSP was 6.5 +/- 0.8 msec. Although this is slightly longer than that reported by Shefchyk and Jordan (1985a) (mean of 5.1 msec), results similar to the present study have recently been reported for chloralose anaesthetized cats (mean of 6.5 msec) (Shefchyk and McCrea, 1986) during electrical stimulation of the area corresponding to the MLR (Shik et al., 1967). The results demonstrate that a fast conducting pathway from the MLR to hindlimb motoneurons is activated by stimulation of the MLR (Jordan, 1983) and

is inconsistent with the proposal that locomotion produced by stimulation of the MLR is mediated by a polysynaptic pathway (Kazennikov et al., 1983a, 1985; Mori et al., 1977, 1978b; Shik, 1983; Shik and Yagodnitsyn, 1978) (see below for further discussion).

The mean latency for the earliest arrival of the descending volley at the cord dorsum recording electrode produced by stimulation of the MLR was 4.0 +/- 0.6 msec (range of 2.9-5.2 msec). The maximum conduction velocity of the reticulospinal neurons projecting to the spinal cord was calculated at a mean of 103.3 m/sec after taking into account the approximate 1 msec delay for activation of these cells by the MLR (Orlovsky, 1969). Similar short-latency orthodromic activation of reticulospinal neurons with stimulation of the MLR at rates used to elicit locomotion have been reported (Garcia-Rill and Skinner, 1987b; Orlovsky, 1970a). It is recognized that the calculations of maximum conduction velocity for the reticulospinal cells are based on the assumption that the first descending volley recorded at the spinal cord dorsum electrode is related to the production of locomotion. As experiments were not conducted to determine the information content of the cord dorsum potentials, the nature of the first volley is not known with certainty. Since cooling of the VLF of the spinal cord to abolish locomotion sometimes decreases the size of the initial volley, it was thought necessary to base our measurements on the arrival of this volley. The results are consistent with conduction velocities up to 100 m/sec (Garcia-Rill and Skinner, 1987b), 90-120 m/sec (Orlovsky, 1969), or 90-130 m/sec (Orlovsky, 1970a) for reticulospinal cells activated by stimulation of the MLR. Similarly, short-latency responses correlated to the discharge of cells in the MLR (Garcia-Rill et al., 1983a) and MRF (Drew et al., 1986; Shimamura et al., 1982) have been observed in EMG recordings from hindlimb muscles.

The maximum segmental latency for the onset of the MLR- evoked EPSPs was calculated to be 2.3 +/- 0.7 msec (range of 0.9-3.0 msec). This indicates that stimulation of the MLR activates at least one or two spinal interneurons interposed between the MRF and spinal motoneurons as suggested by Shefchyk and Jordan (1985a). The fact that the segmental latency for the production of MLR-evoked hyperpolarization in motoneurons (4.4 +/- 1.4 msec: range of 2.2-8.7 msec) is significantly longer than that observed for MLR- evoked EPSPs indicates that additional spinal interneurons are probably involved in the

production of these IPSPs (Shefchyk and Jordan, 1985a). However, as the onset of the MLR-evoked IPSP is not known with certainty, further experimentation would be necessary to proove this. In accordance with this, stimulation of the MRF may produce disynaptic and polysynaptic PSPs in flexor and extensor motoneurons (Peterson et al., 1979).

The present study demonstrates that rhythmically active spinal neurons which are activated by stimulation of the MLR are found within the L6-L7 lumbar segments. These cells are also activated at latencies appropriate for their participation in the production of MLR-evoked PSPs in lumbar motoneurons. MLR- evoked activity during the interburst period has also been observed for some of these neurons, thus accounting for the maintained presence of PSPs throughout the LDP. As the projections of these cells were not determined, their action on motoneurons (if any) are unknown. However, their location in the intermediate zone and ventral horn of the spinal grey coincides with the anatomical projections of reticulospinal pathways (Holstege and Kuypers, 1982; Petras, 1967) and the physiological actions of pathways descending in the ventral funiculus (Skinner and Willis, 1970; Skinner et al., 1970). In addition, recent work using the horseradish peroxidase-wheat germ agglutinin technique (Jankowska, 1985) has revealed last order interneurons in the 5th, 6th and 7th lumbar segments which may be involved in the production of locomotion (Noga et al., 1987b). This indicates that these cells are likely candidates for the mediation of MLR- evoked PSPs. L4 spinal interneurons, which receive group II afferent input and which project monosynaptically to motoneurons in the lower lumbar segments (Edgley and Jankowska, 1987) have recently been found to be rhythmically active during MLR-evoked locomotion (Shefchyk et al., unpublished). In addition, these cells have been shown to produce short-latency responses to stimulation of the region corresponding to the MLR (Edgley et al., unpublished), thus indicating that the MLR activates spinal interneurons at a number of different spinal cord levels. The results listed above are interesting in view of the evidence that the rostral and caudal segments of the lumbar spinal cord can generate fictive scratching sequences (Deliagina et al., 1983). Whether the cells responsible for the generation of the locomotor rhythm are distributed throughout the spinal cord remains to be demonstrated, but it is certainly clear that spinal neurons that are rhythmically active during locomotion (this study; Baev et al, 1979; Feldman and Orlovsky, 1975; McCrea et al., 1980; Orlovsky and Feldman, 1972; Noga et al.,

1987a; Orlovsky, 1972; Pratt and Jordan, 1987) and responsive to stimulation of the MLR (this study; Shefchyk et al., unpublished) are distributed over wide areas of the lumbar spinal cord.

Descending and Segmental Pathway

The results from the present study demonstrate that the MLR produces fictive locomotion by a pathway which projects to the MRF and through the VLF of the spinal cord as proposed previously (Steeves and Jordan, 1980, 1984). This conclusion was based on the observation that cooling of the MRF or the VLF of the spinal cord to temperatures which block synaptic transmission or fiber transmission (Brooks, 1983), respectively, could reversibly abolish MLR-evoked PSPs, LDPs, and locomotion. However, cooling of the dorsal aspect of the spinal cord to temperatures that would block synaptic transmission throughout and decrease fiber transmission in the dorsal half of the cord was without effect. The present study confirms the observations of Shefchyk et al. (1984), who demonstrated that cooling of the MRF could reversibly abolish MLR-evoked treadmill locomotion. Furthermore, the results are inconsistent with the proposal that stimulation of the MLR produces locomotion by a polysynaptic pathway projecting via the lateral tegmentum of the brainstem and the DLF of the spinal cord (Kazennikov et al., 1983a, 1985; Mori et al., 1977, 1978b; Shik, 1983; Shik and Yagodnitsyn, 1978).

The present study demonstrates that stimulation of the MLR activates a pathway that descends bilaterally in the VLF of the spinal cord. This conclusion is based on the observations that relative to the side of motoneuronal recording, cooling of either side of the VLF could reduce or abolish locomotion (ENG rhythmicity or LDP) during stimulation of either the ipsilateral or contralateral MLR. Furthermore, cooling of the contralateral VLF could abolish long-latency EPSPs produced by stimulation of the ipsilateral MLR. Bilateral projections from the MLR to the MRF have been described previously (Garcia-Rill et al., 1983b; Steeves and Jordan, 1984). In addition, unilateral stimulation of the MLR may activate reticulospinal pathways descending in the VLF of the spinal cord, bilaterally (Garcia-Rill and Skinner, 1987b; Orlovsky, 1970a). Anatomical and electrophysiological studies have shown that some reticulospinal axons terminate in the spinal cord on the side contralateral to their site of origin (Peterson et al.,

1979; Petras, 1967). Furthermore, during spontaneous locomotion, the activity of cells in the MLR (Garcia- Rill et al., 1983a) and MRF may be correlated to the activity of hindlimb motor units on either side of the animal (Drew et al., 1986; Shimamura and Kogure, 1983; Shimamura et al., 1982).

The present study also provides evidence that a pathway that crosses at the spinal level (caudal to the T12 cooling site) may contribute to the production of PSPs and LDPs during locomotion produced by stimulation of the MLR. For example, cooling of the contralateral VLF specifically abolished long-latency EPSPs without affecting shorter latency EPSPs (Fig. 36). In addition, a longer latency MLR-evoked EPSP was revealed during cooling of the ipsilateral VLF (Fig. 35). In both cases, the LDP was reduced but not completely abolished. Evidence for a crossed spinal influence in the recovery of locomotor function has been previously obtained following chronic unilateral (Eidelberg, 1981; Eidelberg, et al., 1986; Kamishima, 1978; Sholomenko and Steeves, 1987) or bilateral staggered (Jane et al., 1964; Kato et al., 1984) hemisections. While recovery from spinal cord hemisections leading to the recovery of the capacity for locomotion in limbs caudal to the lesion site is usually not immediate (Eidelberg, 1981), stimulation of the MRF may produce bilateral hindlimb locomotion following acute spinal hemisections (Sholomenko and Steeves, 1987). Rhythmic activity in contralateral limbs has also been reported with unilateral stimulation of the spinal cord (Jacobson and Hollyday, 1982; Lennard and Stein, 1977, Williams et al., 1984; Yamaguchi, 1986, 1987). The fact that MLR stimulation may produce small LDPs by pathways crossing at the spinal level indicates that this pathway may be involved in the normal recovery of locomotor function after chronic spinal lesions and also in the coordination of bilateral limb movements. Previous work has demonstrated that contralateral lamina VIII interneurons may be involved in crossed reflex interactions in the spinal cord (Harrison et al., 1986). Whether these cells mediate some of the crossed spinal effects produced by stimulation of the MLR is unclear, although a recent study utilizing the horseradish peroxidase-wheat germ agglutinin transneuronal labelling technique (Jankowska, 1985) has shown that these cells may participate in the production of overground locomotion (Noga et al., 1987b). Clearly more work is required to establish their role in the production of MLR-evoked locomotion.

Summary

Key questions regarding the brainstem and spinal descending pathways capable of producing locomotion when stimulated were examined using treadmill and fictive-locomotion preparations in decerebrate cats.

In the first group of experiments, we determined the distribution of cells in the medial reticular formation (MRF) and the pontomedullary locomotor strip (PLS) which, when activated, can induce locomotion. Controlled microinjections of neuroactive substances (Goodchild et al., 1982) into the MRF or PLS were made in order to activate cell bodies in those areas. The ability of trigeminal receptive field stimulation to induce locomotion before and after drug infusion into the PLS was also assessed since the PLS and the spinal nucleus of the trigeminal nerve are similar in their anatomical distribution. Experiments were performed on precollicular-postmamillary decerebrate cats walking on a treadmill.

Injections of glutamic acid (GA) (500 nmol) into the MRF produced locomotion which was antagonized by infusion of glutamic acid diethyl ester into the same spot. Decreases in the current threshold for locomotion produced by electrical stimulation of the MRF were observed when the MRF was infused with either GA (40-80 nmol), DL-homocysteic acid (DL-HCA) (200 nmol), or picrotoxin (PIC) (15 nmol). Injections of GA (100 nmol), DL-HCA (700 nmol), PIC (10-50 nmol), and substance P (2 nmol) into the PLS also produced locomotion. Locomotion produced by injections of PIC into the PLS was blocked by infusion of equal amounts of muscimol or gamma amino butyric acid. Effective PLS injection sites were all confined to the trigeminal spinal nucleus or immediately ventral and medial to this in the adjacent lateral reticular formation. Trigeminal nerve peripheral field stimulation evoked locomotion after microinjection of PIC into the PLS, although this same facial stimulus was not effective prior to drug injection.

We conclude that the MRF and PLS regions of the cat brainstem contain cells which produce locomotion when chemically stimulated, and we suggest that the PLS is closely related to or synonymous with the spinal nucleus of the trigeminal nerve. We also suggest that stimulation of trigeminal afferents is analogous to stimulation of segmental afferent pathways in the production of

locomotion (Afelt, 1970; Budakova, 1972; Grillner and Zangger, 1979; Jankowska et al., 1967b; Sherrington, 1910).

In another series of experiments, the descending pathways from the brainstem locomotor areas were investigated by utilizing reversible (cooling to block synaptic or fiber transmission) and irreversible subtotal lesions of the brainstem or spinal cord (C2-C3 level). Experiments were conducted on decerebrate cats induced to walk on a treadmill by electrical stimulation of the brainstem. Locomotion produced by stimulation of the mesencephalic locomotor region (MLR) was not abolished by caudal brainstem lesions which isolated the lateral tegmentum or by extended dorsal hemisections of the spinal cord. Locomotion produced by stimulation of the PLS was blocked by reversible cooling of the MRF or the ventrolateral funiculus (VLF) of the spinal cord. Locomotion could be produced by stimulation of the PLS following the removal of extended portions of the dorsal half of the spinal cord or following the isolation of the lateral tegmentum of the brainstem from the MRF. Lesions of the dorsal spinal cord also resulted in the loss of PLS-evoked locomotion in some cases. These results demonstrate that the MLR does not require a pathway projecting through the lateral tegmentum of the brainstem or the dorsal half of the spinal cord as has been previously suggested (Mori et al., 1977, 1978b ; Kazennikov et al., 1980a, 1983a; Shik, 1983). Rather, the MLR projects through the MRF and the ventral half of the spinal cord, confirming the results obtained by Steeves and Jordan (1980, 1984) and Shefchyk et al. (1984). The results also demonstrate that the PLS can produce locomotion by activation of cells in the MRF and which project via the ventral half of the spinal cord. However, a projection through the dorsal half of the spinal cord (Kazennikov et al. 1980a, 1983a,b; Shik, 1983) cannot be ruled out.

In the final series of experiments, the characteristics of short-latency MLR-evoked excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs) observed in lumbar spinal motoneurons and their relationship to the production of motoneuron locomotor drive potentials (LDPs) and locomotion were examined. Fictive locomotion experiments were conducted according to the procedures previously described (Jordan et al., 1979). MLR-evoked PSPs were classified into a number of groups, with the most common ones being excitatory (E) or excitatory followed by an similarly sized inhibitory PSP (E-I). (Longer

latency EPSPs were sometimes observed following these short-latency PSPs). In flexor motoneurons, the MLR-evoked PSPs were predominantly excitatory in nature although slightly longer latency IPSPs were also observed. MLR-evoked PSPs in extensor motoneurons were predominantly of the E-I or E type. MLR-evoked PSPs were modulated during the step cycle with the EPSPs largest in the depolarized phase and the IPSPs largest in the hyperpolarized phase of the LDP. The mean latency of the EPSPs as measured from the MLR stimulus artifact (total latency) was 6.5 +/- 0.8 msec. No significant difference in the EPSP total latency was found between ipsilateral MLR or contralateral MLR stimulation or between flexor or extensor motoneurons. Segmental latencies were consistent with a minimum segmental delay of one interposed interneuron. The total and segmental latencies to the MLR-evoked hyperpolarization was significantly longer. Similar step cycle-related amplitude transitions for MLR-evoked PSPs and LDPs indicated that the PSPs were strongly related to the duration of each phase of the LDP. The amplitude of the LDPs and PSPs were positively correlated to each other and to the strength of stimulation of the same MLR site. Stimulation of different MLR sites could produce slightly different PSP types and different descending volley and segmental latencies. During locomotion produced by MLR stimulation, stimulus-locked spiking was also observed in motoneurons and electroneurograms (ENGs) of various hindlimb nerves. Motoneuron spikes were initiated on the rising phase of the short-latency EPSPs. A reciprocal relationship between excitation and inhibition was observed for antagonists at the same joint. Rhythmically active spinal neurons that showed latencies appropriate for possible mediation of MLR-evoked excitation or inhibition of motoneurons were recorded from in the same lumbar spinal segments.

Cooling of the MRF or VLF of the spinal cord (T12-13 segment) to temperatures which block synaptic or fiber transmission, respectively, could reversibly abolish MLR-evoked locomotion (ENG rhythmic activity). Loss of locomotion was associated with the reduction or loss of MLR-evoked motoneuron PSPs and LDPs. The complete loss of the LDP was usually associated with the complete loss of PSPs. In cases where the LDP was not abolished, residual MLR-evoked PSPs were seen. Residual IPSPs (in the absence of locomotion) were associated with tonic excitation of the antagonist motoneuron pool (ENG). During cooling or rewarming of the VLF, loss of the depolarizing phase of the LDP was associated with reduced (or abolished) interburst inhibition in the

antagonist ENG. Cooling of the dorsal half of the spinal cord was without effect. Evidence is accumulated that suggest that the signal for the production of locomotion by stimulation of the MLR descends bilaterally in the VLF. Furthermore, evidence for a crossed segmental pathway produced by stimulation of the MLR is provided. In general, the crossed segmental pathway produces EPSPs of slightly longer latency than that seen with the uncrossed descending pathway. A positive correlation was observed between LDP amplitude and the amount of MLR-evoked PSP modulation during cooling of the VLF.

The evidence presented indicates that locomotion, LDPs and PSPs observed in motoneurons by stimulation of the MLR are produced by a pathway which relays in the MRF and descends to the spinal cord in the VLF. Furthermore, they are consistent with the concept that the spinal interneurons activated by MLR stimulation are related to the process of locomotion.

MN	MLR SIDE	E(E)	EI(E)	eI(E)	I(E)
SMAB	i	II	IIIII	III	III
	c	I	IIIII		
LGMG	i		I	I	I
	c	II	IIII	III	I
Vast	c		I		
PBSt	i			I	
	c	III	II		
FDL	i				I
	c	II	II		
Unid Ext	i	III	IIIII	I	
	c	IIIII	IIIII	II	
TA	i	IIII	I		
	c	III	I		
Unid Flex	i	I	I		I

Table I. Distribution of PSP types with respect to motoneuron type and side of MLR stimulation.

		E	total	EI(E)	total	eI(E)	total	I(E)	total
Extensor MN	c MLR	34.2%	28.1%	50%	45.3%	13.2%	17.2%	2.6%	9.4%
	i MLR	19.2		38.5%		23.1%		19.2%	
Flexor MN	c MLR	75%	63.6%	25%	27.3%	-	-	-	9.1%
	i MLR	57%		28.6%		-		14.3%	
Total (PSP Types)		33.3%		42.7%		14.7%		9.3%	

Table II. Occurrence (%) of PSP types for flexor and extensor motoneurons with respect to side of MLR stimulation.

		MLR EPSP n	mean ± SD	CD EPSP n	mean ± SD	MLR CD n	mean ± SD	MLR IPSP n	mean ± SD	CD IPSP n	mean ± SD
iMLR	Ext MNs	15	6.3±0.8	15	2.0±0.6 *	19	4.1±0.6	16	8.0±1.1 ****	14	3.8±1.1 ***
	Flex MNs	7	7.0±1.2	6	2.4±0.7						
	Both	22	6.6±1.0 [*]	21	2.1±0.6 ** ■				[*]		■
cMLR	Ext MNs	30	6.4±0.7	28	2.4±0.6 *	20	3.9±0.6	17	8.9±1.3 ****	14	5.0±1.4 ***
	Flex MNs	4	6.6±0.9	4	3.0±0.8						
	Both	34	6.4±0.7 [**]	32	2.5±0.7 ** ■■				[**]		■■
Both MLRs	Ext MNs	45	6.4±0.7	43	2.3±0.6	39	4.0±0.6	33	8.5±1.3	28	4.4±1.4
	Flex MNs	11	6.9±1.0	10	2.6±0.7						
	Both	56	6.5±0.8 [***]	53	2.3±0.7 ■■■				[***]		■■■

p ≤ 0.05	*	**	***
p ≤ 0.01	[*]	[**]	[***]
	■	■■	■■■

Table III. Descending volley and segmental latency measurements for short-latency EPSPs and motoneuron hyperpolarization produced with stimulation of the MLR.

Figure Legends

Figure 1. Isotherms produced during cooling of the brainstem to temperatures sufficient for local block of synaptic transmission.

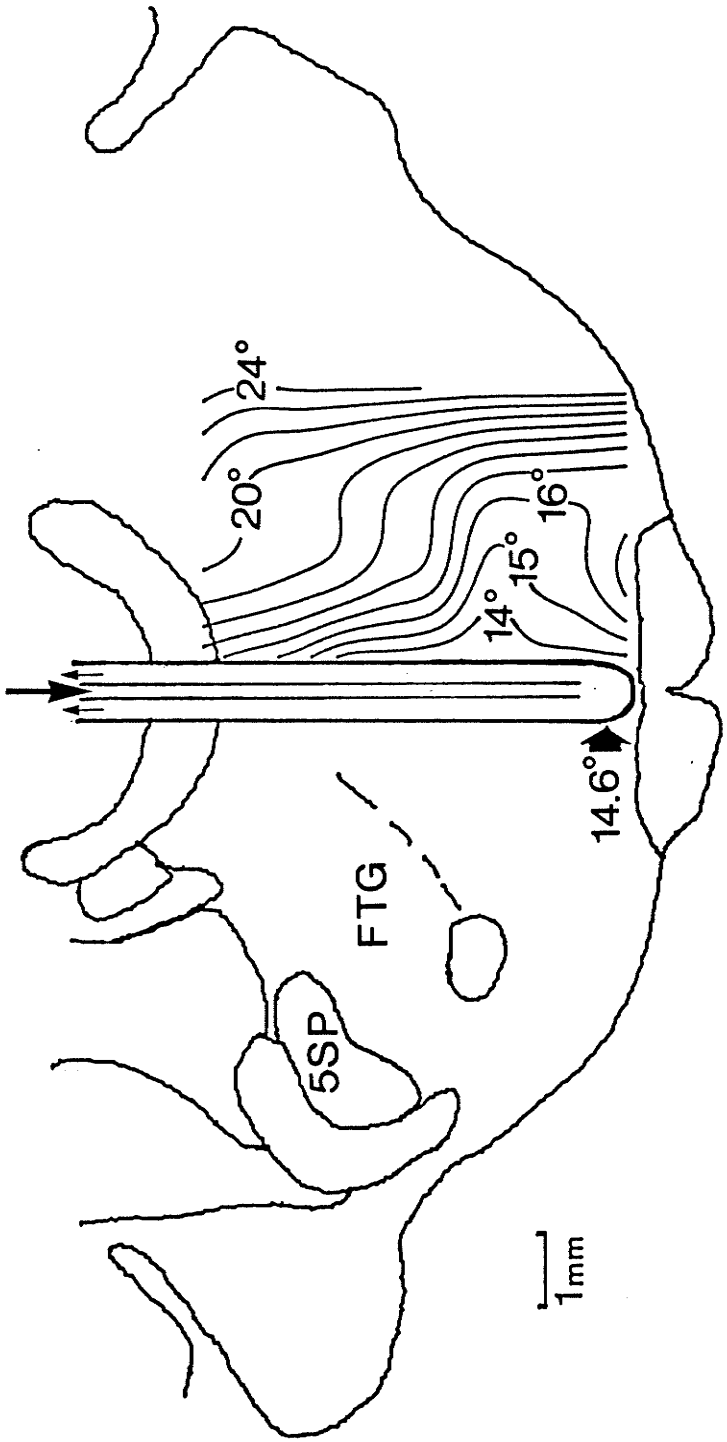

24°
20°
16°
15°
14°
14.6°
FTG
5SP
1mm

Figure 2. Surface cooling of the spinal cord. A shows spinal cord isotherms measured during extradural cooling of the VLF. Cooling probe temperature was maintained at -0.2 degrees C. Temperatures achieved during cooling are sufficient to block fiber transmission in the ipsilateral VLF and synaptic transmission throughout the spinal cord cooling area. B illustrates spinal potentials produced by stimulation (200 uA, 10 Hz, 0.5 msec duration) of the ipsilateral VLF (rostral T12 spinal segment) and recorded at the ventral surface of the L6 spinal segment during cooling of the ipsilateral VLF (caudal T12 spinal cord). Fiber transmission is completely blocked at cooling probe temperatures of 0 to -1 degree C. Partial blockage of signal transmission through the DLF during cooling of the VLF is shown in C. Spinal potentials are produced by stimulation of the ipsilateral DLF (rostral T12 spinal segment) (200 uA, 20 Hz, 0.5 msec duration) and recorded at the dorsal surface of the L6 spinal segment during cooling of the ipsilateral VLF (caudal T12 spinal cord). Calibration bar in B and C is 10 msec. Temperature changes in the ipsilateral and contralateral ventral funiculi during a cooling trial of the VLF (T12 spinal segment) are illustrated in D and E, respectively. Location of thermistor in spinal cord is indicated by dots. Spinal cord and cooling probe temperatures plateau after approximately 2 minutes of cooling.

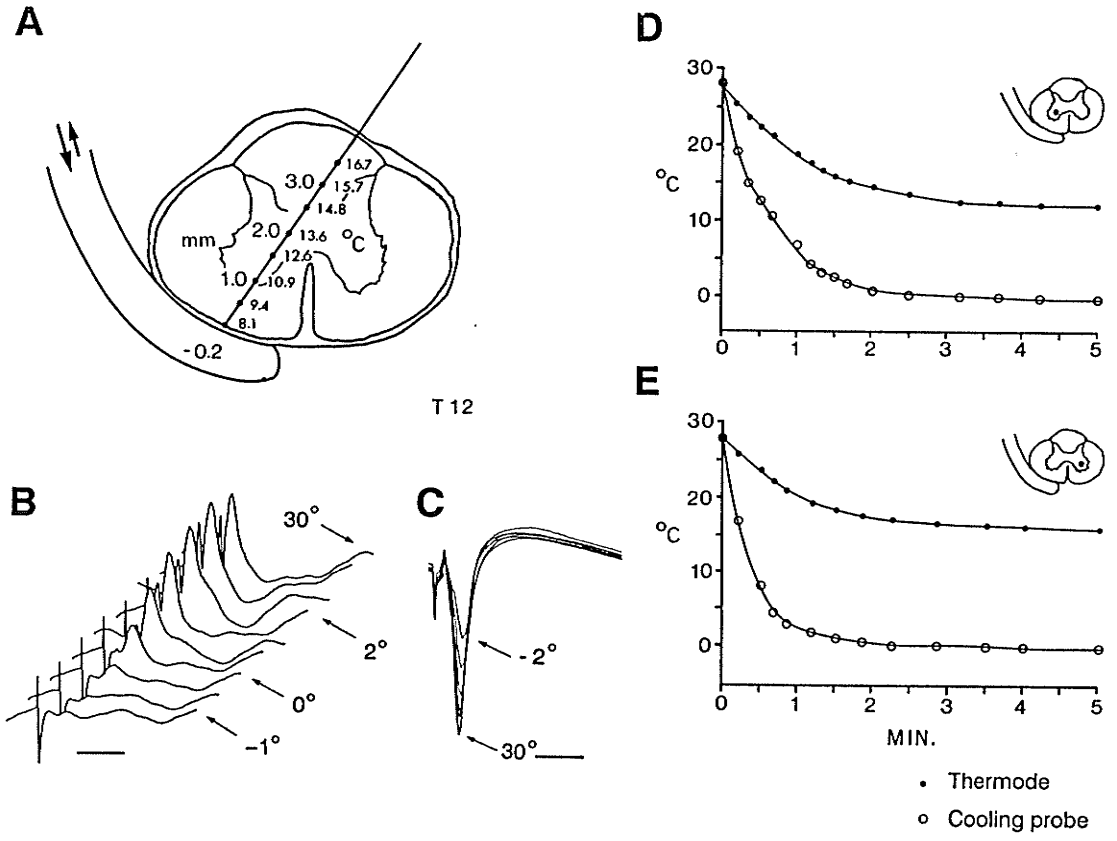

A
3.0
16.7
15.7
14.8
mm
2.0
13.6
°C
12.6
1.0
10.9
9.4
8.1
-0.2
T 12
B
30°
2°
0°
-1°
C
-2°
30°
D
30
20
°C
10
0
0
1
2
3
4
5
E
30
20
°C
10
0
0
1
2
3
4
5
MIN.
• Thermode
o Cooling probe

Figure 3. A: Schematic drawing of the brainstem surface (P3 - P15; L0 - L7) (cerebellum excluded for illustrative purposes) from the area indicated in the inset to demonstrate the rostro- caudal and medio-lateral distribution of sites capable of producing locomotion when electrically stimulated. All electrode tracts are from animals that could locomote with PLS and MRF stimulation. Numbers of trials are indicated by relative size of each circle. Limbs used during locomotion are indicated by the shaded quadrant within each circle; open circles represent areas ineffective in inducing locomotion when stimulated. B: histograms indicating the distribution of locomotor (shaded) and non-locomotor (open) trials at different brainstem P levels (X axis: mm from midline; Y axis: number of trials).

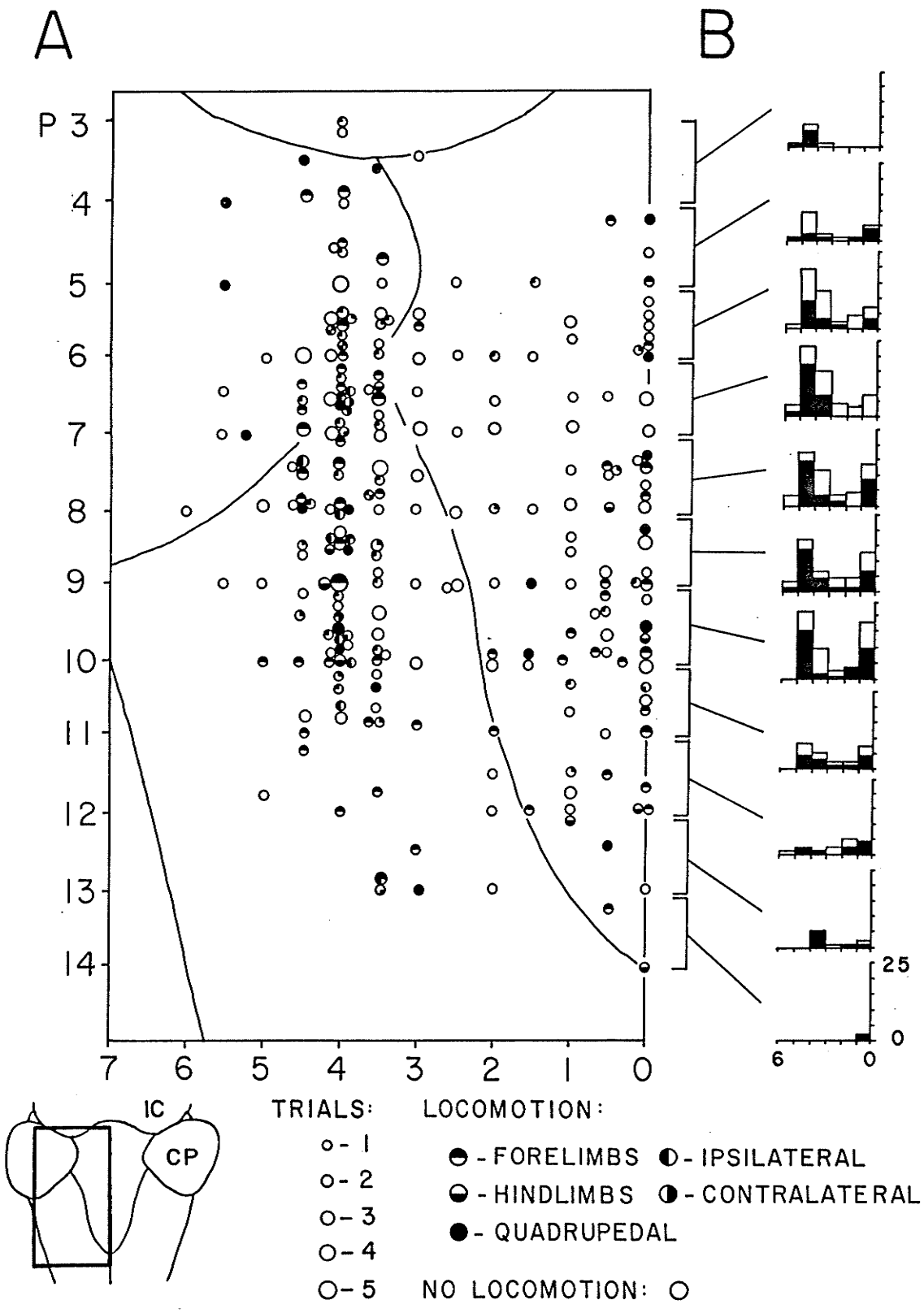

A
B
P 3
4
5
6
7
8
9
10
11
12
13
14
7 6 5 4 3 2 1 0
6 0
25
0
IC
CP
TRIALS:
o - 1
o - 2
o - 3
o - 4
o - 5
LOCOMOTION:
◓ - FORELIMBS ◐ - IPSILATERAL
◒ - HINDLIMBS ◑ - CONTRALATERAL
● - QUADRUPEDAL
NO LOCOMOTION: ○

Figure 4. Distribution of sites within the brainstem that produce locomotion when electrically stimulated. Two general areas are indicated. MRF sites are found in the raphe nuclei or adjacent magnocellular or gigantocellular tegmental fields (FTM or FTG). At more rostral levels the effective sites are located more dorsally. Effective sites corresponding to the PLS are found within the spinal nucleus of the fifth nerve (5SP) or medial and/or ventral to it in the lateral tegmental field (FTL). Symbols indicating limb involvement during locomotion are as illustrated in Fig. 3 except that right and left quadrants indicate right and left sides, respectively.

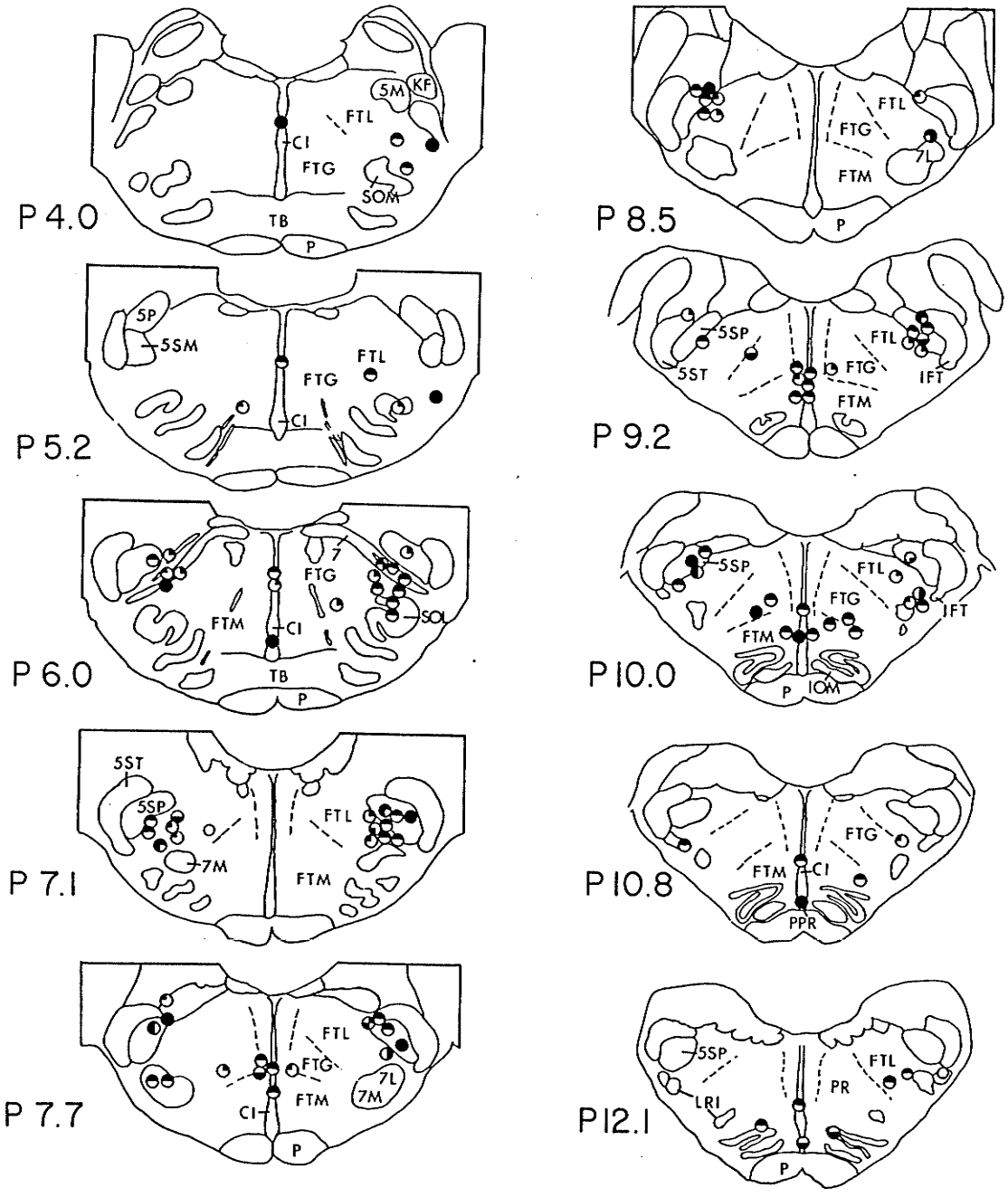

P 4.0
5M
KF
FTL
CI
FTG
SOM
TB
P
P 5.2
5P
5SM
FTL
FTG
CI
P 6.0
FTG
FTM
CI
SOL
TB
P
P 7.1
5ST
5SP
7M
FTL
FTM
P 7.7
FTL
FTG
7L
7M
FTM
CI
P
P 8.5
FTL
FTG
FTM
P
P 9.2
5SP
FTL
5ST
FTG
IFT
FTM
P 10.0
5SP
FTL
FTG
IFT
FTM
P
IOM
P 10.8
FTG
FTM
CI
PPR
P12.1
5SP
FTL
PR
LRI
P

Figure 5. Electrically induced and drug-induced locomotion in the MRF. A illustrates the EMG records during quadrupedal locomotion produced by electrical stimulation of the site indicated in E. Injection of 500 nmol glutamic acid (GA) (in 5 ul, 0.1 M) into the MRF also induces coordinated quadrupedal locomotion (B). A change in phasic activity of some muscles is seen during locomotion produced by GA infusion (see text). Bouts of locomotion were induced by swinging the forelimbs (C) during periods of quiescence. D shows cessation of walking due to a 500 nmol (in 5 ul, 0.1 M) injection of glutamic acid diethyl ester (GDEE) into the same site. Only tonic muscle activity is evident after forelimb swing (arrow). EMG traces in B - D are in same order as listed in A.

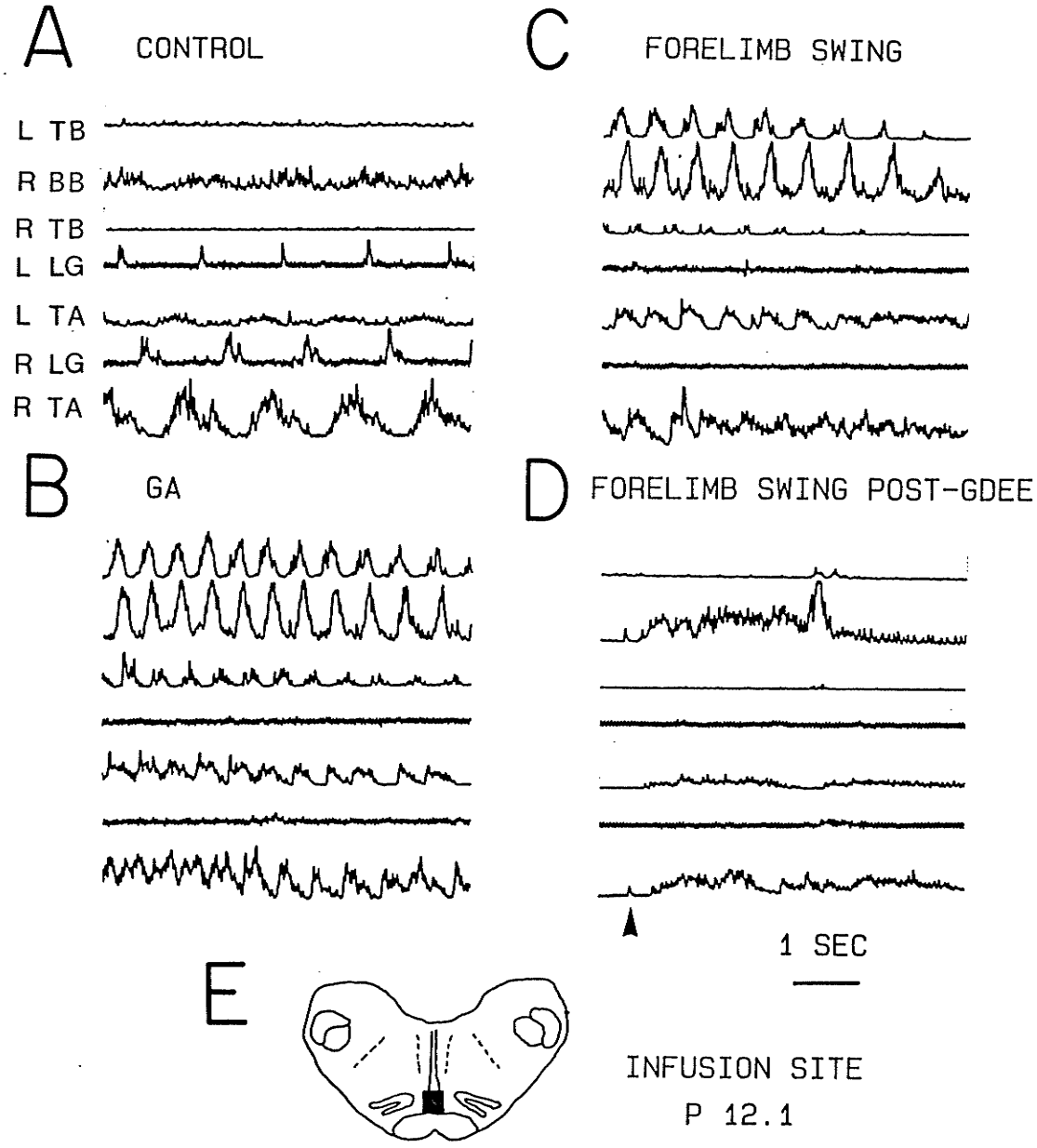

A
CONTROL
L TB
R BB
R TB
L LG
L TA
R LG
R TA
C
FORELIMB SWING
B
GA
D
FORELIMB SWING POST-GDEE
1 SEC
E
INFUSION SITE
P 12.1

Figure 6. Electrically induced and drug-induced locomotion in the PLS. A illustrates typical EMG records during forelimb locomotion produced by electrical stimulation of the PLS. B, C, D, and F illustrate locomotion produced by injection into the PLS of GA (100 nmol in 1 ul, 0.1 M), D-L-Homocysteic acid (DL-HCA) (700 nmol in 7 ul, 0.1 M), PIC (25 nmol in 5 ul, 0.005 M) and substance P (SP) (2 nmol in 3 ul, 0.74 mM), respectively. E shows the loss of sustained PIC-induced locomotion produced with forelimb swinging following infusion of an equivalent quantity (25 nmol in 5 ul, 0.005 M) of GABA into the same site. G shows the location of infusion sites as obtained from electrolytic lesions in the different animals tested. Symbols as in Fig. 7.

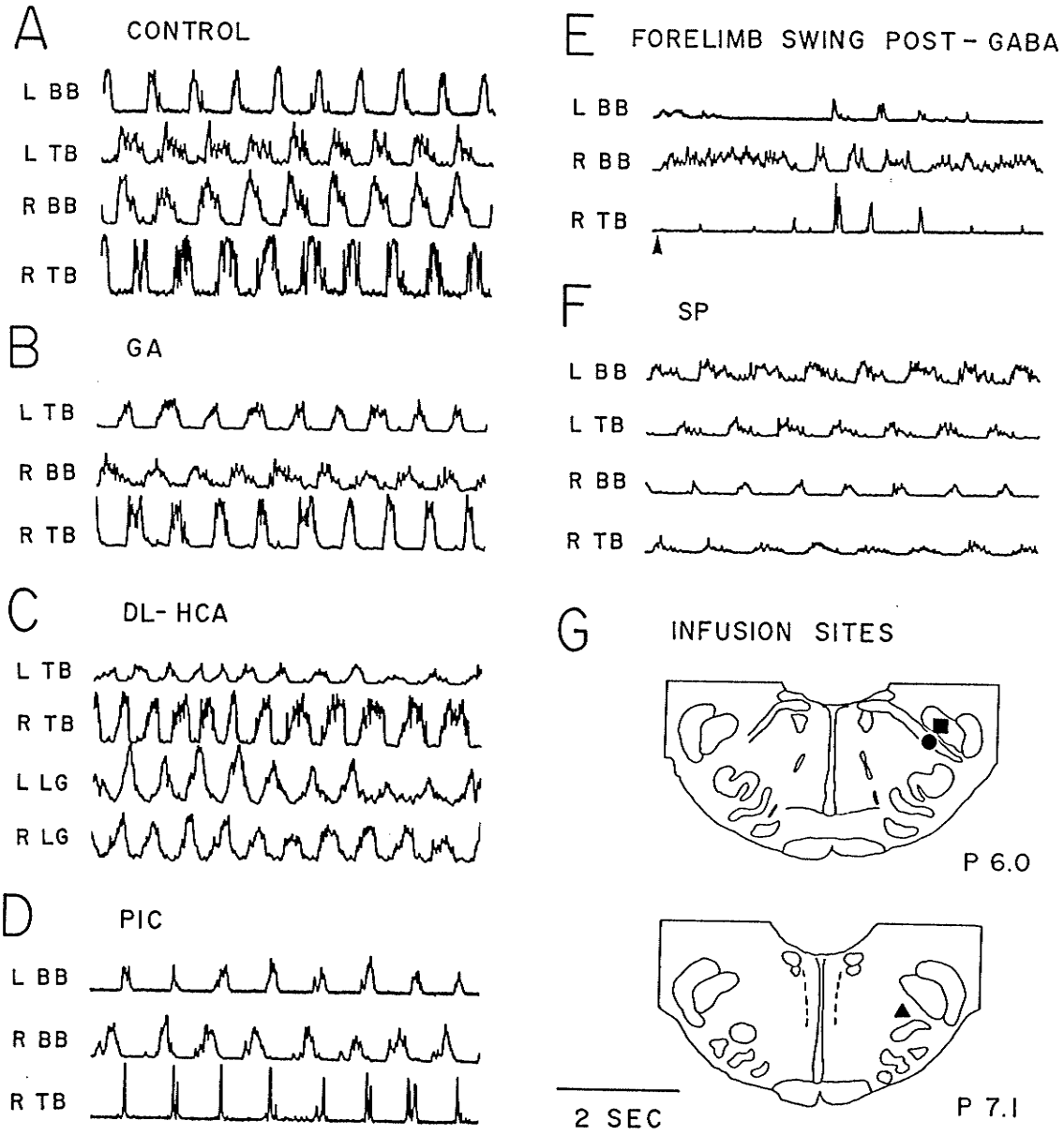

A
CONTROL
L BB
L TB
R BB
R TB
B
GA
L TB
R BB
R TB
C
DL- HCA
L TB
R TB
L LG
R LG
D
PIC
L BB
R BB
R TB
E
FORELIMB SWING POST - GABA
L BB
R BB
R TB
F
SP
L BB
L TB
R BB
R TB
G
INFUSION SITES
P 6.0
P 7.1
2 SEC

Figure 7. Schematic drawing of the surface of the brainstem from area (P3-P15) indicated in the inset to demonstrate the rostro- caudal and medio-lateral distribution of sites capable of producing locomotion after drug infusion. Sites producing decreases in the threshold for electrically induced locomotion or no response (NR) are also indicated. Sites capable of inducing locomotion are found close to the midline and also in the classically defined PLS. Injection sites producing decreased thresholds are found somewhat more lateral to the midline or within the PLS.

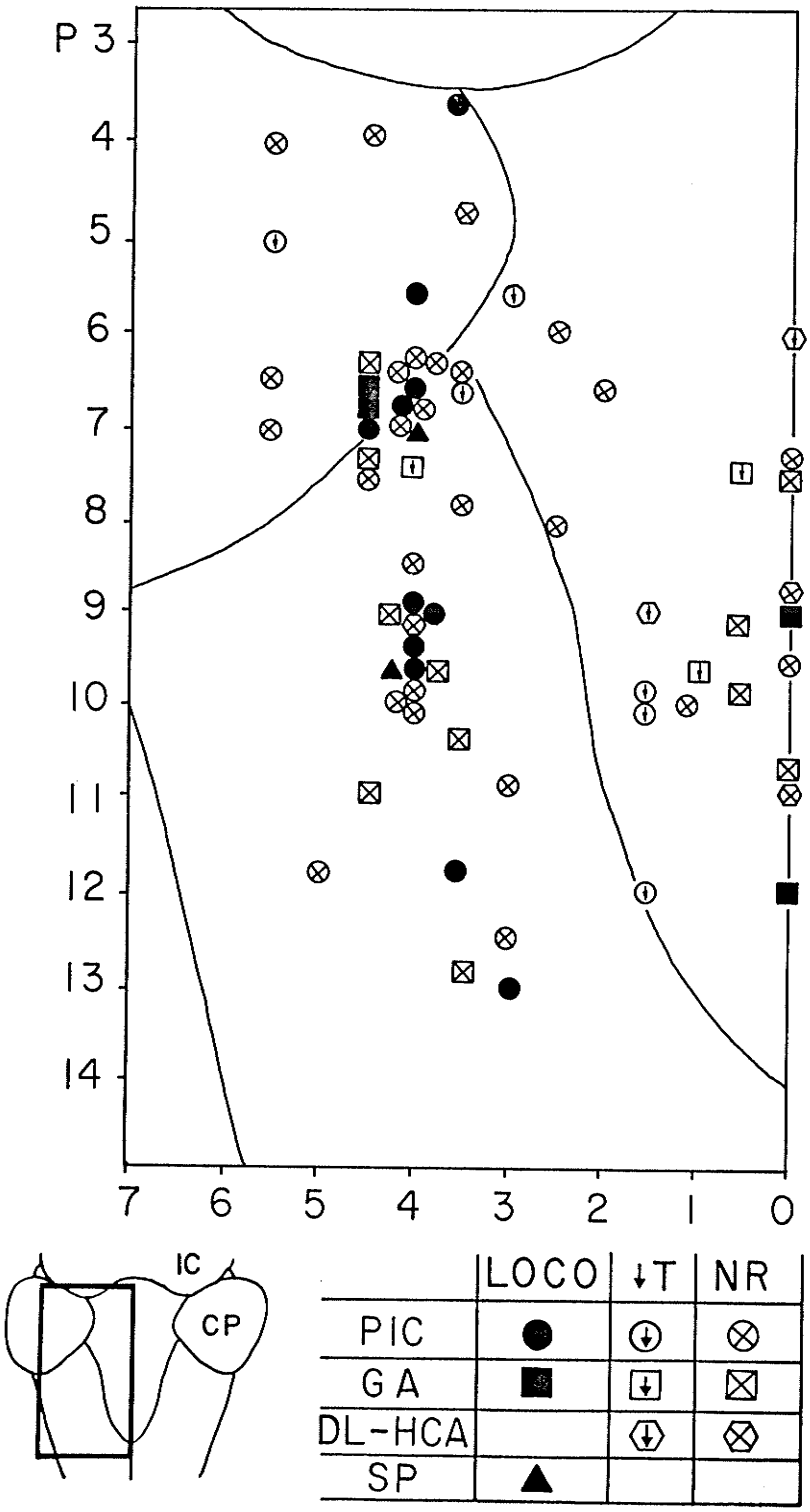

P 3
4
5
6
7
8
9
10
11
12
13
14
7
6
5
4
3
2
1
0
IC
CP
LOCO
↓T
NR
PIC
GA
DL-HCA
SP

Figure 8. Distribution of drug injection sites at different levels of the brainstem. Areas producing locomotion or decreases in the threshold for electrically induced locomotion following drug infusion are found within the raphe nuclei, FTG, and FTM or within the trigeminal spinal nucleus or adjacent FTL. Ineffective sites are located ventral and medial to the classically defined PLS. Injection sites producing locomotion with stimulation of the trigeminal receptive field (see text for explanation) are indicated by asterics. Symbols as in Fig. 7.

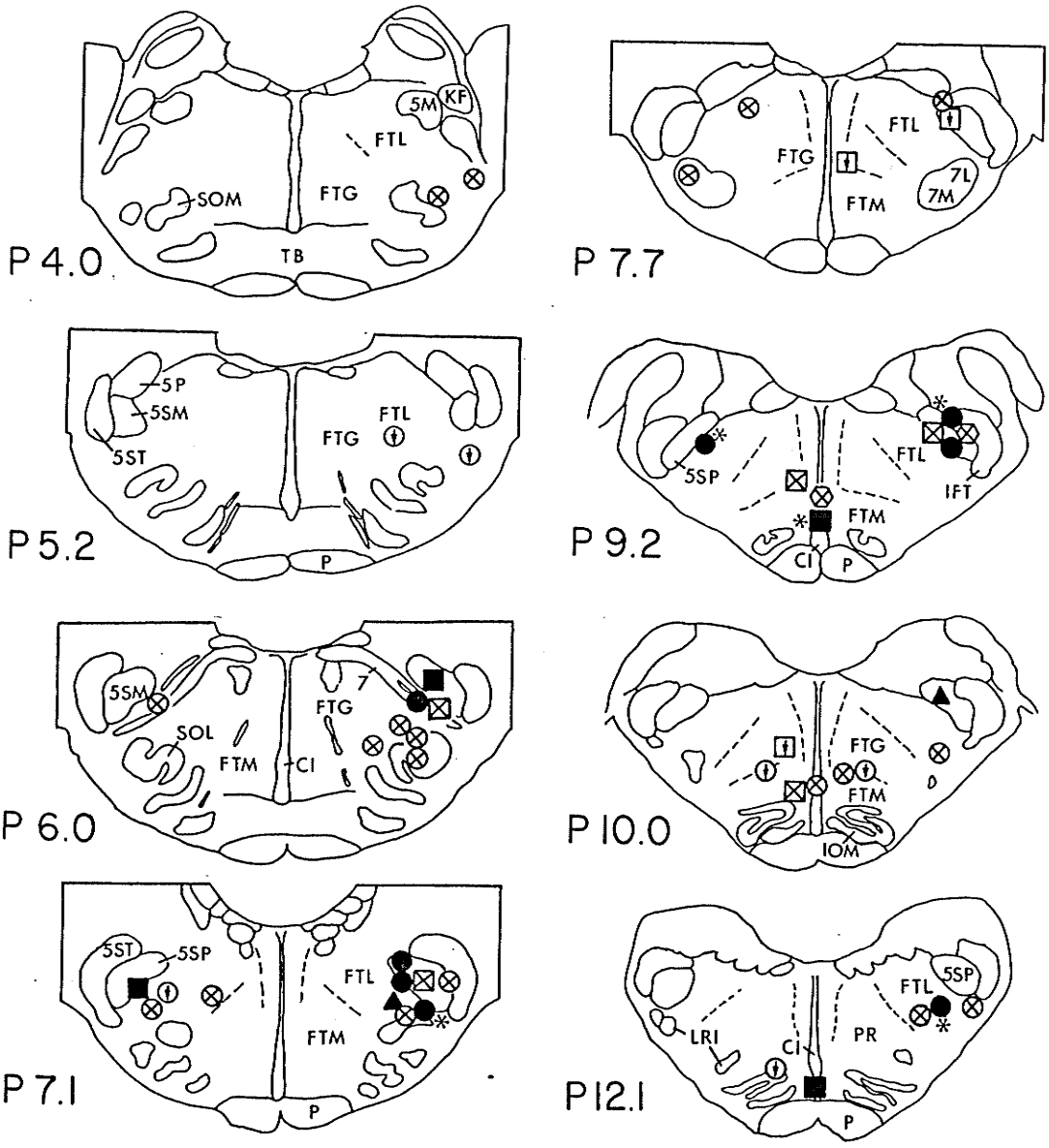

P 4.0
5M
KF
FTL
SOM
FTG
TB
P 7.7
FTG
FTL
7L
7M
FTM
P 5.2
5P
5SM
5ST
FTL
FTG
P
P 9.2
5SP
FTL
IFT
FTM
CI
P
P 6.0
5SM
SOL
FTM
CI
FTG
P 10.0
FTG
FTM
IOM
P 7.1
5ST
5SP
FTL
FTM
P
P 12.1
LRI
CI
PR
FTL
5SP
P

Figure 9. Picrotoxin infusion into the PLS facilitates locomotion produced by stimulation in the receptive field of the fifth nerve. A illustrates locomotion produced by electrical stimulation of the right PLS (P 12.1) in a site illustrated in N (filled circle). B shows spontaneous locomotion produced by injection of 15 nmol (in 3 ul, 0.005M) PIC into this site, which could be initiated (C) by passive forelimb movement (swing) during periods of quiescence. Panels D thru I show locomotion induced after PIC due to trigeminal receptive field stimulation (as indicated in each panel). Onset of stimulus indicated by the arrow heads. See text for details of onset latency for locomotion following the receptive field stimulation. Areas ineffective for the initiation of locomotion included the left pinna (J), left cornea (K), nape of neck (L), and chest (M). N shows that electrical stimulation or PIC injection failed to produce any observable effects in a site located 1.5 mm lateral (open circle) to the site producing locomotion with PIC infusion (filled circle).

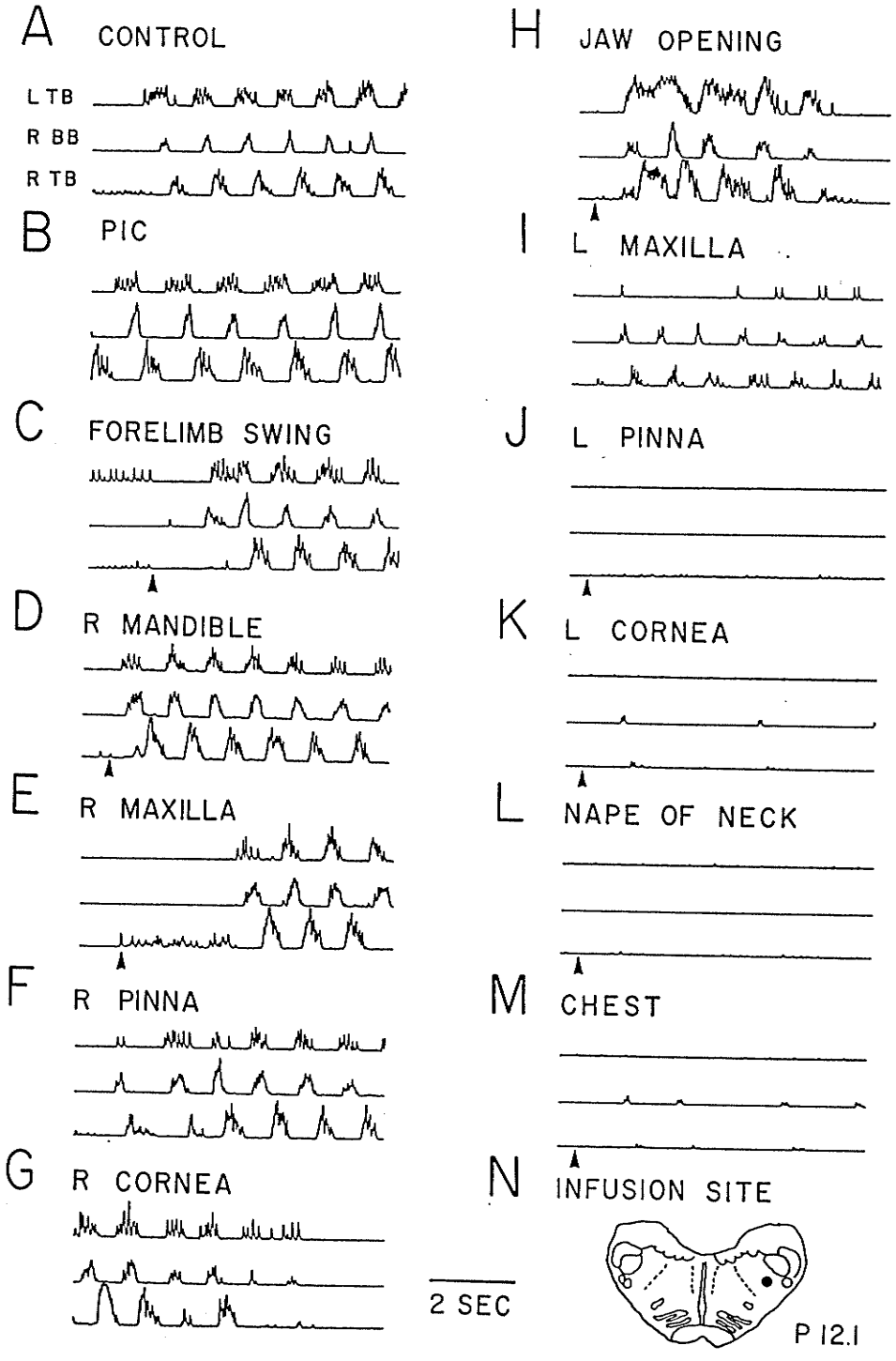

A CONTROL
L TB
R BB
R TB
B PIC
C FORELIMB SWING
D R MANDIBLE
E R MAXILLA
F R PINNA
G R CORNEA
H JAW OPENING
I L MAXILLA
J L PINNA
K L CORNEA
L NAPE OF NECK
M CHEST
N INFUSION SITE
2 SEC
P 12.1

Figure 10. MLR-evoked locomotion does not require the integrity of the dorsal spinal cord. A and C illustrate control runs of left and right MLR-evoked quadrupedal treadmill locomotion, respectively. Following a C2-C3 dorsal hemisection (F), left and right MLR sites (E) maintain their ability to produce locomotion (B and D, respectively) and at similar stimulation parameters. The spinal cord surface shown at the C3 level indicates the maximum extent of the lesion determined histologically. The lesion spanned 8 mm in total length. MLR stimulation parameters were: 40 Hz, 0.5 msec duration (all trials); 175 uA (A and C); 150 uA (B); and 200 uA (D). EMG recordings in A-D are in same order as listed in A (see methods for abbreviations).

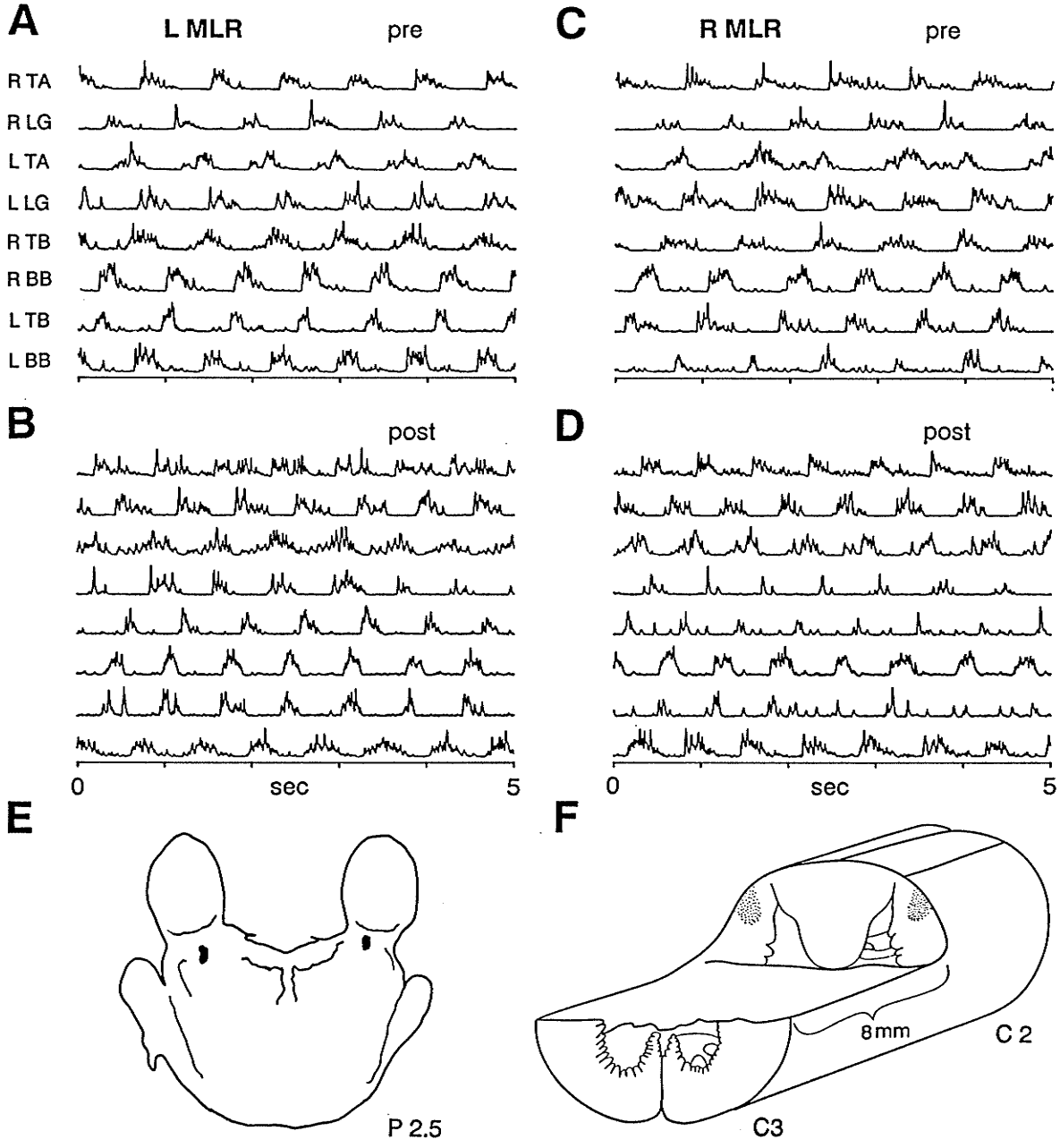

A
L MLR
pre
R TA
R LG
L TA
L LG
R TB
R BB
L TB
L BB
C
R MLR
pre
B
post
0
sec
5
D
post
0
sec
5
E
P 2.5
F
8mm
C 2
C3

Figure 11. Stimulation of the PLS may produce locomotion following an extensive dorsal hemisection of the spinal cord at the C2-C3 level. A and D show control bouts of locomotion produced by stimulation of the left and right PLS, respectively. Following the C2-C3 dorsal hemisection indicated in Fig. 10F, the left and right PLS still maintain their ability to produce hindlimb or forelimb locomotion (B and E, respectively) when electrically stimulated. Stimulation sites are illustrated in C and F. PLS stimulation parameters were: 40 Hz, 0.5 msec duration (all trials); 175 uA (A); 135 uA (B); 180 uA (C); and 200 uA (D).

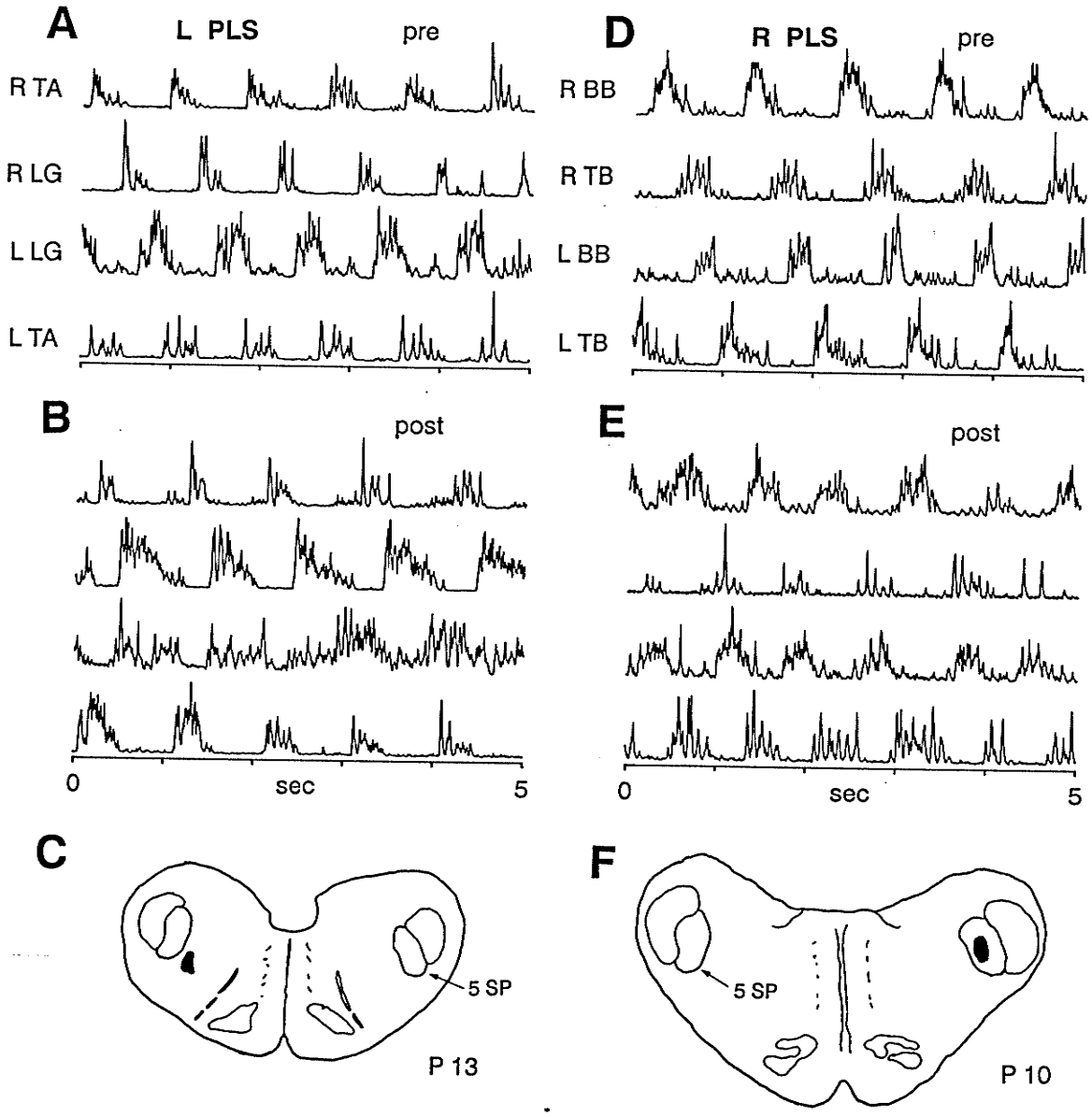

A
L PLS
pre
R TA
R LG
L LG
L TA
D
R PLS
pre
R BB
R TB
L BB
L TB
B
post
0
sec
5
E
post
0
sec
5
C
5 SP
P 13
F
5 SP
P 10

Figure 12. Summary of MLR and PLS-evoked locomotion before and after various subtotal spinal cord lesions (C2 or C3 levels). Each experiment indicated alphabetically. Lesions in A-C (and bottom lesion in D) are simple transections of the indicated regions. All other lesions are extended (rostral-caudal) for various indicated lengths. The maximum extent of each lesion is indicated for each experiment. The locomotion produced by MLR or PLS stimulation before and after each lesion is shown in separate columns on the right of each lesion. Stimulation of the left (L) or right (R) MLR or PLS is also indicated. Each limb is represented by quadrants of a circle: forelimbs are located in upper quadrants, hindlimbs in lower quadrants, left and right limbs by the left and right halves, respectively. Limbs illustrating locomotor movements are indicated schematically by the open areas; limbs not participating in locomotion are indicated by filled (black) quadrants. Limbs which show disrupted (but not completely abolished) alternation between flexion and extension movements (visually and as seen in EMG recordings) are indicated by the stippled quadrants.

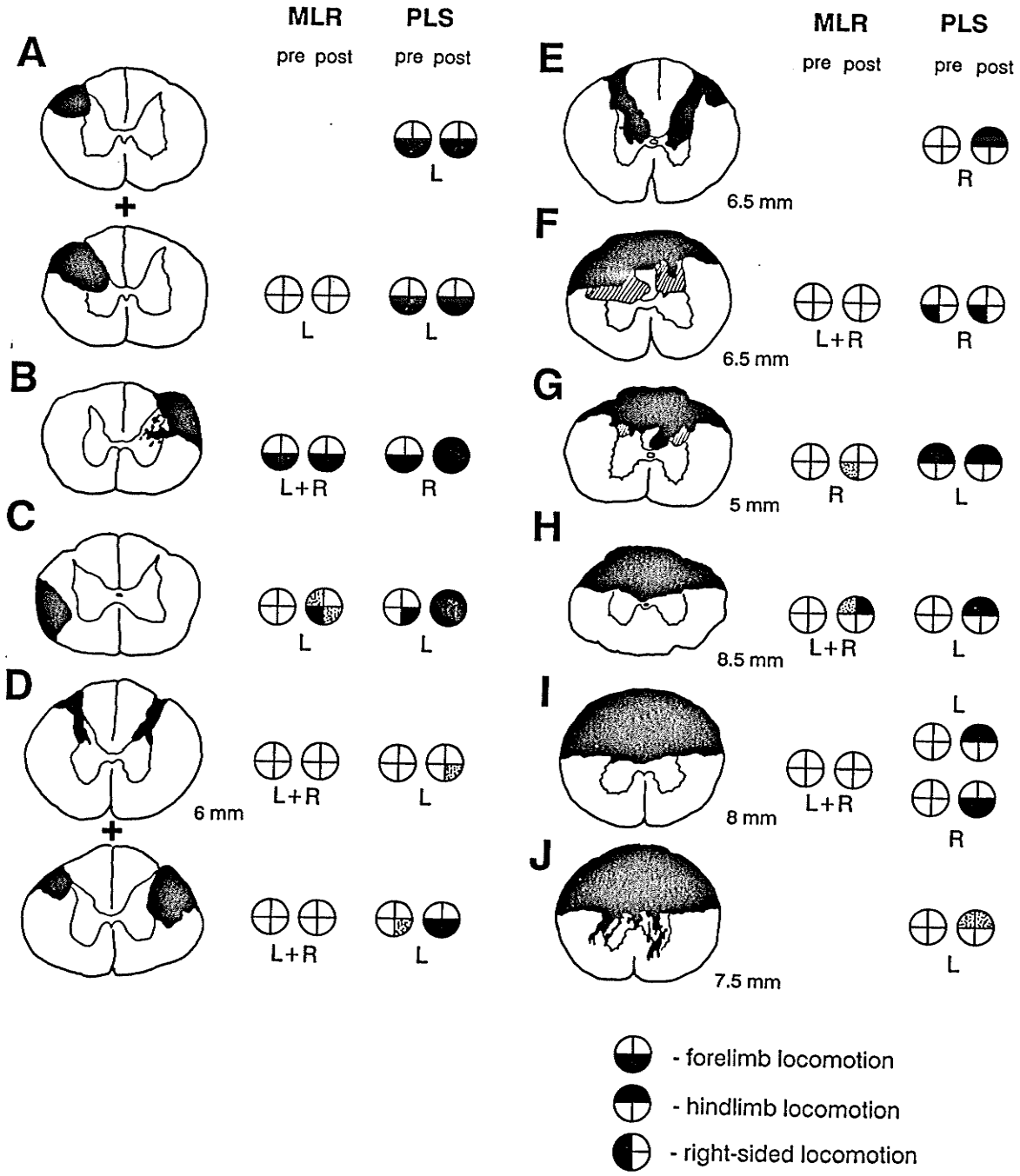

MLR
PLS
pre post
pre post
A
L
L
B
L+R
R
C
L
L
D
6 mm
L+R
L
L+R
L
E
6.5 mm
R
F
6.5 mm
L+R
R
G
5 mm
R
L
H
8.5 mm
L+R
L
I
8 mm
L+R
L
R
J
7.5 mm
L
- forelimb locomotion
- hindlimb locomotion
- right-sided locomotion

Figure 13. Cooling of the MRF to temperatures sufficient for block of synaptic transmission can block locomotion produced by stimulation of the PLS. Panels A-C illustrate the responses produced by stimulation of the right PLS site in D (P 9.5), prior to, during, and following local cooling of the MRF, respectively. MRF cooling site is indicated in D (P 12.7). PLS stimulation parameters were: 40 Hz, 0.5 msec duration (all trials); 160 uA (A); 200 uA (B); 195 uA (C). All EMGs are at the same gain.

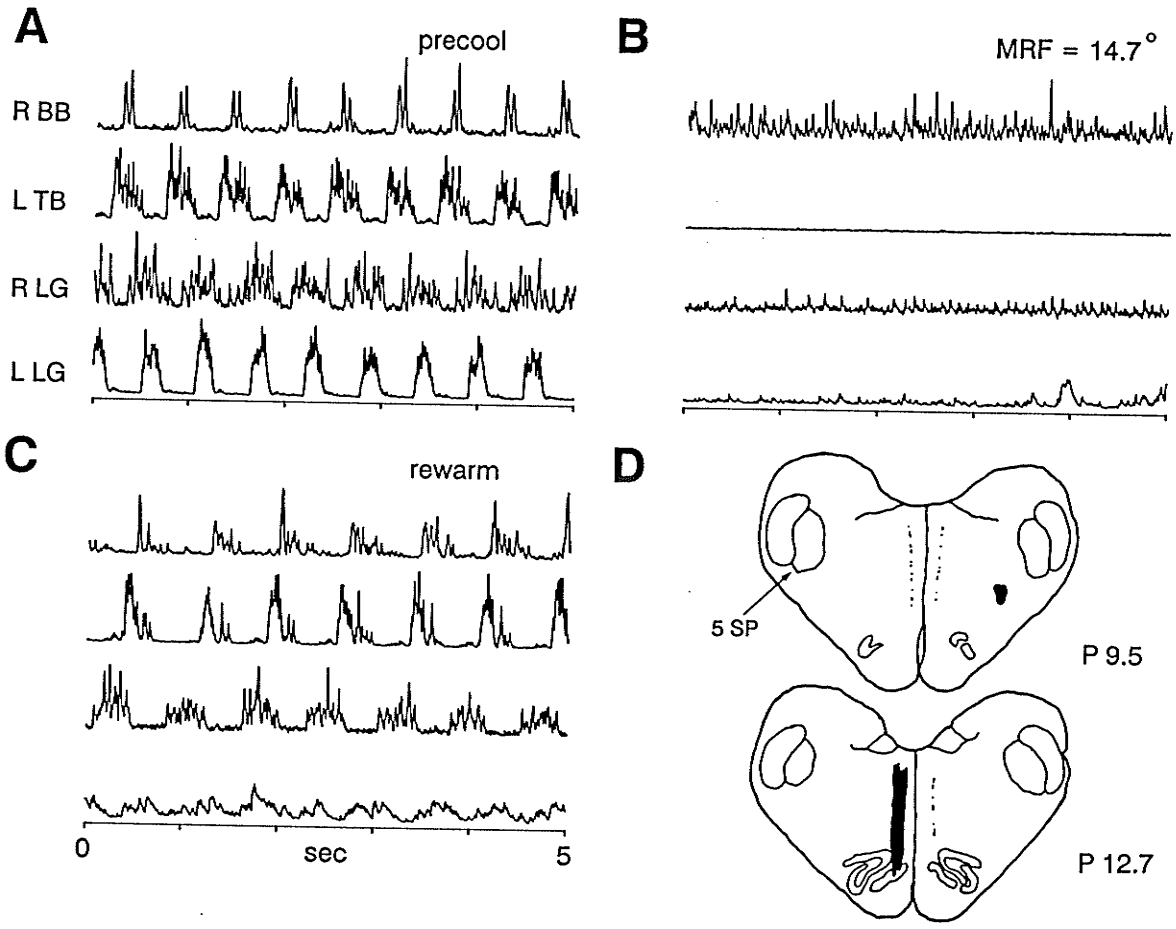

A
precool
R BB
L TB
R LG
L LG
B
MRF = 14.7°
C
rewarm
0
sec
5
D
5 SP
P 9.5
P 12.7

Figure 14. Isolation of the lateral portion of the brainstem does not abolish the ability of the MLR or the PLS to produce locomotion when stimulated. Locomotion was monitored using EMG recordings from both forelimb and hindlimb muscles. Panels A and C show the control bouts of locomotion produced by stimulation of the right MLR (125 uA) and the right PLS (100 uA), respectively. Stimuli delivered in both control trials were at 30 Hz and 1 msec duration. Following an extensive (P 4-12) longitudinal lesion placed approximately 2 mm lateral to the midline (with a transverse section extending to the lateral edge of the brainstem at the rostral end) stimulation of the MLR or the PLS was still capable of producing locomotion (B and D, respectively). The brainstem lesion is shown in E. Location of the stimulation sites for the MLR (P 2) and the PLS (P 9) are shown in E. Stimulation strengths for the production of locomotion following the brainstem lesion are 150 uA (B) and 190 uA (D) (both at 30 Hz and 1.0 msec duration).

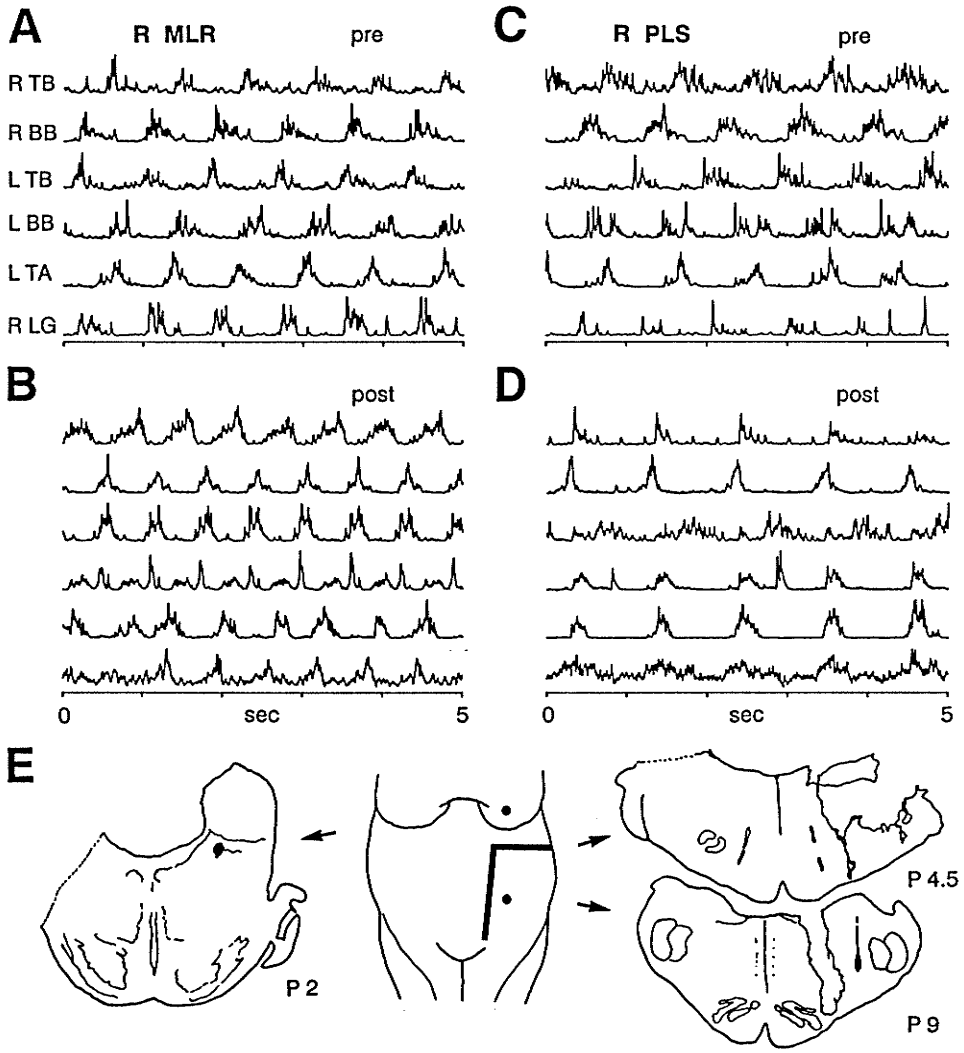

A
R MLR
pre
R TB
R BB
L TB
L BB
L TA
R LG
C
R PLS
pre
B
post
0
sec
5
D
post
0
sec
5
E
P 2
P 4.5
P 9

Figure 15. Short-latency EPSPs in a TA motoneuron (antidromic activation in A) produced by stimulation of the MLR. An 8 second trial of locomotion recorded intracellularly (top trace) and from an extensor ventral root filament (bottom trace) produced by stimulation of the right MLR (200uA, 17 Hz, 0.5 msec) is seen in panel B. The averaged LDP triggered at the onset of each rising phase of the LDP (self trigger) from 11 step cycles is illustrated in C (step cycle duration of 1272 +/- 128 msec). Modulation of the short-latency EPSP over 1 step cycle can be seen in panel D (MLR stimuli indicated by dots). Calibration pulse in D is 2 mV. In this example, the EPSP may be as large as 6 mV and varies in size throughout the depolarized phase of the LDP, even though the membrane potential remains at similar levels. Panels E and F show averaged signals (20 or more traces) (over a 30 msec window triggered just prior to the delivery of the MLR stimulus) obtained from the cord dorsum (E) and the motoneuron AC trace (F) grouped into 10 bins on the basis of their occurrence during the normalized step cycle. Lines 1, 2, 3, and 4 in panels E and F represent the MLR stimulus artifact, arrival of the descending volley at the cord dorsum recording electrode, onset, and peak amplitude of the MLR-evoked EPSP respectively. Lines 1 and 2 are unlabelled in panel E. Descending volley and segmental latencies are 4.0 and 2.3 msec, respectively. Calibration pulse in F and in all subsequent AC trace averages (unless otherwise indicated) is 2mV, 1 msec. The amplitude modulation (mean and standard deviation) of the EPSP (difference between onset and peak amplitude) with respect to the normalized step cycle is illustrated in G. H illustrates the peak EPSP amplitude measured on the basis of Em ($r=0.97$).

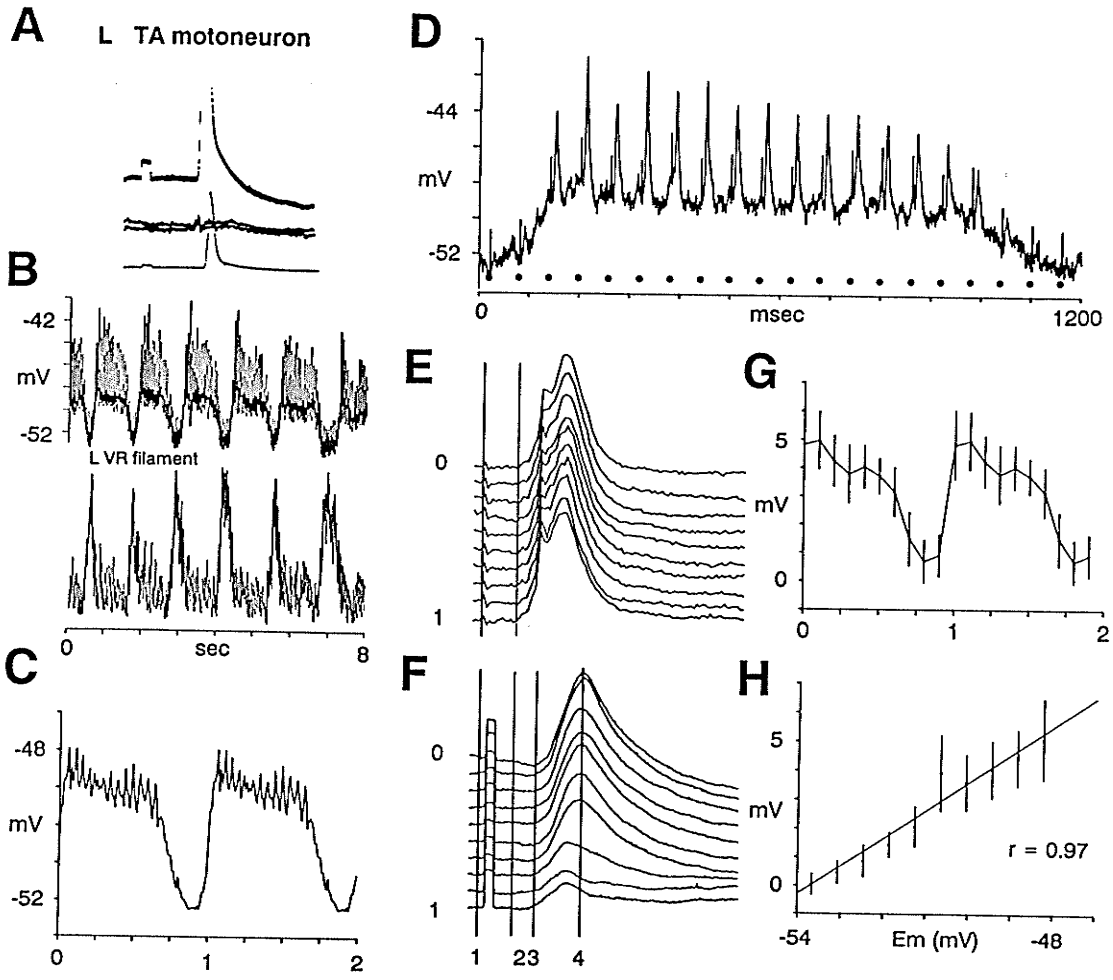

A
L TA motoneuron
B
-42
mV
-52
L VR filament
0
sec
8
C
-48
mV
-52
0
1
2
D
-44
mV
-52
0
msec
1200
E
0
1
F
0
1
1
23
4
G
5
mV
0
0
1
2
H
5
mV
0
r = 0.97
-54
Em (mV)
-48

Figure 16. Short-latency EPSP-IPSPs in a MG motoneuron (antidromic identification in A) produced by stimulation of the contralateral MLR. B illustrates the LDPs produced by stimulation of the right MLR (150 uA, 20 Hz, 1 msec) for a 5 second trial of locomotion. The averaged LDP (self trigger) from 18 step cycles is illustrated in C (step cycle duration of 786 +/- 46 msec). D illustrates averages of the AC-coupled intracellular recording over a 30 msec window triggered just prior to the delivery of the MLR stimulus. Each trace represents the average of 12-15 frames segregated on the basis of their occurrence during the normalized step cycle. Lines 1,2,3,4,5 represent the MLR stimulus artifact, arrival of the descending volley at the cord dorsum recording electrode, onset and peak amplitude of the EPSP, and peak amplitude of the IPSP, respectively. Descending volley and segmental latencies are 3.0 and 2.3 msec, respectively. Calibration bars in D are 1 mV, 5 msec. Modulation of the short-latency EPSP-IPSP over approximately one step cycle can be seen in panel E (MLR stimuli indicated by dots). In this example, the EPSP shows its largest amplitude in the depolarized phase of the LDP, while the IPSP is largest during the hyperpolarized phase of the LDP. Calibration pulse preceding the EPSP is 2 mV. The amplitude modulation (mean and standard deviation) of the EPSP (difference between onset and peak amplitude) with respect to the normalized step cycle and the Em ($r=0.86$) is illustrated in G and H, respectively. Amplitude variation of the IPSP (difference between lines 3 and 5 in panel D) with respect to the step cycle and Em ($r=0.93$) is seen in panels H and I, respectively.

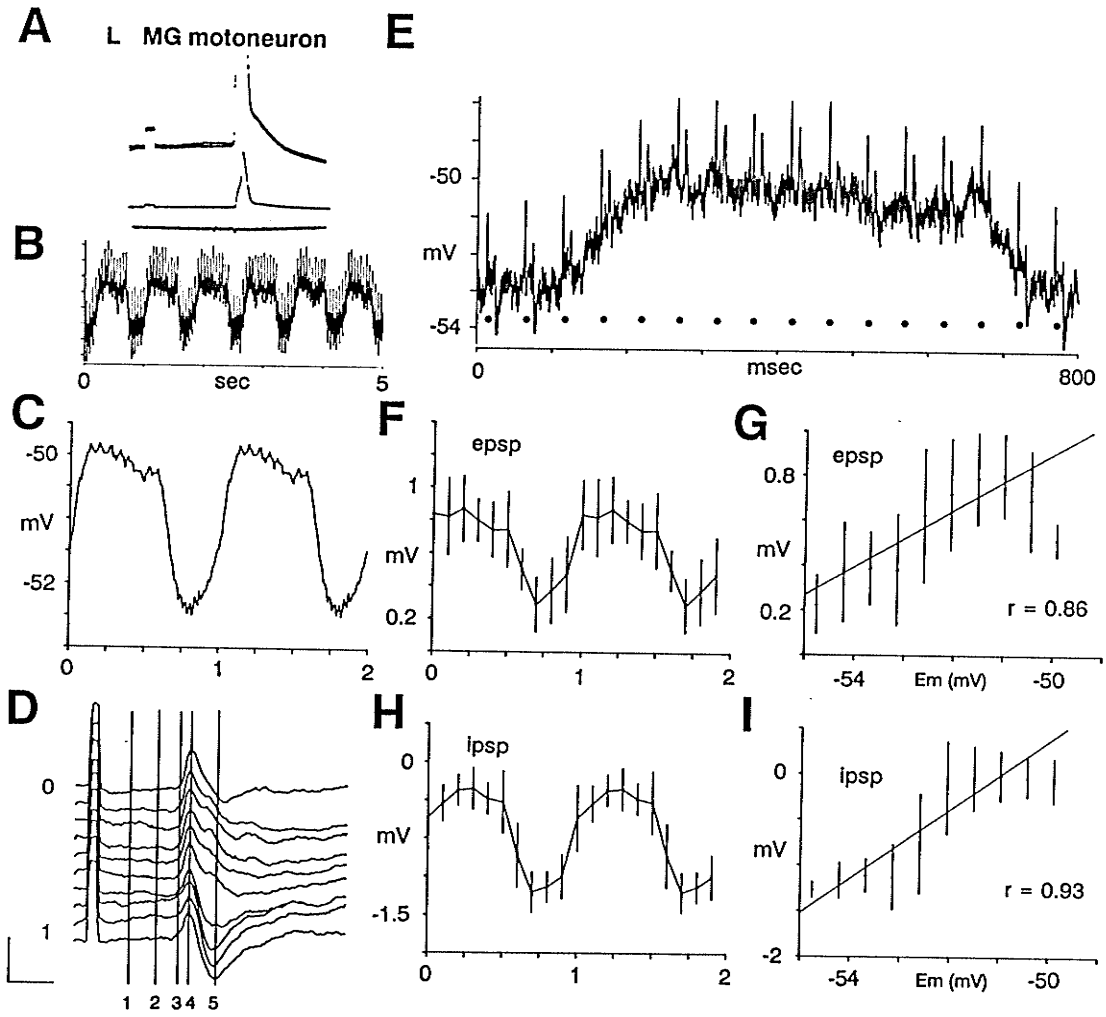

A
L MG motoneuron
B
0
sec
5
E
-50
mV
-54
0
msec
800
C
-50
mV
-52
0
1
2
F
epsp
1
mV
0.2
0
1
2
G
epsp
0.8
mV
0.2
r = 0.86
-54
Em (mV)
-50
D
0
1
1 2 3 4 5
H
ipsp
0
mV
-1.5
0
1
2
I
ipsp
0
mV
-2
r = 0.93
-54
Em (mV)
-50

Figure 17. Locomotor drive potentials and PSPs elicited in a TA motoneuron (antidromic activation in J) with variation in the strengths of stimulation of the same ipsilateral MLR site indicated in E. Stimulation strengths were 140 uA (16 Hz, 0.5 msec duration) and 180 uA (13 Hz, 1 msec duration) for the LDPs and PSPs illustrated in A-D and F-I, respectively. Five second periods of locomotion are shown in A and F. The averaged LDP (self trigger) from 7 step cycles is illustrated in B and G. The average step cycle durations for A and F are 1850 and 1702 msec, respectively. The depolarized phase of the LDP in panel B occupies 26 +/- 2 % of the normalized step cycle, whereas that illustrated in in panel G occupies 85 +/- 11% of the normalized step cycle. Averages of the PSPs (AC trace) (30 msec window) with respect to their occurrence during the normalized step cycle are illustrated in panels C and H. Lines 1,2,3,4 represent the MLR stimulus artifact, arrival of the descending volley at the cord dorsum recording electrode, onset, and peak amplitude of the EPSP, respectively. The descending volley latency and segmental latency for stimulation of this MLR site are 5.2 and 2.8 msec, respectively. Calibration bars in C and H are 1 mV, 5 msec and 2 mV, 5 msec, respectively. The amplitude modulation (mean and standard deviation) of the EPSP (difference between onset and peak amplitude) with respect to the normalized step cycle is illustrated in panels D and I. Increasing the strength of stimulation of the same MLR site prolongs the depolarized phase of the LDP, the occurrence of the stimulus-locked EPSPs, and increases the amplitude of the MLR- evoked EPSPs.

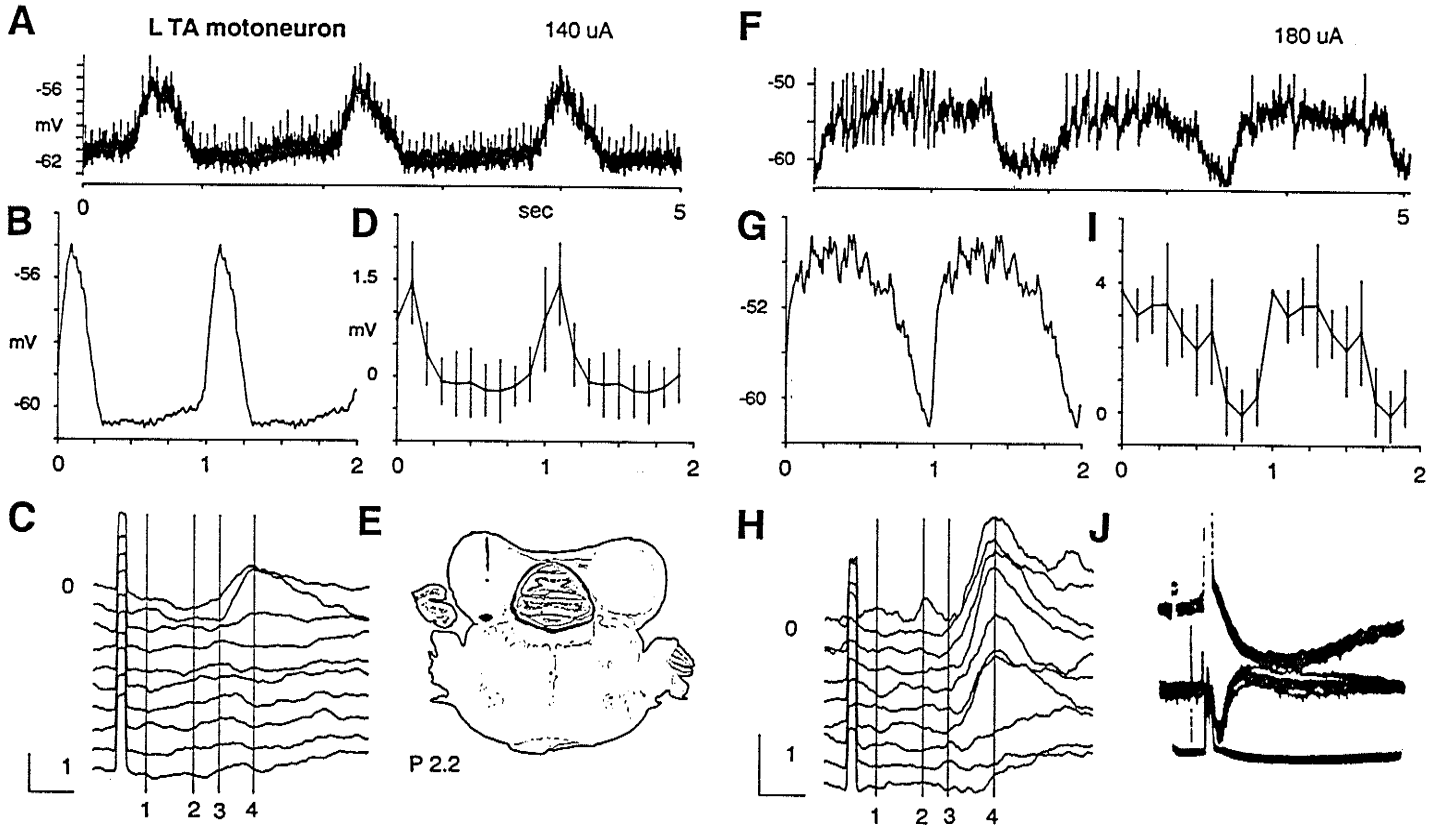

A
L TA motoneuron
140 uA
-56
mV
-62
0
sec
5
B
-56
mV
-60
0
1
2
D
1.5
mV
0
0
1
2
C
0
1
1 2 3 4
E
P 2.2
F
180 uA
-50
-60
5
G
-52
-60
0
1
2
I
4
0
0
1
2
H
0
1
1 2 3 4
J

Figure 18. Locomotor drive potentials and PSPs elicited in the same TA motoneuron as in Fig. 18, but with stimulation of a different MLR site on the same side of the brainstem (220 uA, 0.5 msec duration, 19 Hz). A five second period of locomotion is illustrated in A. The averaged LDP (self trigger) from 12 step cycles (step cycle duration of 1025 msec) is illustrated in B. The depolarized phase of the LDP occupies 54 +/- 10% of the normalized step cycle. C illustrate averages of the AC-coupled intracellular recording over a 30 msec window triggered just prior to the delivery of the MLR stimulus. Each trace represents the average of 20-28 frames segregated on the basis of their occurrence during the normalized step cycle. Lines 1,2,3,4 represent the MLR stimulus artifact, arrival of the descending volley at the cord dorsum recording electrode, onset, and peak amplitude of the EPSP, respectively. Total latency for the MLR site indicated in panel E is 1.0 msec shorter than that obtained for the site in Fig. 17E. A longer latency IPSP (arrow) is also present in the hyperpolarized phase of the LDP (bottom five traces) in panel C. Calibration bars in C are 2mV, 5 msec. The amplitude modulation (mean and standard deviation) of the EPSP (difference between onset and peak amplitude) with respect to the normalized step cycle is illustrated in panel D.

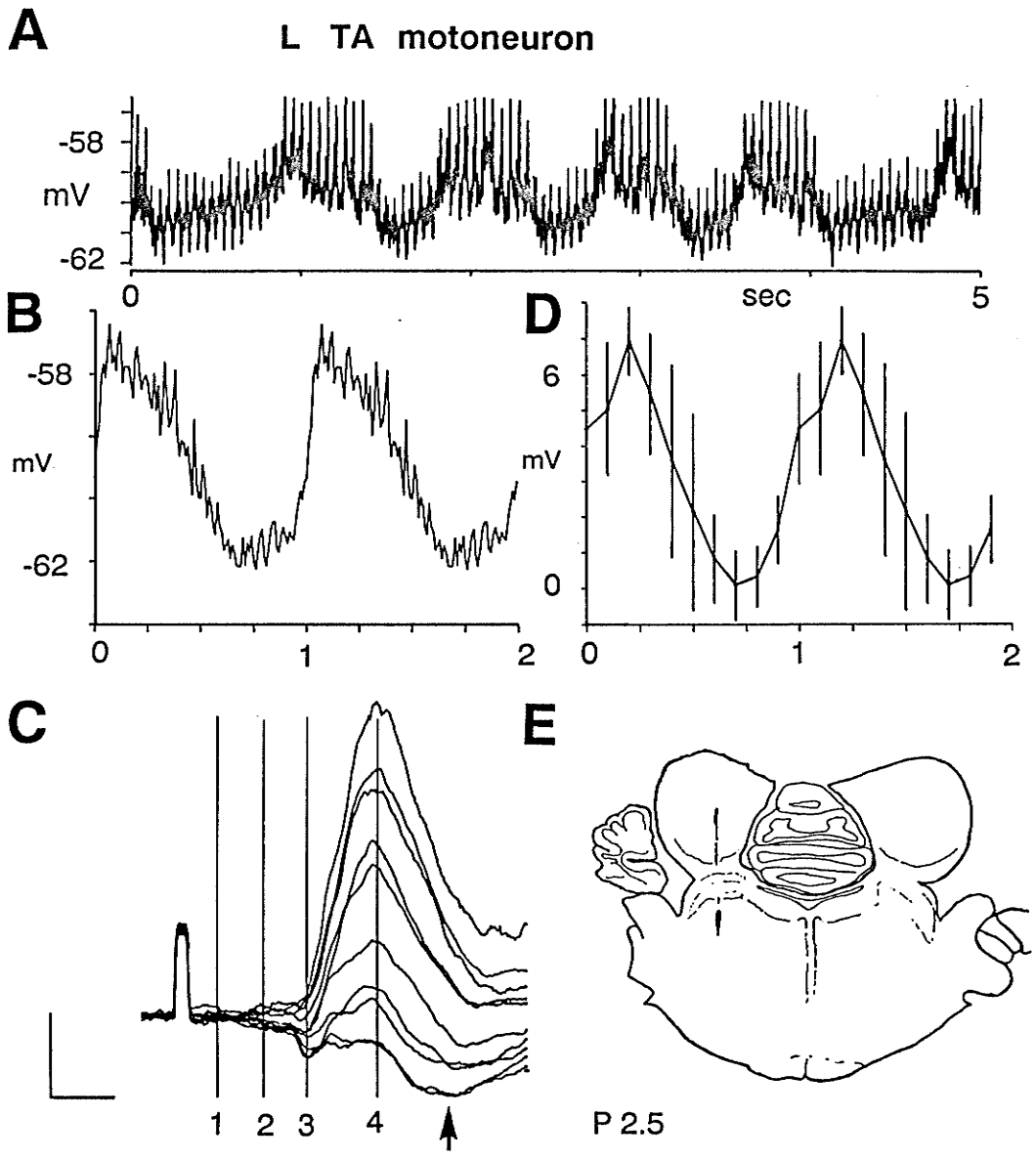

A
L TA motoneuron
-58
mV
-62
0
sec
5
B
-58
mV
-62
0
1
2
D
6
mV
0
0
1
2
C
1
2
3
4
E
P 2.5

Figure 19. Locomotor drive potentials and PSPs elicited in a TA motoneuron by separate stimulation of bilateral MLR sites. A-C illustrates the averaged PSPs and LDPs during a period (approximately 15 seconds) of locomotion produced by stimulation of the contralateral MLR site indicated in D (12 Hz, 220 uA, 1 msec). E-G illustrates the averaged PSPs and LDPs during a period (approximately 15 seconds) produced by stimulation of the ipsilateral MLR site indicated in H (10 Hz, 180 uA, 1 msec). The average step cycle durations for A-C and E-G are 1145 +/- 274 and 631 +/- 101 msec, respectively. A and E illustrate averages of the AC-coupled intracellular recording over a 30 msec window triggered just prior to the delivery of the MLR stimulus (approximately 30 frames/bin). Lines 1,2,3,4 represent the MLR stimulus artifact, arrival of the descending volley at the cord dorsum recording electrode, onset, and peak amplitude of the EPSP, respectively. Calibration bars in A and E are 2mV, 5 msec. Stimulation of the contralateral MLR results in a 0.3 msec longer segmental latency and different EPSP shape in this motoneuron. The amplitude modulation (mean and standard deviation) of the first EPSP with respect to the normalized step cycle is illustrated in panels B and F. The averaged LDP (self trigger) from each trial is illustrated in C and G. While both LDPs have similar burst durations, the amplitude of the LDP produced by stimulation of the contralateral MLR (panel C) is slightly larger.

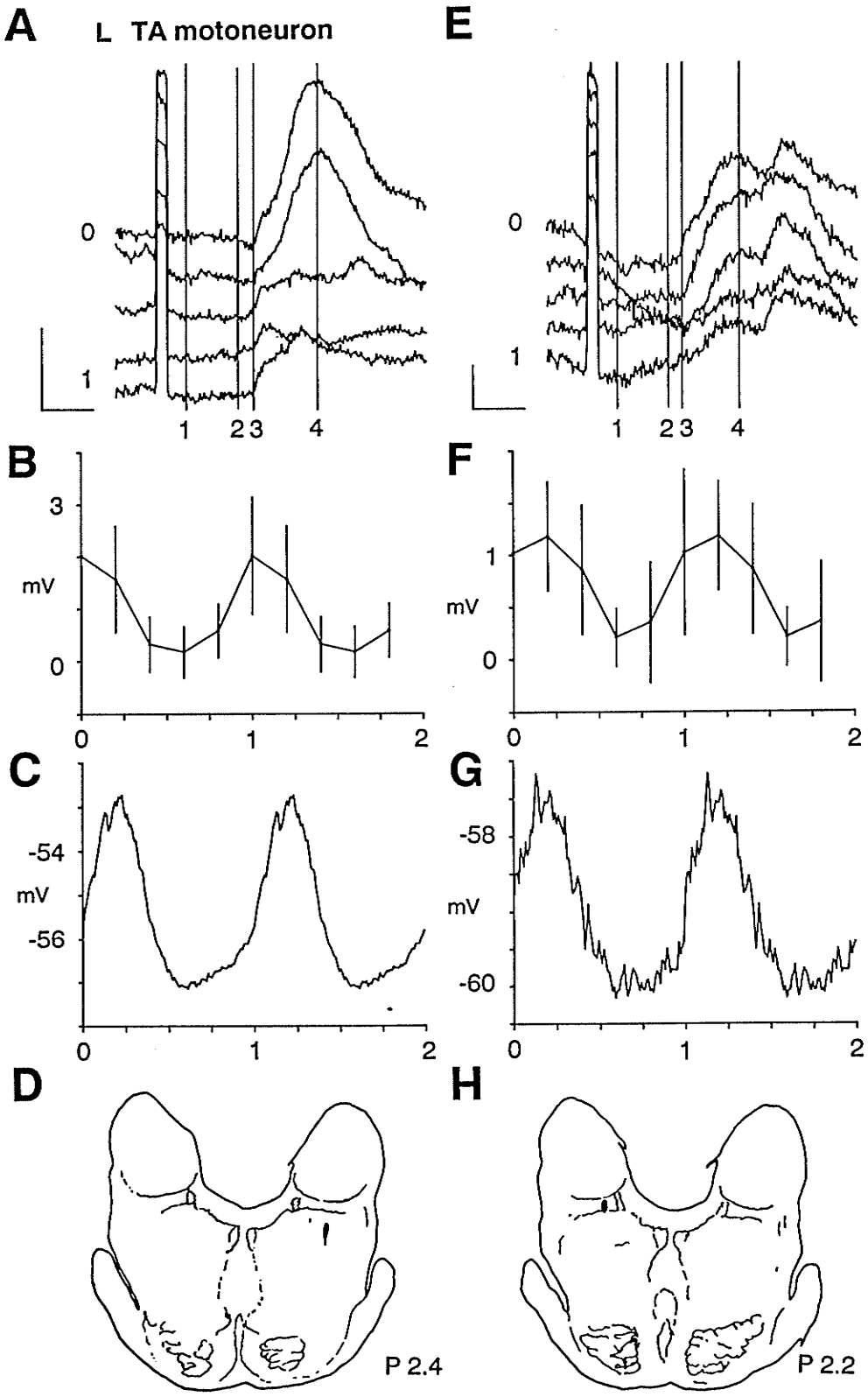

A
L TA motoneuron
0
1
1 2 3 4
E
0
1
1 2 3 4
B
3
mV
0
0 1 2
F
1
mV
0
0 1 2
C
-54
mV
-56
0 1 2
G
-58
mV
-60
0 1 2
D
P 2.4
H
P 2.2

Figure 20. Locomotor drive potentials and PSPs elicited in a SM motoneuron by separate stimulation of bilateral MLR sites. Two different PSP types are observed in this cell. Responses of this cell to stimulation (16 Hz, 220 uA, 1 msec) of the ipsilateral MLR site (E) are indicated in A-D. Responses to stimulation (15.6 Hz, 200 uA, 1 msec) of the contralateral MLR (J) are seen in panels F-I. Panels A and F illustrate the LDPs over periods of 600 msec or 4 sec (inset). The MLR-evoked PSPs can be seen following each calibration pulse (2 mV, 1 msec). During the depolarizing phase of the LDP (A) short-latency EPSPs are only occasionally seen (*) following each MLR stimulus (dots). B and G illustrate averages of the AC-coupled intracellular recording over a 30 msec window triggered just prior to the delivery of the MLR stimulus (25-30 frames/bin). Averages are segregated on the basis of their occurrence during the normalized step cycle. Lines 1,2,3,4 represent the MLR stimulus artifact, onset of the EPSP, and peak amplitude of the EPSP and IPSP, respectively. Total latency to the onset of the first EPSP was shorter (0.6 msec) for the ipsilateral MLR stimulus. Calibration pulses in A, B, F and G are 2 mV, 1 msec. Calibration bars in B and G are 2 mV, 5 msec. The amplitude modulation (mean and standard deviation) of the first EPSP with respect to the normalized step cycle is illustrated in panels C and H. No significant modulation of the EPSP with respect to the step cycle is present in C. D and I illustrate the peak IPSP amplitude measured on the basis of Em. The IPSP produced by stimulation of the ipsilateral (left) MLR is largest in the depolarized phase in contrast to the IPSP seen with stimulation of the contralateral (right) MLR.

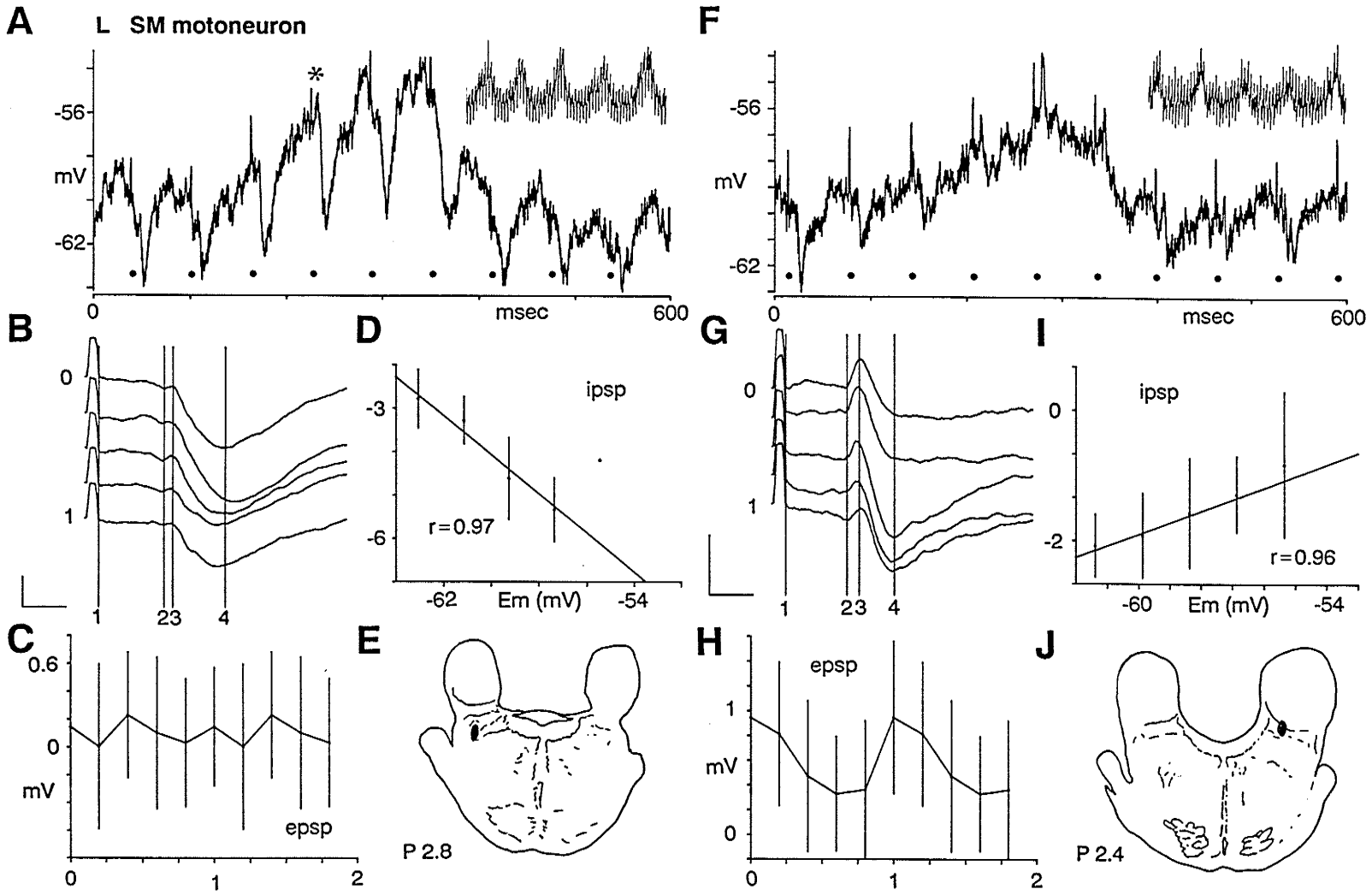

A
L SM motoneuron
-56
mV
-62
0
msec
600
F
-56
mV
-62
0
msec
600
B
0
1
1
23
4
D
ipsp
-3
-6
r=0.97
-62
Em (mV)
-54
G
0
1
1
23
4
I
ipsp
0
-2
r=0.96
-60
Em (mV)
-54
C
0.6
0
mV
epsp
0
1
2
E
P 2.8
H
epsp
1
mV
0
0
1
2
J
P 2.4

Figure 21. Locomotor drive potentials and PSPs elicited in an AB motoneuron by stimulation (17 Hz, 200 uA, 1 msec) of the ipsilateral MLR. 600 msec of the onset of the depolarized phase of one LDP is illustrated in A (MLR stimuli indicated with dots). Action potentials may be seen occurring from the shortest latency EPSP (5.9 msec) and also from a longer latency EPSP (approximately 27-30 msec following the MLR stimulus). Large IPSPs may also be seen following the short-latency EPSP in some cases. The averaged LDP (self trigger) during a 6 second trial of locomotion illustrated in B. Panel C illustrates the averages of the AC-coupled intracellular recording over a 30 msec window triggered just prior to the delivery of the MLR stimulus. Each bin represents the average of 20-32 frames segregated on the basis of their occurrence during the normalized step cycle. Lines 1,2, and 3 represent the onset, and peak amplitude of the EPSP, and the peak amplitude of the longer latency IPSP, respectively. The MLR stimulus artifact is indicated with an arrow. The averaged short latency EPSP is very small even though large short-latency EPSPs may be seen in raw records (see A). See text for further explanation. Bottom trace in C is averaged (155 frames) extracellular recording to illustrate field potentials produced during MLR stimulation. Calibration pulse in A and C is 2mV, 2 msec. Calibration bars in C are 2 mV, 5 msec. The amplitude modulation (mean and standard deviation) of the EPSP with respect to the normalized step cycle is illustrated in panels D. The EPSP is modulated but with high variability. Panel E illustrate the peak IPSP amplitude measured on the basis of Em. The IPSP is largest in the depolarized phase (r=.91).

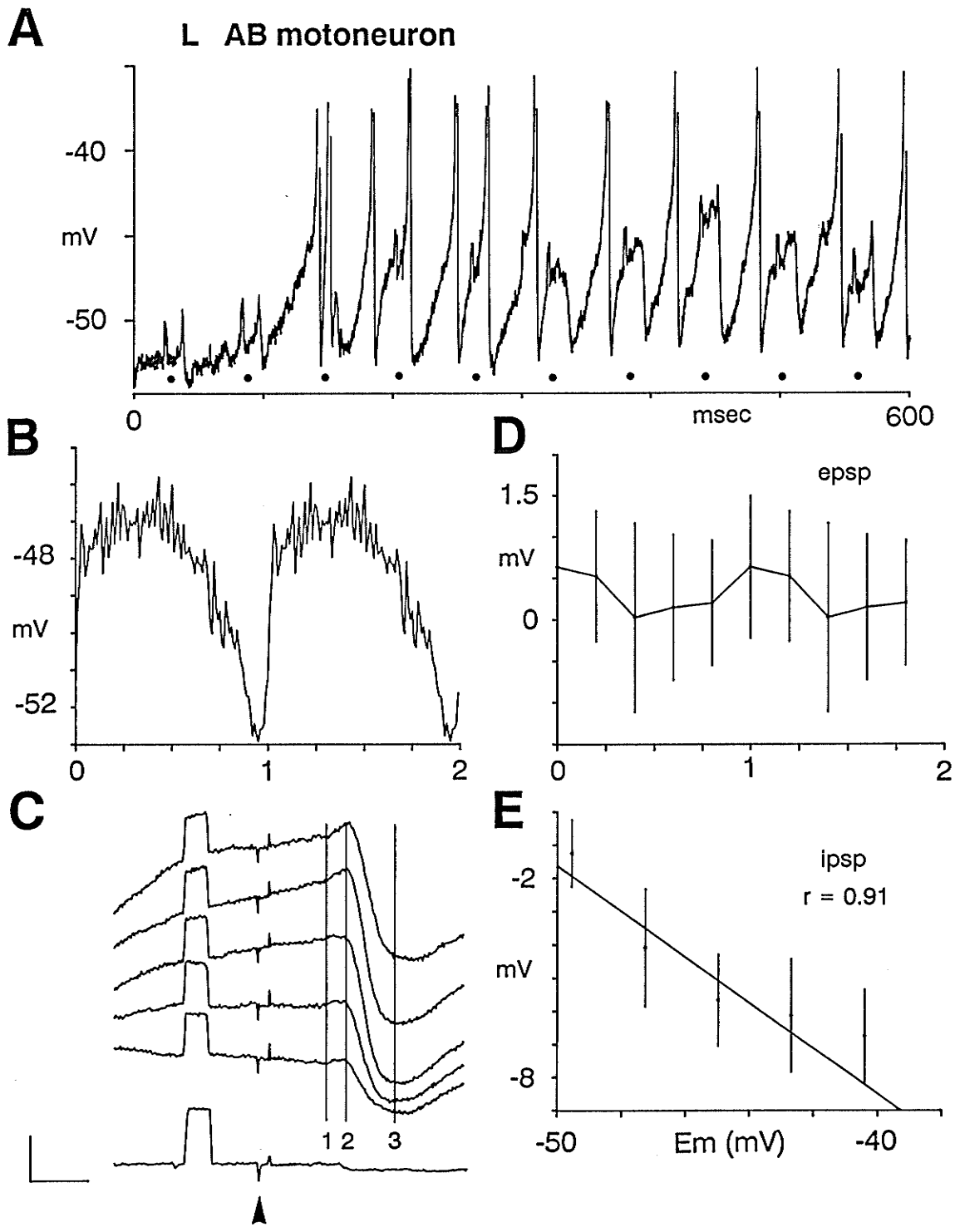

A
L AB motoneuron
-40
mV
-50
0
msec
600
B
-48
mV
-52
0
1
2
C
1 2 3
D
epsp
1.5
mV
0
0
1
2
E
ipsp
r = 0.91
-2
mV
-8
-50
Em (mV)
-40

Figure 22. MLR stimulus-dependent and independent motoneuronal spiking as observed with intracellular (A, B) and extracellular (ENG) (C- E) recording electrodes. MLR stimuli (dots) are indicated in each panel. Locomotion trials are from different experiments. Recordings are from a SMAB (A) and a TA (B) motoneuron, and from TA ENGs (C-E). In panel A, the action potentials primarily occur at variable latencies following the MLR stimuli (independent). In panel B, however, each action potential occurs at a fixed latency (triggered from the rising phase of the short-latency EPSP) following stimulation of the MLR. A gradation from independent to dependent spiking in a group of motoneurons (ENG) is observed in panels C-E. In D and E, spiking begins to occur in discreet bursts of activity. Calibration pulses in A and B are 2 mV, 1 msec.

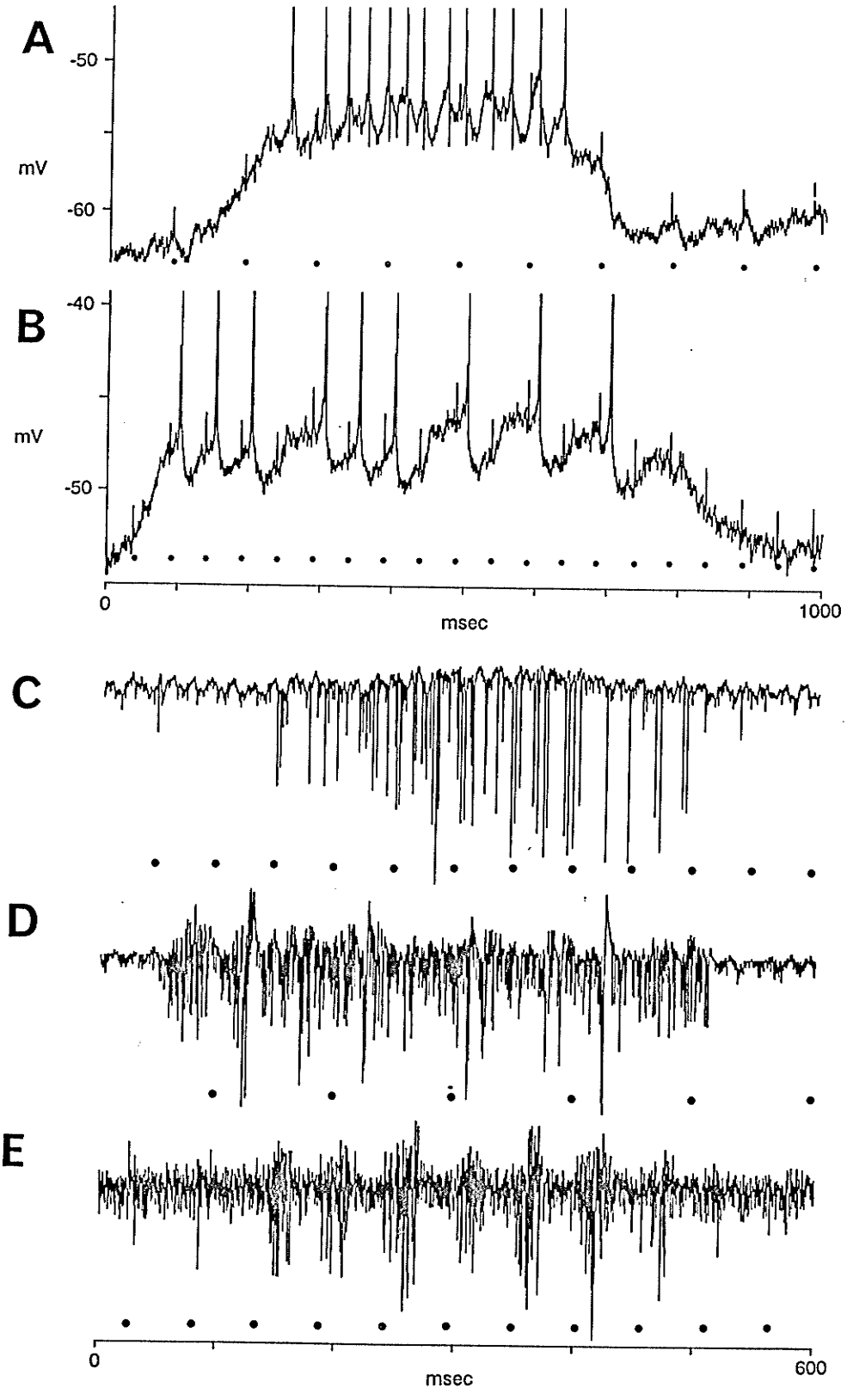

A
-50
mV
-60
B
-40
mV
-50
0
1000
msec
C
D
E
0
600
msec

Figure 23. Timing relationships between MLR stimulus-locked spiking (ENG) and PSPs (intracellular) for unilateral agonists and antagonists. The top trace (A-C) is the intracellular recording from an unidentified extensor motoneuron which received monosynaptic EPSPs from stimulation of the PBST nerve. The bottom trace illustrates the raw ENG from L TA. A 10 sec trial of locomotion is illustrated in A. B shows the activity observed for a 800 msec period during the complete hyperpolarized phase of one LDP. Small stimulus-locked EPSP-IPSPs can be seen following each MLR stimulus (dots). The activity of the largest single unit from the antagonist ENG is locked to the MLR stimulus. A 50 msec window is illustrated in C for one stimulus (arrow). Latencies from the MLR stimulus to the onset of the EPSP, IPSP (estimated), and single unit (indicated by lines 1, 2, 3, respectively) are 7.4, 9.0, and 10.2 msec, respectively. Note in B that the activity of some smaller (non stimulus-locked) TA units occur at similar times that IPSPs are observed in the intracellular record. Panels D-F illustrate the activity of an unidentified extensor motoneuron (top trace) (received EPSPs from stimulation of the FDL nerve) and the raw ENGs from ipsilateral MG (second trace) and SM (bottom trace) nerves. An 8 sec trial of locomotion is illustrated in D. E shows the activity observed for a 800 msec period and includes the depolarized phase of the one LDP. Large stimulus-locked EPSPs can be seen following each MLR stimulus (dots). A 30 msec window is illustrated in F for one stimulus (arrow). Latencies from the MLR stimulus to the onset of the EPSP, and single units in MG and SM (indicated by lines 1, 2, 3, respectively) are 7.6, 10.5, and 9.4 msec, respectively.

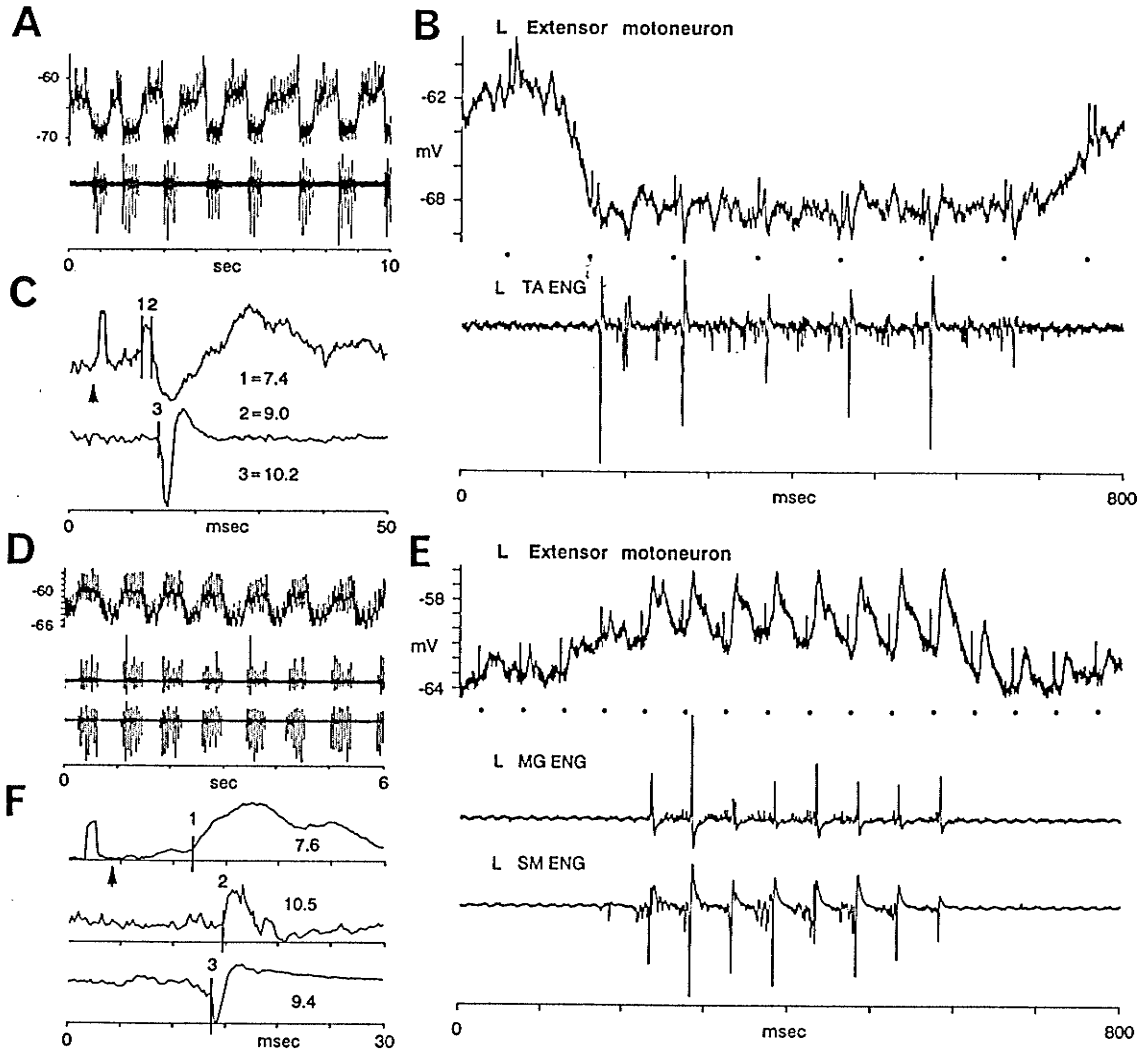

A
-60
-70
0
sec
10
B
L Extensor motoneuron
-62
mV
-68
L TA ENG
0
msec
800
C
12
1=7.4
3
2=9.0
3=10.2
0
msec
50
D
-60
-66
0
sec
6
E
L Extensor motoneuron
-58
mV
-64
L MG ENG
L SM ENG
0
msec
800
F
1
7.6
2
10.5
3
9.4
0
msec
30

Figure 24. Reciprocal organization between excitation and inhibition of strict antagonists during locomotion induced by stimulation of the MLR. Top and bottom waveforms are intracellular recording from a left MG motoneuron (ankle extensor) and extracellular recording from a left TA nerve (ankle flexor) during locomotion produced by stimulation of the right MLR (10 Hz, 140 uA, 1 msec duration), respectively. Loss (or reduction) of rhythmic inhibition (hyperpolarizing phase of the LDP) in the MG motoneuron is associated with the loss (or reduction) of rhythmic excitatory output of the TA motoneuronal pool as recorded in the TA nerve. Rhythmic excitation (depolarizing phase of the LDP) of the MG motoneuron is still present during these sustained plateaus of depolarization since the cell still continues to spike in a rhythmic fashion separated by periods of non-spiking.

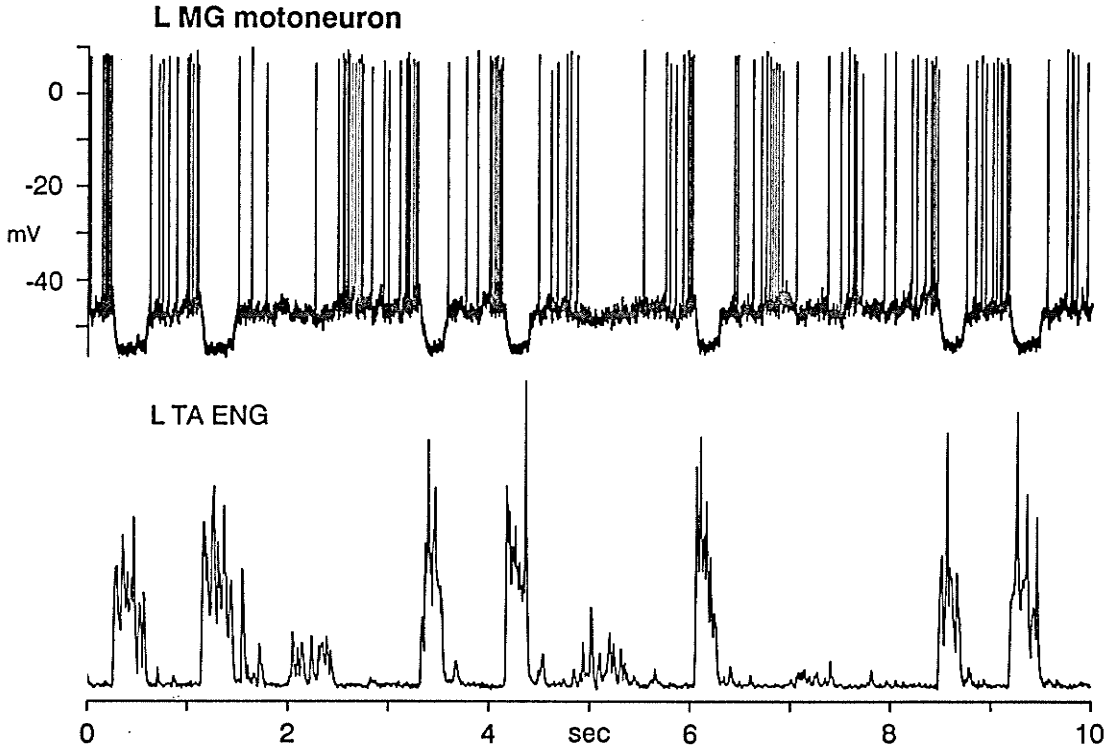

L MG motoneuron
0
-20
mV
-40
L TA ENG
0
2
4
sec
6
8
10

Figure 25. Recording from a spinal interneuron during fictive locomotion produced by stimulation of the contralateral MLR (18.8 Hz, 160 uA, 0.5 msec duration). Location of cell (lamina 7) in the rostral L7 spinal segment is indicated in A. The cell was rhythmically active and out of phase with the ipsilateral TA ENG (A). The activity of this neuron during one of the bursts for a period of 200 msec is illustrated in B. C shows an averaged response over a 60 msec window from 210 triggered sweeps occurring within the rhythmic bursts. MLR stimulus is indicated by dots in B and C. As observed in B and C, spiking is induced in this cell at a fixed latency (6.6 msec) following the delivery of each stimulus to the MLR. Other, variable latency action potentials are also seen in this cell following the MLR stimulus. Calibration pulses in B and C are 2 mV, 1 msec.

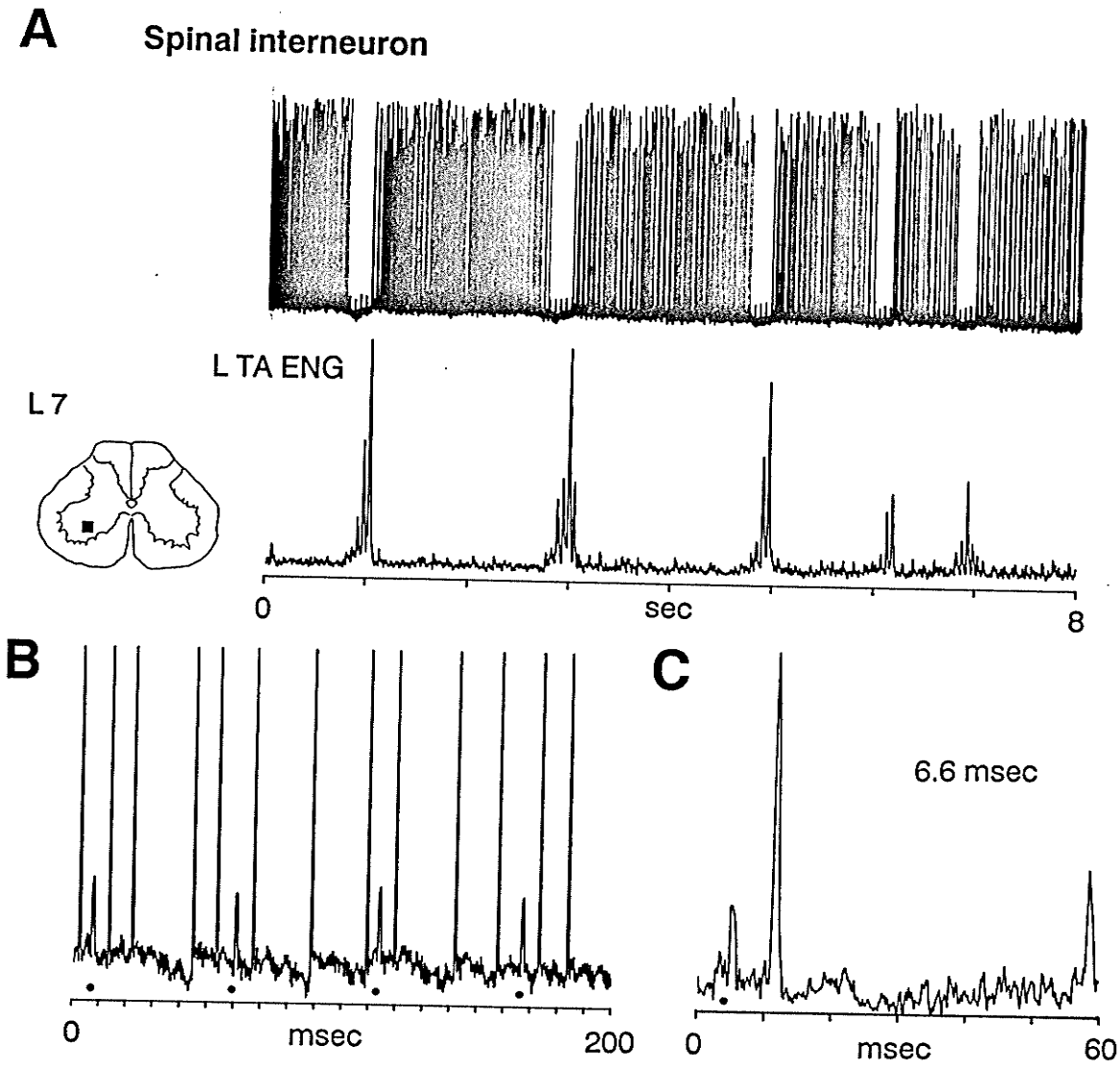

A
Spinal interneuron
L TA ENG
L 7
0
sec
8
B
0
msec
200
C
6.6 msec
0
msec
60

Figure 26. Responses of various spinal interneurons to stimulation of the MLR. Locomotion induced by stimulation of the ipsilateral MLR (1) (10 Hz, 110 uA, 1 msec) can be seen in A for a flexor-related spinal neuron and the ipsilateral (left) TA ENG. The averaged response (mean total latency of 8.1 msec) of the neuron to stimulation of the MLR is shown in frame 2. Frame 3 in panel A illustrates the response of the cell to stimulation (10 T) of the ipsilateral sural nerve. This cell also responded to stimulation of the ipsilateral FDL (10 T) and posterior tibial nerves and to stimulation of the contralateral sural and superficial peroneal nerves. The cell shown in panel B discharged in long bursts (200-400 msec) of activity to the delivery of a single train of 4 stimuli MLR (90 Hz, 190 uA, 0.5 msec pulse duration) every second (frame 1). These bursts were separated by periods of low activity. A single burst is illustrated in frame 2. MLR stimuli are indicated in the lower trace in B1 and B2. The cell was also capable of displaying rhythmic activity with a tonic stimulation (20 Hz, 190 uA, 0.5 msec duration) of the MLR (not illustrated). Location of the cell is shown in panel D (open box). C illustrates the responses of a flexor-related spinal neuron to stimulation of the MLR (20 Hz, 200 uA, 1 msec duration). The interbursts periods were separated by decreased tonic activation of this neuron. Short-latency (6.7 +/- 1.3 msec), stimulus-locked spikes can be seen following some of the MLR stimuli (dots) (C2) during one of these interburst periods. The locations of 9 rhythmically active spinal neurons in the L7 spinal segment is shown in D.

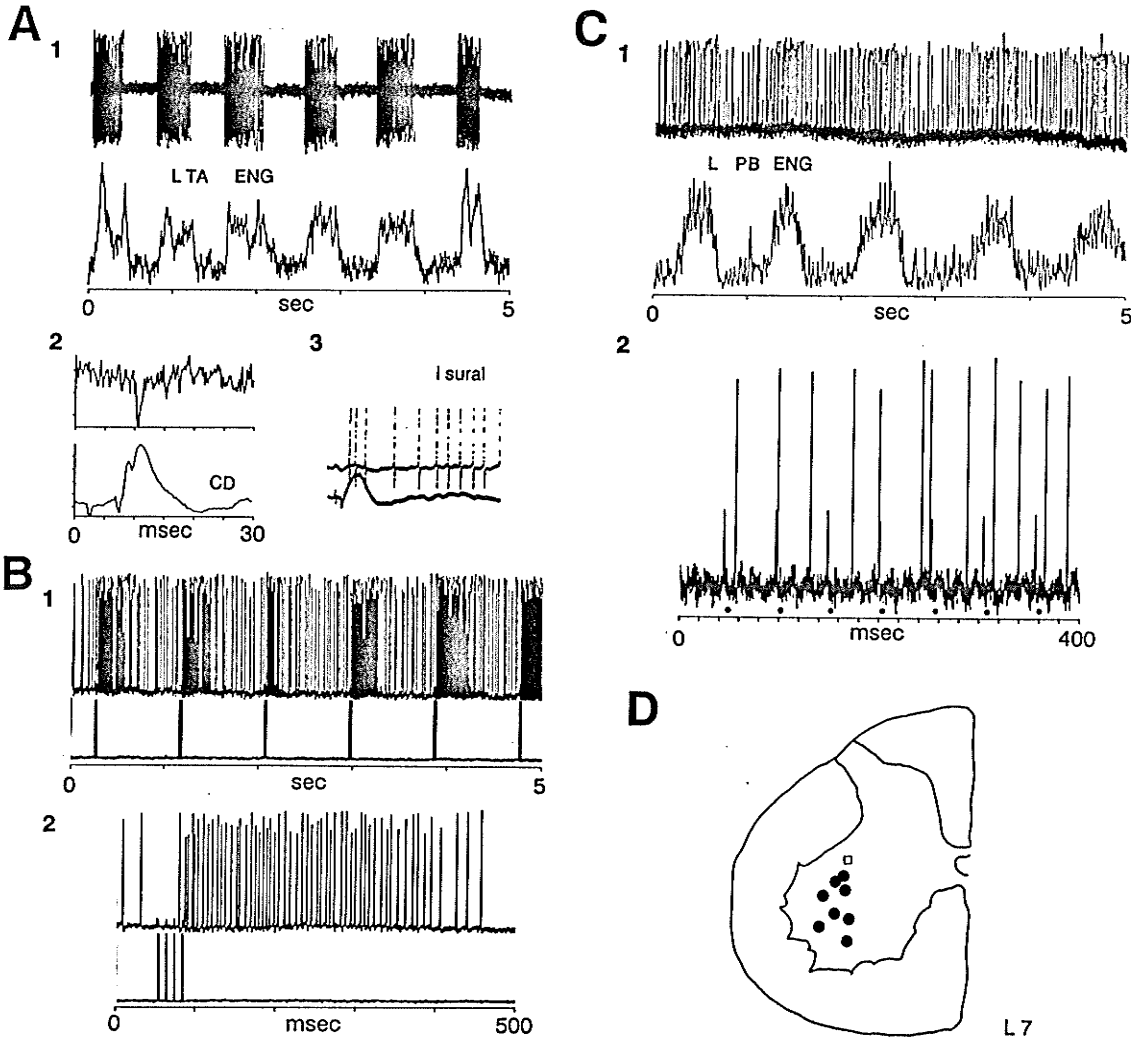

A
1
L TA ENG
0
sec
5
2
CD
0
msec
30
3
I sural
B
1
0
sec
5
2
0
msec
500
C
1
L PB ENG
0
sec
5
2
0
msec
400
D
L 7

Figure 27. Loss of MLR-evoked fictive locomotion by cooling of the MRF. Locomotion is monitored by intracellular recording from a left SMAB motoneuron (top trace), an ipsilateral extensor- related L7 ventral root (VR) filament (middle trace), and a left TA ENG (bottom trace). Locomotion is reduced (B) and finally abolished (C) by cooling of the MRF (P9, L0, probe tip 1.5 mm from bottom) to 13 and 11 degrees C, respectively. All traces are at constant gain, throughout. Motoneuron action potentials are truncated for illustrative purposes. Locomotion was produced by stimulation of the right MLR (20 Hz, 180 uA, 0.5 msec duration; stimulation strength in C was 200 uA) (P2, L4, H=-1.6).

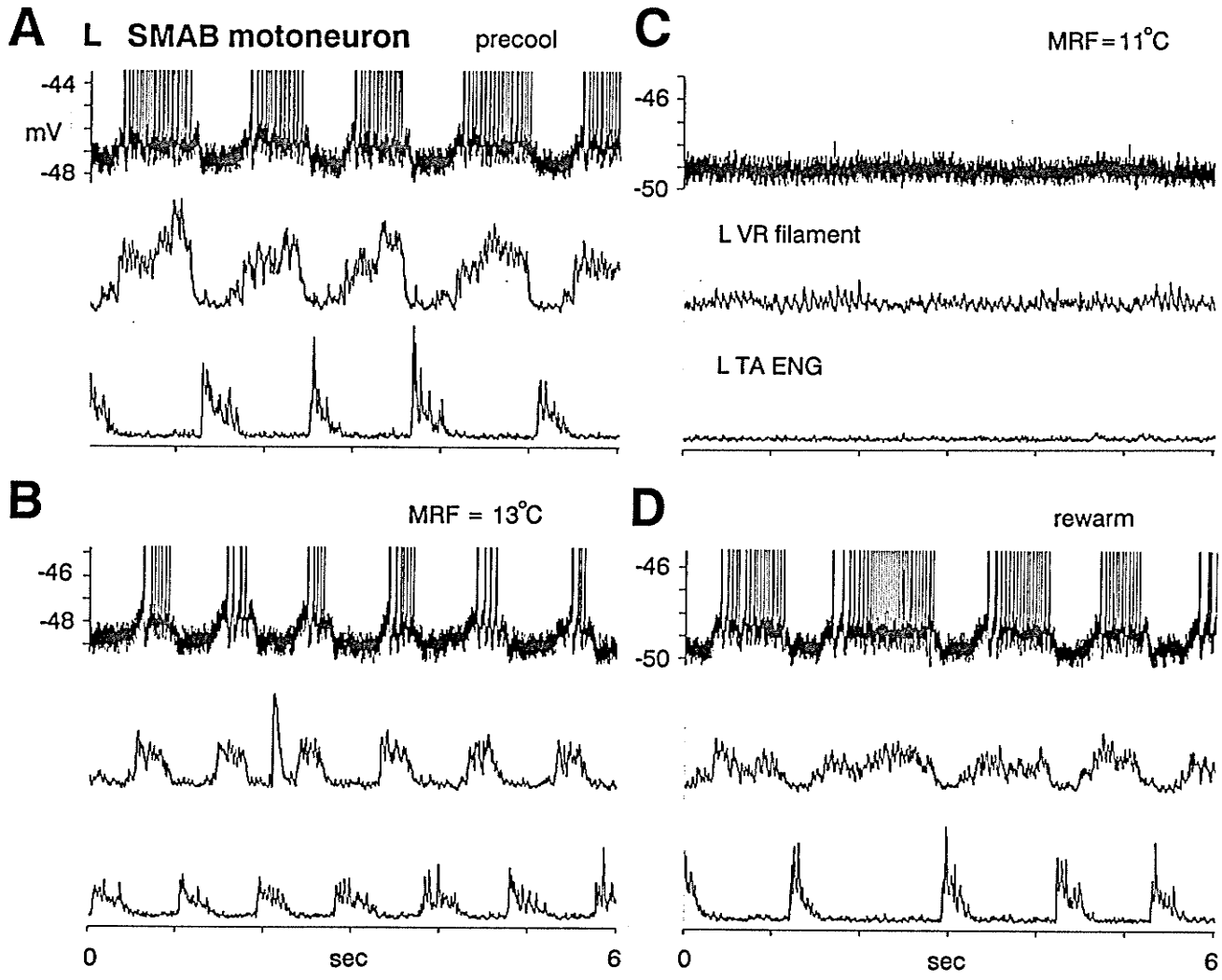

A
L SMAB motoneuron
precool
-44
mV
-48
C
MRF=11°C
-46
-50
L VR filament
L TA ENG
B
MRF = 13°C
-46
-48
D
rewarm
-46
-50
0
sec
6
0
sec
6

Figure 28. Concurrent reduction in the amplitude of the LDP and MLR-evoked short-latency PSPs in a left FDL motoneuron during cooling of the MRF. Locomotion produced by stimulation of the right MLR (P2.8, L3.7, H=-0.8) is shown in A, B, C (top trace: left FDL motoneuron; middle trace: ipsilateral TA ENG; bottom trace: contralateral SM ENG). All traces are at constant gain, throughout. MLR-evoked PSPs averaged into 5 bins on the basis of their occurrence during the normalized step cycle is seen in D, E, and F for the MRF precool, cool, and rewarm trials, respectively. A reduction in the amplitude of the ENGs, LDPs and PSPs is evident with cooling of the MRF (P 10, L 0) to probe temperatures of 14 degrees. Calibration bars in D-F are 1 mV, 5 msec.

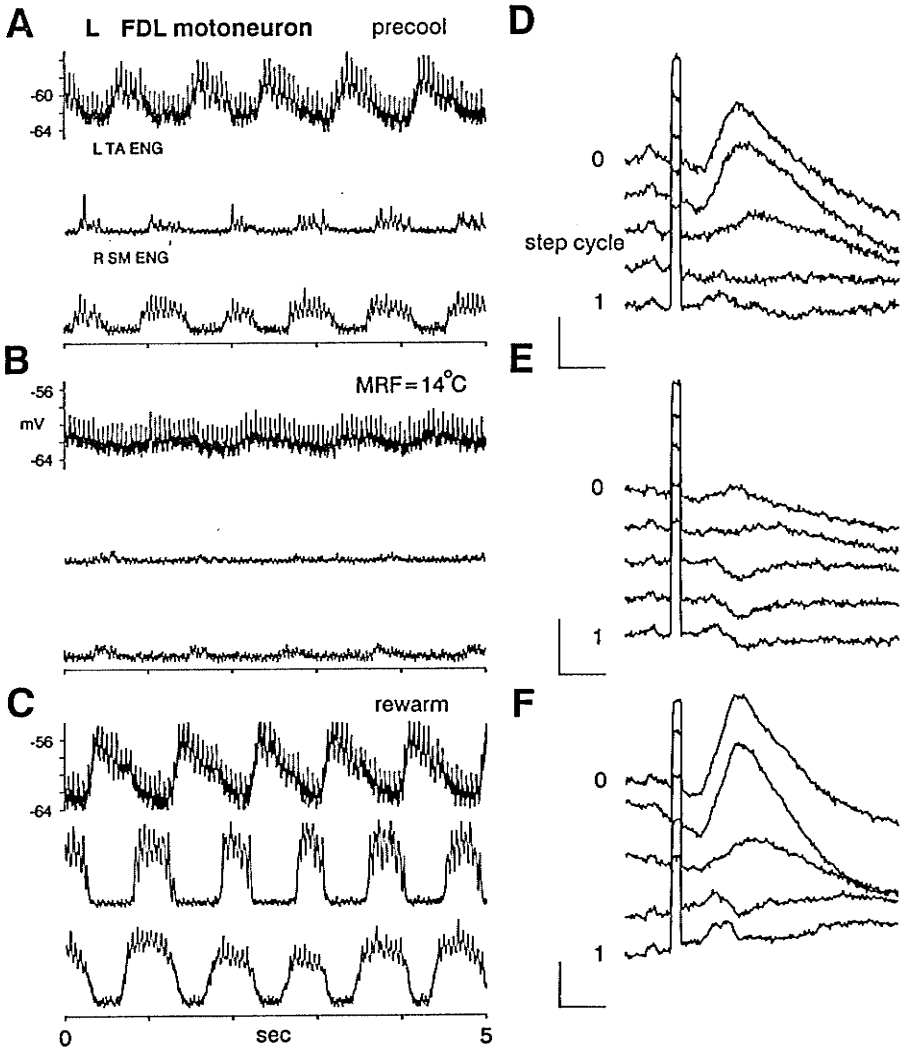

A
L FDL motoneuron
precool
-60
-64
L TA ENG
R SM ENG
B
MRF=14°C
-56
mV
-64
C
rewarm
-56
-64
0
sec
5
D
0
step cycle
1
E
0
1
F
0
1

Figure 29. MRF cooling demonstrates that the appearance of the hyperpolarized phase of the motoneuron LDP is linked to excitation of the antagonist motoneuron pool across the same joint. Top traces in A and B illustrate the intracellular record of a left LG motoneuron, whereas middle and bottom traces show the extracellular records of a left extensor-related L7 VR filament and left TA ENG, respectively. Panel A and B are control and MRF cooling (10.6 degrees C), respectively. For illustrative purposes the gains of each trace are not equal (amplitudes for each trace were reduced during cooling). Locomotion was produced by stimulation (20 Hz, 120 uA, 0.5 msec duration) of the right MLR (P2, L4, H=-1.6).

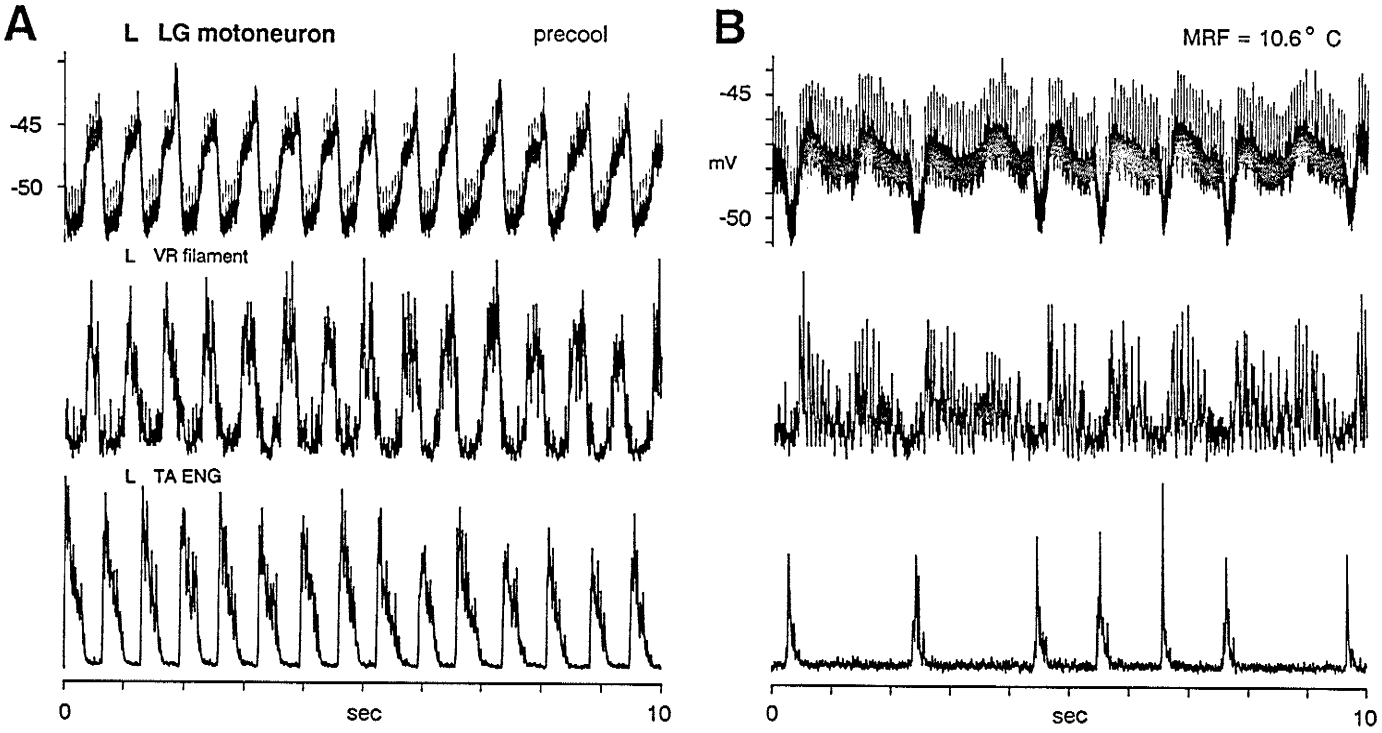

A
L LG motoneuron
precool
-45
-50
L VR filament
L TA ENG
0
sec
10
B
MRF = 10.6° C
-45
mV
-50
0
sec
10

Figure 30. Cooling of the VLF of the spinal cord at T12 can reversibly abolish MLR-evoked locomotion ipsilaterally. Fictive locomotion (A) produced by stimulation (220 uA, 20 Hz, 1 msec) of the right MLR (P2, L4, H=-0.5) was monitored by right and left ENGs during extradural cooling (B) and rewarming (C) of the left VLF. All ENG traces are at constant gain throughout. Decreased levels of activity were observed in some ENGs on the left side during cooling. Loss of rhythmic activity in the left MG ENG was also seen. Upon rewarming, locomotion on the right side returned.

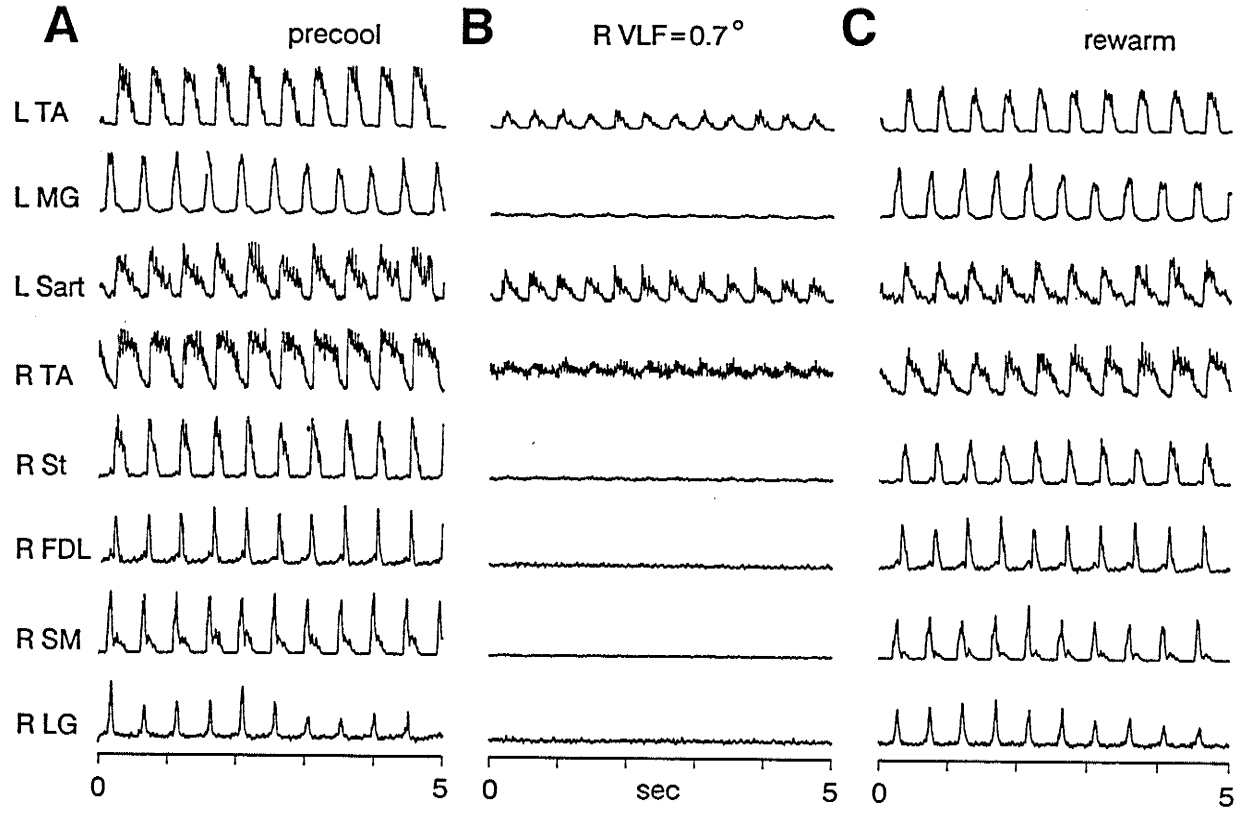

A
precool
B
R VLF=0.7°
C
rewarm
L TA
L MG
L Sart
R TA
R St
R FDL
R SM
R LG
0
5
0
sec
5
0
5

Figure 31. Loss of short-latency MLR-evoked EPSPs is concurrent with the loss of the LDP during cooling of the VLF. Upper panel in A and C shows the average amplitude of the LDP recorded from a left TA motoneuron during locomotion produced by stimulation of the left MLR (P 2.2, L4.5, H=0). Middle panel in A and C illustrate the amplitude modulation of the MLR-evoked EPSP (shown in bottom panel) averaged into 5 bins on the basis of its occurrence during the normalized step cycle. Extradural cooling of the left VLF at the T12 spinal level (probe temperature of 1.7 degrees) abolishes MLR-evoked locomotion as monitored in the DC trace and left Vastus ENG (top and middle panel in B). The MLR- evoked EPSPs (averaged on the basis of Em) are abolished with cooling (lower panel in B). MLR stimulation parameters: A: 180 uA, 13 Hz, 1 msec; B: 180 uA, 11 Hz, 1 msec; C: 175 uA, 13 Hz, 1 msec. Calibration bars are 1 mV/5 msec (A and C) or 0.5 mV/5 msec (B).

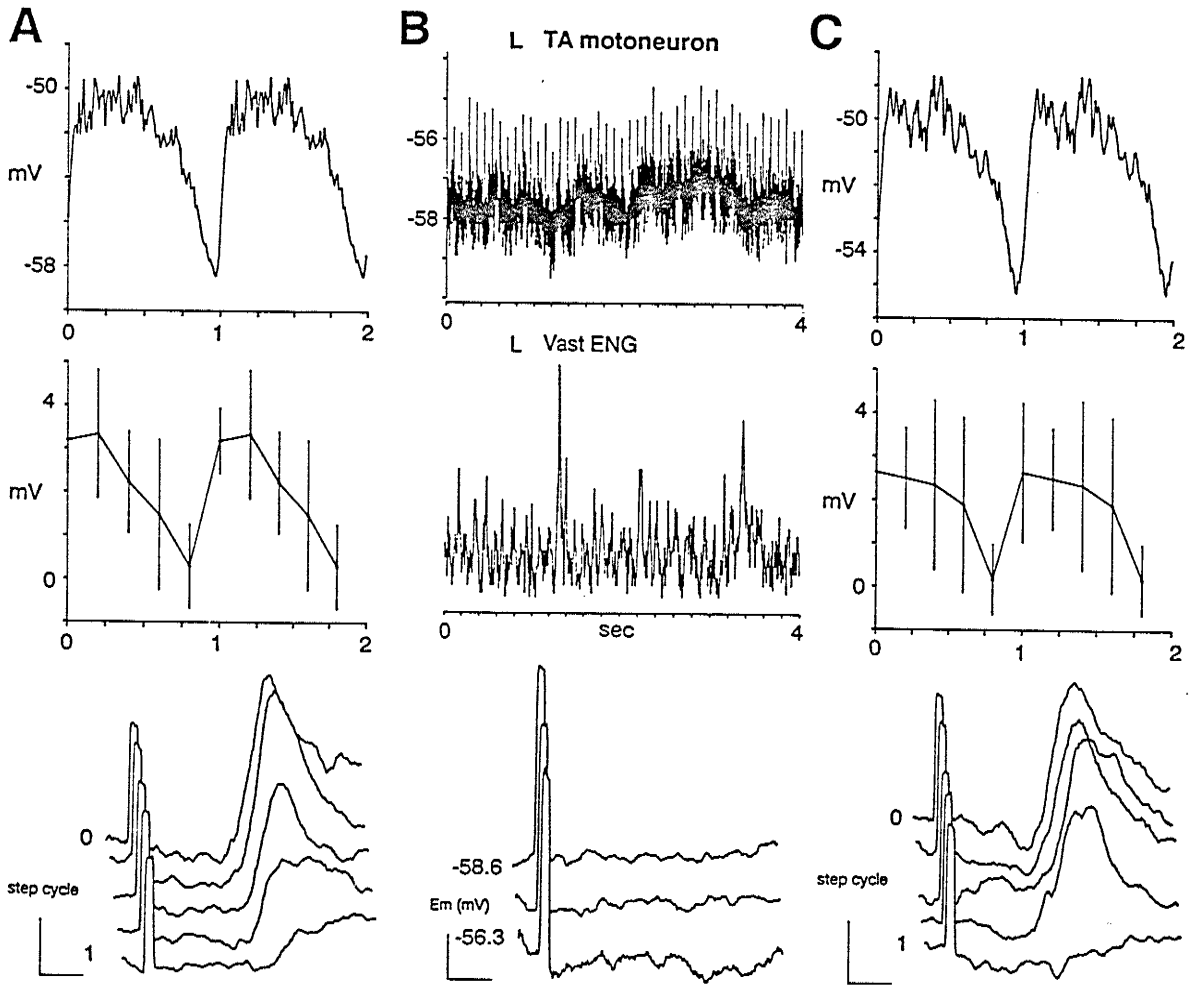

A
-50
mV
-58
0
1
2
4
mV
0
0
1
2
0
step cycle
1
B
L TA motoneuron
-56
-58
0
4
L Vast ENG
0
sec
4
-58.6
Em (mV)
-56.3
C
-50
mV
-54
0
1
2
4
mV
0
0
1
2
0
step cycle
1

Figure 32. Loss of the LDP during cooling of the VLF (T12) is sometimes associated with the loss of the MLR-evoked EPSP and the presence of a residual IPSP. Five second bouts of locomotion recorded from a left LG motoneuron (upper trace) and the left TA ENG are illustrated in A-D for the control, cooled, partial rewarm, and complete rewarm trials, respectively (gain is the same throughout). At left VLF probe tip temperatures of 0 to -1 degree (B) no locomotion (LDPs or ENG modulation) or MLR-evoked EPSPs were observed. Only tonic excitation of the ipsilateral TA and tonic inhibition (IPSPs) of the LG motoneuron remained. Bottom panel in A illustrates the amplitude of the IPSP as a function of Em during the control bout of locomotion. The amplitude of the residual IPSP seen during the cooling trial (with respect to Em) is also plotted in A. C shows the failure of production of the depolarized phase of the LDP and corresponding reduction of inhibition in the antagonist (TA ENG) during the cord rewarming trial (probe temperature of 17-20 degrees). With rewarming of the cord (D), normal LDPs, ENG modulation, and PSP modulation during the normalized step cycle (bottom panel) is apparent. Locomotion was produced by stimulation (210 uA, 12 Hz, 1msec) of the L MLR (P 2.5, L 4, H= 0). Calibration bars in A, B, and D are 1 mV/5 msec.

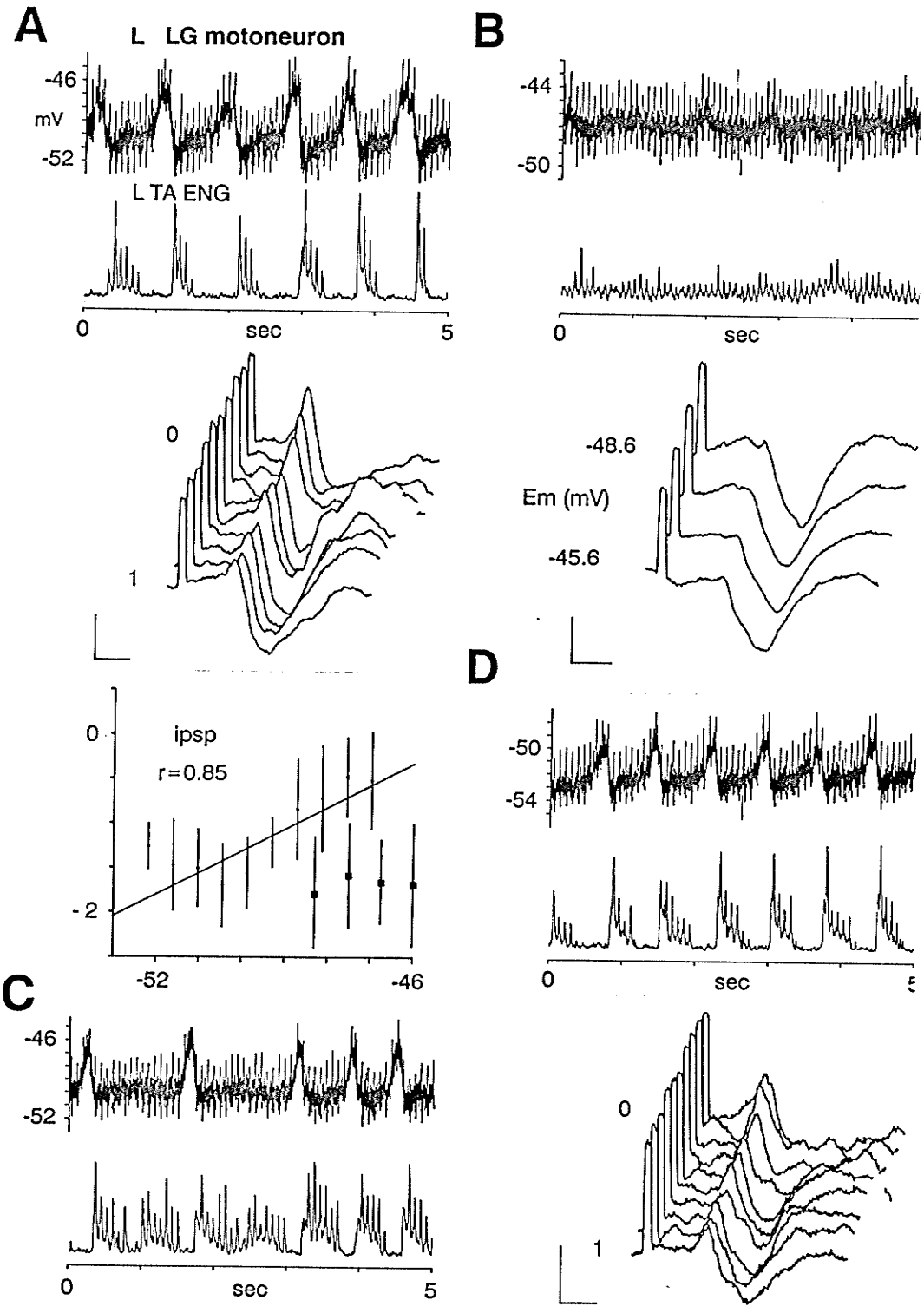

A
L LG motoneuron
-46
mV
-52
L TA ENG
0
sec
5
0
1
ipsp
r=0.85
0
-2
-52
-46
B
-44
-50
0
sec
-48.6
Em (mV)
-45.6
D
-50
-54
0
sec
0
1
C
-46
-52
0
sec
5

Figure 33. Effect of cooling of the VLF on MLR-evoked eI PSPs in a SM motoneuron. A, B, and C illustrate responses in the control, cooled and rewarm trials, respectively. Panels 1 show bouts of locomotion produced by stimulation (220 uA, 16 Hz, and 1.0 msec) of the L MLR (P 2.8, L 4.5, H=-1.5). Panels 2 illustrate the averaged PSPs segregated on the basis of their occurrence during the normalized step cycle. Lines 1, 2, and 3 represent the stimulus artifact, onset of the EPSP, and peak amplitude of the IPSP, respectively. Calibration bars are 2 mV, 5 msec. Panels 3 and 4 are peak amplitude measurements of the IPSP (difference between lines 2 and 3) with respect to the normalized step cycle and Em, respectively. In the control bout of locomotion (A), the MLR-evoked IPSP shows an amplitude modulation which is highly dependent upon Em (largest during depolarization). After cooling and rewarming of the L VLF, the amplitude modulation is reversed: the IPSP is largest during the hyperpolarized phase and smaller with Em depolarization.

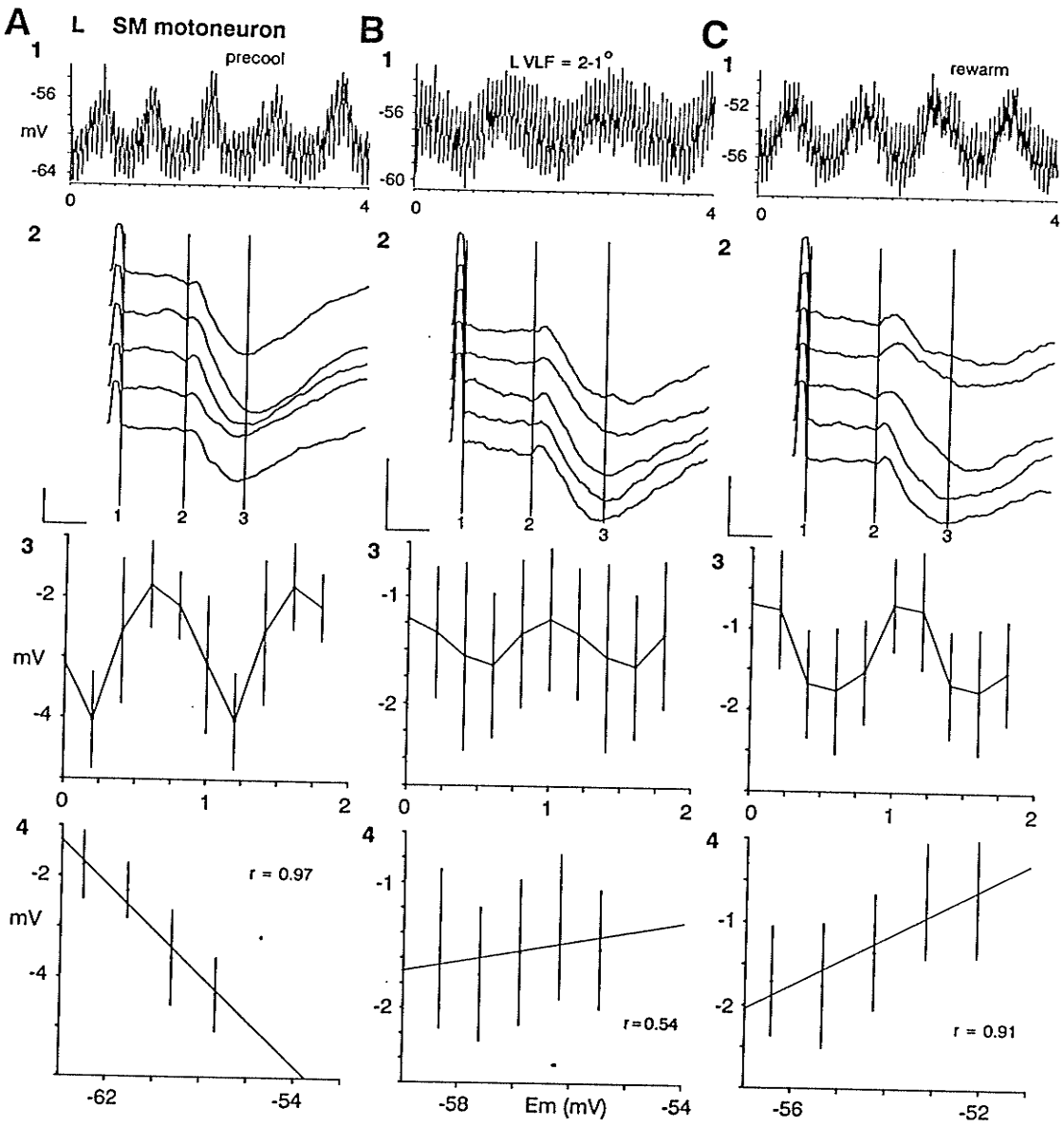

A
L SM motoneuron
precool
B
L VLF = 2-1°
C
rewarm
mV
r = 0.97
r=0.54
r = 0.91
Em (mV)

Figure 34. Longer latency MLR-evoked EPSPs are sometimes revealed upon cooling of the VLF ipsilateral to the intracellular recording site. Panel A illustrates the averaged LDP of a left TA motoneuron with respect to the normalized step cycle recorded during fictive locomotion produced by stimulation of the right MLR (P 2.5, L 4, H=-1). Cooling of the left VLF extradurally (probe temperature: 1 to 0 degrees) reduces the size of the LDP (bottom trace). LDPs are plotted on the same scale. Panel B shows the averaged responses of the cord dorsum (30 msec window) during the control (top trace) and cooling (middle trace) locomotion trials. The bottom trace shows the difference between both cord dorsum averages. Lines 1 and 2 indicate the onset of the stimulus artifact and that portion of the cord dorsum signal removed during VLF cooling, respectively. No change in the descending volley latency is apparent during cooling of the VLF. Panel C illustrates the averaged MLR-evoked EPSPs sorted on the basis of their occurrence during the normalized step cycle. After cooling of the left VLF (T12), the latency of the MLR-evoked EPSP increased by approximately 1 msec (line 4, bottom) from that seen during the control bout of locomotion (line 3, top). MLR stimulation parameters were 190 uA, 20 Hz, 0.5 msec.

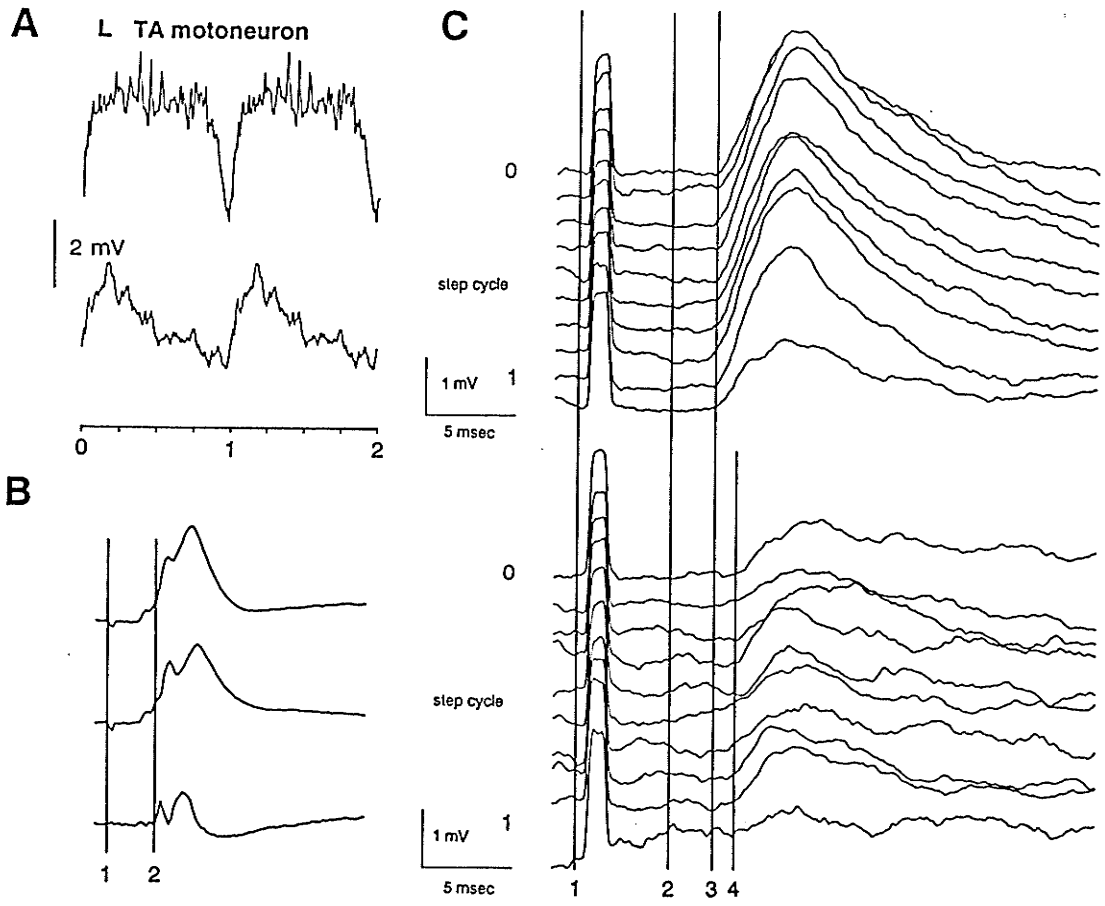

A
L TA motoneuron
2 mV
0
1
2
B
1
2
C
0
step cycle
1 mV
1
5 msec
0
step cycle
1 mV
1
5 msec
1
2
3
4

Figure 35. Cooling of the VLF contralateral to the recording side abolishes longer latency MLR-evoked EPSPs. Cooling of the dorsal cord has no effect on MLR-evoked locomotion or PSPs. Intracellular recording from a left unidentified motoneuron is illustrated in top traces in A-D. Averaged MLR-evoked PSPs sorted on the basis of the normalized step cycle are illustrated in lower panels. Locomotion on the left side of the animal (as monitored by intracellular recording or left TA ENG) produced by stimulation (220 uA, 13.8 Hz, 1 msec duration) of the left MLR (P1.5, L4, H=-0.5) is decreased but not abolished after cooling of the contralateral (right) VLF (R VLF) (probe temperature of -1 degree C) (panel B). Cooling of the dorsal cord (DC) (probe temperature 0 to -2 degree C) has no effect (panel D). Lines 1, 2, and 3 indicate onset of stimulus, arrival of descending volley at the cord dorsum recording electrode, and onset of the short- latency EPSP, respectively. Slight increases in the descending volley latency are seen during cooling of the VLF (0.4 msec) or DC (0.2 msec). No change of the segmental latency to the first EPSP was observed during cooling. Calibration bars in A are 0.5 mV, 5 msec. Calibration bars in B,C,and D are 1.0 mv, 5 msec.

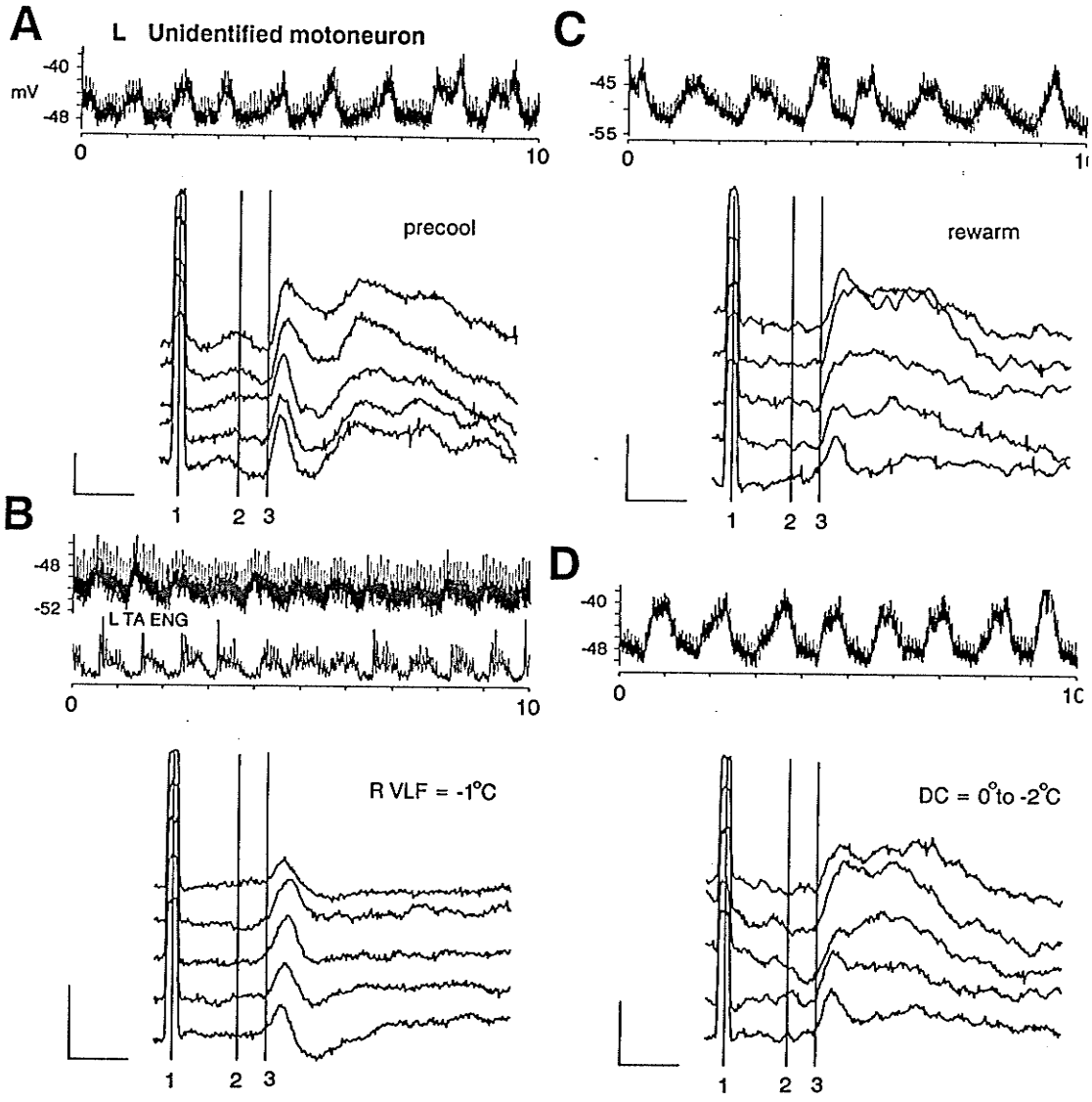

A
L Unidentified motoneuron
-40
mV
-48
0
10
precool
1 2 3
B
-48
-52
L TA ENG
0
10
R VLF = -1°C
1 2 3
C
-45
-55
0
rewarm
1 2 3
D
-40
-48
0
DC = 0°to -2°C
1 2 3

Figure 36. MLR-evoked PSP amplitude modulation plotted with respect to LDP amplitude modulation. Larger LDP amplitudes are associated with greater amplitude modulation of the PSPs. Values obtained from motoneurons which showed simple E or EI MLR-evoked PSPs. PSP modulation determined by measurement of the absolute change in amplitude of the largest EPSP or IPSP seen during the step cycle (minimum amplitude subtracted from the maximum amplitude for each PSP type). In cases of mixed PSPs (EI), the absolute values for the EPSP and the IPSP modulation were added together. LDP amplitude determined by the difference between maximum and minimum Em values (depolarized phase and hyperpolarized phase, respectively).

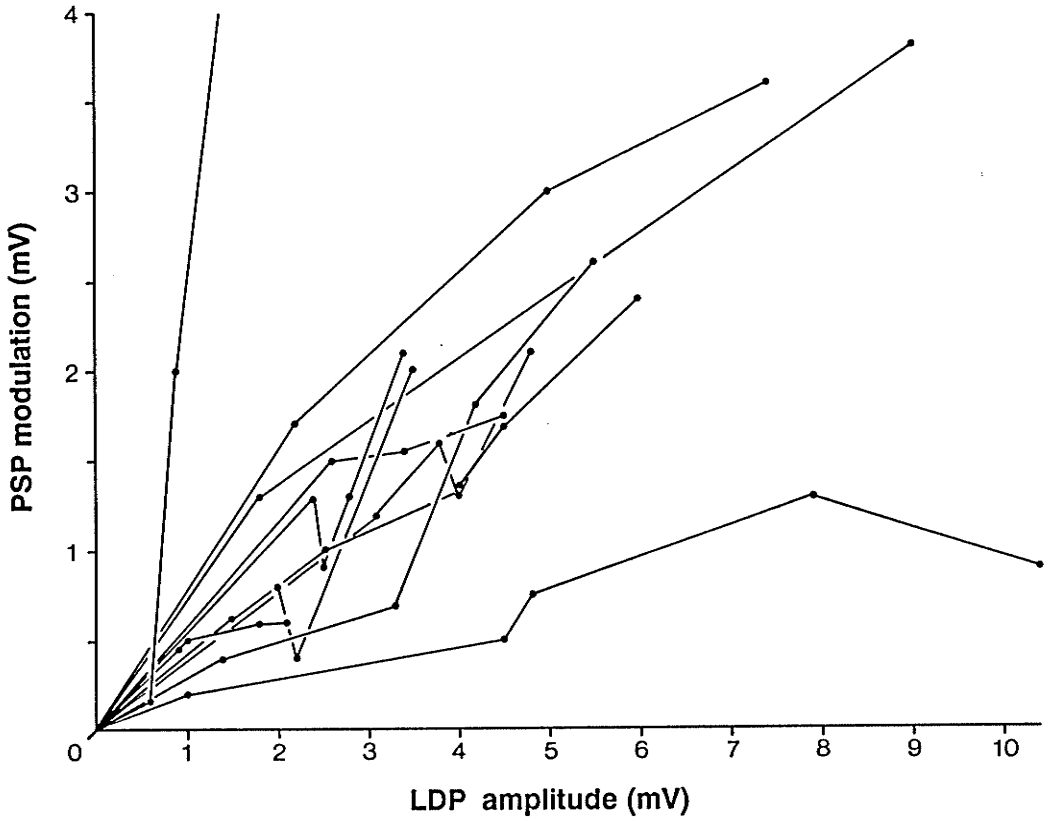
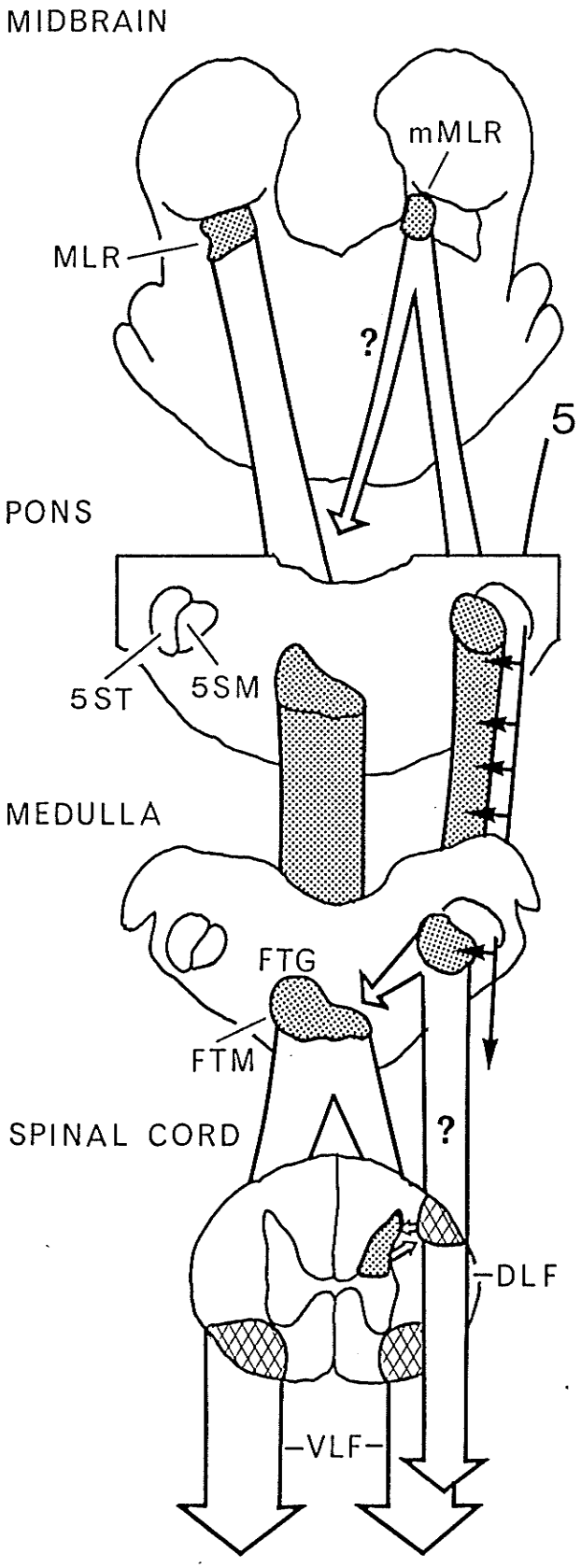

PSP modulation (mV)
LDP amplitude (mV)
0
1
2
3
4
5
6
7
8
9
10

Figure 37. Organization of locomotor pathways descending from the midbrain to the spinal cord and their possible interrelationships (see text for details). Two different pathways are illustrated. Locations of cell bodies is shown by stippled areas, fiber tracts by clear or hatched areas.


MIDBRAIN
mMLR
MLR
?
5
PONS
5ST
5SM
MEDULLA
FTG
FTM
SPINAL CORD
?
DLF
VLF

Abbreviations

5 - trigeminal nerve
5 M - motor trigeminal nucleus
5 P - principal sensory trigeminal nucleus
5 SM - spinal trigeminal nucleus- magnocellular division
5 SP - spinal trigeminal nucleus- parvocellular division
5 ST - spinal trigeminal tract
7 - facial nerve
7L - facial nucleus, lateral division
7M - facial nucleus, medial division
CI - inferior central nucleus
CP - cerebellar peduncle
FTG - gigantocellular tegmental field
FTL - lateral tegmental field
FTM - magnocellular tegmental field
IC - inferior colliculus
IFT - infratrigeminal nucleus
IOM - medial accessory nucleus of the inferior olive
KF - Kolliker-Fuse nucleus
LRI - lateral reticular nucleus - internal division
P - pyramidal tract
PPR - postpyramidal nucleus of the pyramidal tract
PR - paramedian reticular nucleus
SOL - lateral nucleus of the superior olive
SOM - medial nucleus of the superior olive
TB - trapezoid body

References

Afelt, Z. (1970) Reflex activity in chronic spinal cats. Acta Neurobiol. Exp. 30: 129-144

Afelt, Z. (1974) Functional significance of ventral descending tracts of the spinal cord in the cat. Acta Neurobiol Exp. 34: 393-407

Afelt, Z., Kasicki, S., Sybirska, E., and Zagrodzka, J. (1975) Limb coordinations during locomotion in amygdalar, rubral and funicular cats. Acta. Neurobiol. Exp. 35: 379-388

Aoki, M., and Mori, S. (1981) Locomotion elicited by pinna stimulation in the acute precollicular-post-mamillary decerebrate cat. Brain Res. 214: 424-428

Azerad, J., Woda, A., and Able-Fessard, D. (1982) Physiological properties of neurons in different parts of neurons in different parts of the cat trigeminal sensory complex. Brain Res. 246: 7-21

Baev, K.V., Degtyarenko, A.M., Zavadskaya, T.V., and Kostyuk, P.G., (1979) Activity of lumbar interneurons during fictitious locomotion in thalamic cats. Neurophysiol. 11: 244-250

Berman, A.L. (1968) **The Brain Stem of the Cat**, University of Wisconsin Press, Madison

Biedenbach, M.A. (1977) Response of trigeminal neurons in pars interpolaris to non-noxious and noxious stimulation. In: **Pain in the Trigeminal Region**, D.J. Anderson and B. Matthews, eds., pp. 233-244, Elsevier, Amsterdam

Brooks, V.B. (1983) Study of brain function by local, reversible cooling. Rev. Physiol. Biochem. Pharmacol. 95: 1-109

Brudzynski, S.M., Houghton, P.E., Brownlee, R.D., and Mogenson, G.J., (1986) Involvement of neuronal cell bodies of the mesencephalic locomotor region in the initiation of locomotor activity of freely behaving rats. Brain Res. 16: 377-381

Budakova, N.N. (1972) Stepping movements evoked by repetitive dorsal root stimulation in a mesencephalic cat. Neurosci. Behav. Physiol. 5: 355-363

Budakova, N.N., and Shik, M.L. (1980) Continuity of the bulbar locomotor strip is not essential for walking. Bull. Exp. Biol. Med. 89: 1-4

Burton, H. (1968) Somatic sensory properties of caudal bulbar reticular neurons in the cat (Felis domestica). Brain Res. 11: 357-372

Corbin, K.B. (1942) Probst's tract in the cat. J. Comp. Neurol. 77: 455-467

Cuello, A.C., DelFiacco, M., and Paxinos, G. (1978) The central and peripheral ends of the substance P-containing sensory neurons in the rat trigeminal system. Brain Res. 152: 499- 509

Deliagina, T.G., Orlovsky, G.N., and Pavlova, G.A. (1983) The capacity for generation of rhythmic oscillations is distributed in the lumbosacral spinal cord of the cat. Exp. Brain Res. 53: 81-90

Drew, T., and Rossignol, S. (1984) Phase-dependent responses evoked in limb muscles by stimulation of medullary reticular formation during locomotion in thalamic cats. J. Neurophysiol. 52: 653-675

Drew, T., Dubuc, R., and Rossignol, S. (1986) Discharge patterns of reticulospinal and other reticular neurons in chronic, unrestrained cats walking on a treadmill. J. Neurophysiol. 55: 375-401

Eccles, J.C. (1968) **The Physiology of Nerve Cells**, Johns Hopkins Press, Baltimore

Edgerton, V.R., Grillner, S., Sjostrom, A., and Zangger, P. (1976) Central generation of locomotion in vertebrates. In: **Neural control of locomotion**, R.M.

Herman, S.Grillner, P.S.G. Stein and D. Stuart, eds., Vol. 18, pp. 439-464, Plenum Press, New York,

Edgley, S.A., and Jankowska, E. (1987) Interneuronal relay for group I and II muscle afferents in the middle lumbar segments of the cat spinal cord. J. Physiol. (Lond.), 385: 393-414

Eidelberg, E. (1980) Locomotor control in macaque monkeys. In: **Regulatory Functions of the CNS. Motion and Organization Principles**, Adv. Physiol. Sci., Vol. 1, pp. 187-188, J.Szentagothai, M. Palkovits, and J. Hamori, eds., Pergamon Press, Akademiai Kiado

Eidelberg, E. (1981) Consequences of spinal cord lesions upon motor function, with special reference to locomotor activity. Prog. Neurobiol. 17: 185-202

Eidelberg, E., Nguyen, L.H., and Deza, L.D. (1986) Recovery of locomotor function after hemisection of the spinal cord in cats. Brain Res. Bull. 16: 507-515

Eidelberg, E., Story, J.L., Walden, J.G., and Meyer, B.L. (1981a) Anatomical correlates of return of locomotor function after partial spinal cord lesions in cats. Exp. Brain Res. 42: 81-88

Eidelberg, E., Walden, J.G., and Nguyen, L.H. (1981b) Locomotor control in macaque monkeys. Brain 104: 647-663

Feldman, A.G., and Orlovsky, G.N. (1975) Activity of interneurons mediating reciprocal Ia inhibition during locomotion. Brain Res. 84: 181-194

Garcia-Rill, E., Houser, C.R., Skinner, R.D., Smith, W., and Woodward, D.J. (1987) Locomotion-inducing sites in the vicinity of the pediculopontine nucleus. Brain Res. Bull. 18: 731-738

Garcia-Rill, E., and Skinner, R.D. (1987a) The mesencephalic locomotor region. I. Activation of a medullary projection site. Brain Res. 411: 1-12

Garcia-Rill, E., and Skinner, R.D. (1987b) The mesencephalic locomotor region. II. Projections to reticulospinal neurons. Brain Res. 411: 13-20

Garcia-Rill, E., Skinner, R.D., Conrad, C., Mosley, D., and Campbell, C. (1986) Projections of the mesencephalic locomotor region in the rat. Brain Res. Bull., 17: 33-40

Garcia-Rill, E., Skinner, R.D., and Fitzgerald, J.A. (1983a) Activity in the mesencephalic locomotor region during locomotion. Exp. Neurol. 82: 609-622

Garcia-Rill, E., Skinner, R.D., and Fitzgerald, J.A. (1985) Chemical activation of the mesencephalic locomotor region. Brain Research 330: 43-54

Garcia-Rill, E., Skinner, R.D., Gilmore, S.A., and Owings, R. (1983b) Connections of the mesencephalic locomotor region (MLR). II. Afferents and efferents. Brain Res. Bull. 10: 63-71

Goodchild, A.K., Dampney, R.A.L., and Bandler, R. (1982) A method for evoking physiological responses by stimulation of cell bodies, but not axons of passage, within localized regions of the central nervous system. J. Neuroscience Meth. 6: 351-363

Greene, R.W., and Carpenter, D.O. (1985) Actions of neurotransmitters on pontine medial reticular formation of the cat. J. Neurophysiol. 54: 520-531

Grillner, S. (1981) Control of locomotion in bipeds, tetrapods, and fish. In: **Handbook of Physiology, The Nervous System, Part II**, Chapter 26, pp 1179-1236

Grillner, S., and Shik, M.L. (1973) On the descending control of the lumbosacral spinal cord from the "mesencephalic locomotor region". Acta Physiol. Scand. 87: 320-333

Grillner, S., and Zangger, P. (1979) On the central generation of locomotion in the low spinal cat. Exp. Brain Res. 34: 241- 261

Harrison, P.J., Jankowska, E., and Zytnicki, D. (1986) Lamina VIII interneurons interposed in crossed reflex pathways in the cat. J. Physiol. (Lond.) 371: 147-166

Henry, J.L., Hu, J.W., Lucier, G.E., and Sessle, B.J. (1977) Responses of units in the trigeminal sensory nuclei to oral- facial stimuli and to substance P. In: **Pain in the Trigeminal Region**, D.J. Anderson and B. Matthews, eds., pp. 295-306, Elsevier, Amsterdam

Holstege, G., and Kuypers, H.G.J.M. (1982) The anatomy of the brain stem pathways to the spinal cord in a cat. A labeled amino acid tracing study. Prog. Brain Res. 57: 145-175

Huang, C.C., Lau, C.M., and Peng, M.T. (1970) Elicitation of scratching movements by mechanical stimulation to spinal cord in decerebrate cats. Jap. J. Physiol. 20: 365-369

Hunt, C.C., and Kuno, M. (1959) Properties of spinal interneurones. J. Physiol. (Lond.) 147: 346-363

Ingram, W.R., Ranson, S.W., Hannett, F.I. Zeiss, F.R., and Terwilliger, E.H. (1932) Results of stimulation of the tegmentum with the Horsley-Clarke stereotaxic apparatus. Arch. Neurol. Psychiat. 28: 513-541

Ishimine, S., Hikosaka, O., and Nakamura, Y. (1980) Spatio- temoporal patterns of pre- and postsynaptic inhibition induced by primary afferent activation in the trigeminal sensory nucleus in cats. Exp. Brain Res. 40: 154-163

Jacobson, R.D., and Hollyday, M. (1982) Electrically evoked walking and fictive locomotion in the chick. J. Neurophysiol., 48: 257-270

Jane, J.A., Evans, J.P., and Fisher, L.E. (1964) An investigation concerning the restitution of motor function following injury to the spinal cord. J. Neurosurg. 21: 167-171

Jankowska, E. (1985) Further indications for enhancement of retrograde transneuronal transport of WGA-HRP by synaptic activity. Brain Res. 341: 403-408

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

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

Jessell, T.M., and Iversen, L.L. (1977) Opiate analgesics inhibit substance P release from rat trigeminal nucleus. Nature 268: 549-551

Jordan, L.M. (1981) Comment: Gating effects and constraints on the central pattern generators for rhythmic movements. Can. J. Physiol. Pharmacol. 59: 727-732

Jordan, L.M. (1983) Factors determining motoneuron rhythmicity during fictive locomotion. Soc. Exp. Biol. Symp. 37: 423- 444

Jordan, L.M. (1986) Initiation of locomotion from the mammalian brainstem. In: **Neurobiology of Vertebrate Locomotion**, S. Grillner, P. S. G. Stein, D. G. Stuart, H. Forssberg, and R. M. Herman, eds., pp 21-37, Macmillan Press, Hong Kong

Jordan, L.M., Pratt, C.A., and Menzies, J.E. (1979) Locomotion evoked by brainstem stimulation: occurrence without phasic segmental afferent input. Brain Res. 177: 204-207

Jordan, L.M., Pratt, C.A., and Menzies, J.E. (1981) Intraspinal mechanisms for the control of locomotion. In: **Advances in Physiological Sciences. Motion and Organizational Principles**, Vol. 1, pp. 183-185, J. Szentagothai, M Palkovits, and J. Hamori, eds., Pergamon, New York

Kamishima, Y. (1978) Physiological and pathophysiological investigations on neuronal mechanisms of locomotion in the cats. Hokkaido Igaku Zasshi. 53: 41-48

Kato, M., Murakami, S., Yasuda, K., and Hirayama, H. (1984) Disruption of fore- and hindlimb coordination during overground locomotion in cats with bilateral serial hemisection of the spinal cord. Neurosci. Res., 2: 27-47

Kazennikov, O.V., Selionov, V.A., Shik, M.L., and Yakovleva, G.V., (1979) Neurons of upper cervical segments responding to stimulation of the bulbar "locomotor strip". Neurophysiol., 11: 179-185

Kazennikov, O.V., Selionov, V.A., Shik, M.L., and Yakovleva, G.V., (1980b) The rhombencephalic "locomotor region" in turtles. Neurophysiol. 12: 251-257

Kazennikov, O.V., Shik, M.L., and Yakovleva, G.V. (1980a) Two pathways for the brainstem "locomotor influence" on the spinal cord. Fiziol. Zh (SSSR) 66: 1260-1263

Kazennikov, O.V., Shik, M.L., and Yakovleva, G.V. (1983a) Responses of upper cervical spinal neurons in cats to stimulation of the brain-stem locomotor region at different frequencies. Neurophysiol. 15: 256-261

Kazennikov, O.V., Shik, M.L., and Yakovleva, G.V. (1983b) Stepping movements induced in cats by stimulation of the dorsolateral funiculus of the spinal cord. Bull. Exp. Biol. Med. 96: 1036-1039

Kazennikov, O.V., Shik, M.L., and Yakovleva, G.V. (1985) Synaptic responses of propriospinal neurons to stimulation of the stepping strip of the dorsolateral funiculus in cats. Neurophysiol.,17: 195-202

Kettler, J., and Jordan, L.M. (1984) Metabolic mapping of the brainstem during fictive locomotion. Soc. Neuroscience Abstr., Vol 10: 633

Kluver, H., and Barrera, E. (1953) A method for the combined staining of cells and fibres in the nervous system. J. Neuropath. Exp. Neurol. 12: 400-403

Kriellaars, D.J., Brownstone, R.M., Noga, B.R., and Jordan, L.M. (1987) Quantitative analysis of the temporal relationships among hindlimb motoneurons during fictive locomotion in the cat. Soc. Neurosci. Abstr., Vol. 13: 875

Kuypers, H.G.F.M., and Maiskey, V.A. (1977) Funicular trajectories of descending brainstem pathways in the cat. Brain Res. 136: 159-165

Lamarche, G., Langlois, J.M., and Heon, M. (1960) Unit study of the trigeminal projections in the reticular formation of the medulla oblongata in the cat. Can. J. Biochem. Physiol. 38: 1163-1166

Lee, H.J., Rye, D.B., and Wainer, B.H. (1986) Combined retrograde-immunohistochemical evidence that the pediculopontine tegmental nucleus does not project to extrapyramidal motor structures. Soc. Neuroscience Abstr., Vol. 12: 1546

Legendre, P., McKenzie, J.S., Dupouy, B., and Vincent, J.D. (1985) Evidence for bursting pacemaker neurons in cultured spinal cord cells. Neurosci. 16: 753-767

Leger, L., Charnay, Y., Chayvialle, J.A., Berod, A., Dray, F., Pujol, J.F., Jouvet, M., and Dubois, P.M. (1983) Localization of substance P and enkephalin-like immunoreactivity in relation to catecholamine-containing cell bodies in the cat dorsolateral pontine tegmentum: an immunoflourescence study. Neurosci. 8: 525-546

Lennard, P.R., and Stein, P.S.G. (1977) Swimming movements elicited by electrical stimulation of turtle spinal cord. I. Low spinal and intact preparations. J. Neurophysiol. 40: 768-778

Livingston, C.A. (1986) The descending pathways that control locomotion in the Atlantic stingray, Dasyatis sabina. Ph.D. Thesis, University of Texas: Galvaston

Lovick, T.A., and Wolstencroft, J.H. (1983) Projections from brain stem nuclei to the spinal trigeminal nucleus in the cat. Neurosci. 9: 411-420

Martin, R.F., Jordan, L.M., and Willis, W.D. (1978) Differential projections of cat medullary raphe neurons demonstrated by retrograde labelling following spinal cord lesions. J. Comp. Neurol. 182: 77-88

Matsushita, M., Ikeda, M., and Okado, N. (1982) The cells of origin of the trigeminothalamic, trigeminospinal, and trigeminocerebellar projections in the cat. Neurosci. 7: 1439-1454

Matsushita, M., Okado, N., Ikeda, M., and Hosoya, Y. (1981) Descending projections from the spinal and mesencephalic nuclei of the trigeminal nerve to the spinal cord in the cat. A study with the horseradish peroxidase technique. J. Comp. Neurol. 196: 173-187

Mayer, M.L., and Westbrook, G.L. (1987) The physiology of excitatory amino acids in the vertebrate central nervous system. Prog. Neurobiol. 28: 197-276

McCrea, D.A., Pratt, C.A., and Jordan, L.M. (1980) Renshaw cell activity and recurrent effects on motoneurons during fictive locomotion. J. Neurophysiol. 44: 475-488

Mori, S., Aoki, M., Kawahara, K., and Sakamoto, T. (1980a) Level setting of postural tonus and initiation of locomotion by MLR stimulation. In: Adv. Physiol. Sci., Vol. 1., pp. 179- 182, J. Szentagothai, M. Palkovits, and J. Hamori, eds. Budapest, Pergamon Press/Akademiai Kiado

Mori, S., Kawahara, K., Sakamoto, T., Aoki, M., and Tomiyama, T., (1982) Setting and resetting of level of postural muscle tone in decerebrate cat by stimulation of brain stem. J. Neurophysiol. 48: 737-748

Mori, S., Nishimura, H., and Aoki, M. (1980b) Brain stem activation of the spinal stepping generator. In: **Reticular Formation Revisited**, J.A. Hobson and M.A.B. Brazier, eds., IBRO Monograph Ser., Vol. 6, pp. 241-259, Raven Press, New York

Mori, S., Nishimura, H., Kurakami, C., Yamamura, T., and Aoki, M., (1978a) Controlled locomotion in the mesencephalic cat: distribution of facilitatory and inhibitory regions within pontine tegmentum. J. Neurophysiol. 41: 1580-1591

Mori, S., Nishimura, H., Kurakami, C., Yamamura, T., and Aoki, M., (1978b) Lower brain-stem "locomotor region" in the mesencephalic cat. In: **Integrative Control**

Function of the Brain, M. Ito, ed., Vol. 1, pp. 209-211, Elsevier/North Holland, New York

Mori, S., Selionov, V.A., and Shik, M.L. (1986) Responses of bulbar neurons to stimulation of locomotor and inhibitory sites in the cat brainstem. Neurophysiol. 18: 386-393

Mori, S., Shik, M.L., and Yagodnitsyn, A.S. (1977) Role of pontine tegmentum for locomotor control in mesencephalic cat. J. Neurophysiol. 40: 284-295

Nakamura, Y., and Wu, C.Y. (1970) Presynaptic inhibition of jaw- opening reflex by high-threshold afferents from the masseter muscle of the cat. Brain Res. 23: 193-211

Nauta, W.J.H., and Kuypers, H.G.J.M. (1958) Some ascending pathways in the brainstem reticular formation. **Reticular Formation of the Brainstem**, pp. 3-30, Little, Brown and Co., Boston

Noga, B.R., Shefchyk, S.J., Jamal, J., and Jordan, L.M. (1987a) The role of Renshaw cells in locomotion: antagonism of their excitation from motor axon collaterals with intravenous mecamylamine. Exp. Brain Res., 66: 99-105

Noga, B.R., Shefchyk, S.J., and Jordan, L.M. (1987b) Localization of last-order spinal interneurons participating in the production of locomotion in the cat. Soc. Neuroscience Abstr., Vol. 13: 826

Noga, B.R., Shefchyk, S.J., McCrea, D.A., and Jordan, L.M. The effects of intravenous mecamylamine and atropine on treadmill locomotion evoked by stimulation of the mesencephalic locomotor region in the decerebrate cat. In preparation

Nord, S.G., and Kyler, H.J. (1968) A single unit analysis of trigeminal projections to bulbar reticular nuclei of the rat. J. Comp. Neurol. 134: 485-499

Nord, S.G., and Ross, G.S. (1973) Responses of trigeminal units in the monkey bulbar lateral reticular formation to noxious and non-noxious stimulation of the face: experimental and theoretical considerations. Brain Research 58: 385-399

Olszewski, J. (1950) On anatomical and functional organization of spinal trigeminal nucleus. J. Comp. Neurol. 92: 401-413

Orlovsky, G.N. (1969) Electrical activity in the brainstem and descending pathways in guided locomotion. Sechenov Physiol. J. (USSR), 55: 437-444

Orlovsky, G.N. (1970a) Connexions of the reticulo-spinal neurons with the "locomotor sections" of the brainstem. Biophysics 15: 178-186

Orlovsky, G.N. (1970b) Work of the reticulo-spinal neurons during locomotion. Biophysics 15: 761-771

Orlovsky, G.N. (1972) The effect of different descending systems on flexor and extensor activity during locomotion. Brain Res. 40: 359-371

Orlovsky, G.N., and Feldman, A.G. (1972) Classification of lumbosacral neurons by their discharge pattern during evoked locomotion. Neurophysiol. 4: 311-317

Orsal, D., Perret, C., and Cabelguen, J.-M. (1986) Evidence of rhythmic inhibitory synaptic influences in hindlimb motoneurons during fictive locomotion in the thalamic cat. Exp. Brain Res. 64: 217-224

Perret, C. (1976) Neural control of locomotion in the decorticate cat. In: **Neural Control of Locomotion**, R.M. Herman, S.Grillner, Stein, P.S.G., D.G. Stuart, eds., pp. 587-615, Plenum Press, New York

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

Peterson, B.W., Maunz, R.A., Pitts, N.G., and Mackel, R.G. (1975) Patterns of projection and branching of reticulospinal neurons. Exp. Brain Res.23: 333-351

Peterson, B.W., Pitts, N.G., and Fukushima, K. (1979) Reticulospinal connections with limb and axial motoneurons. Exp. Brain Res. 36: 1-20

Petras, J.M. (1967) Cortical, tectal, and tegmental fiber connections in the spinal cord of the cat. Brain Res. 6: 275-324

Pratt, C.A., and Jordan, L.M. (1987) Ia inhibitory interneurons and Renshaw Cells as contributors to the spinal mechanisms of fictive locomotion. J. Neurophysiol. 57: 56-71

Ross, G.S., and Sinnamon, H.M. (1984) Forelimb and hindlimb stepping by the anesthetized rat elicited by electrical stimulation of the pons and medulla. Physiol. Behav. 33: 201-208

Rye, D.B., Saper, C.B., Lee, H.J., and Wainer, B.H. (1987) Pedunculopontine tegmental nucleus of the rat: cytoarchitecture, cytochemistry, and some extrapyramidal connections of the mesopontine tegmentum. J. Comp. Neurol. 259: 483-528

Salt, T.E., Morris, R., and Hill, R.G. (1983) Distribution of substance P-responsive and nociceptive neurons in relation to substance P-immunoreactivity within the caudal trigeminal nucleus of the rat. Brain Res. 273: 217-228

Segundo, J.P., Takenaka, T., and Encabo, H. (1967) Somatic sensory properties of bulbar reticular neurons. J. Neurophysiol. 30: 1221-1238

Selionov, V.A., and Shik, M.L. (1981) Responses of medullary neurons to microstimulation of the "locomotor strip" in cats. Neurophysiol. 13: 199-205

Selionov, V.A., and Shik, M.L. (1982) Synaptic responses of medullary neurons of turtles to stimulation of the locomotor strip. Neurophysiol. 14: 91-97

Selionov, V.A., and Shik, M.L. (1984) Medullary locomotor strip and column in the cat. Neurosci. 13: 1267-1278

Severin, F.V., Shik, M.L., and Orlovskii, G.N. (1967) Work of the muscles and single motor neurons during controlled locomotion. Biophysics 12: 762-772

Shefchyk, S.J., Jell, R.M., and Jordan, L.M. (1984) Reversible cooling of the brainstem reveals areas required for mesencephalic locomotor region evoked treadmill locomotion. Exp. Brain Res. 56: 257-262

Shefchyk, S.J., and Jordan, L.M. (1985a) Excitatory and inhibitory postsynaptic potentials in alpha-motoneurons produced during fictive locomotion by stimulation of the mesencephalic locomotor region. J. Neurophysiol. 53: 1345- 1355

Shefchyk, S.J., and Jordan, L.M. (1985b) Motoneuron input- resistance changes during fictive locomotion produced by stimulation of the mesencephalic locomotor region. J. Neurophysiol. 54: 1101-1108

Shefchyk, S.J., and McCrea, D.A. (1986) Electrophysiological investigation of a descending pathway from the cuneiform nucleus to alpha motoneurons in cats. Soc. Neurosci. Abstr., Vol. 12: 883

Shefchyk, S.J., McCrea, D.A., Kriellaars, D.J., Noga, B.R., and Jordan, L.M. (1987) Activity of L4 interneurons with group II input during fictive locomotion in the mesencephalic cat. Soc. Neurosci. Abstr., Vol. 13: 826

Sherrington, C.S. (1906) **The Integrative Action of the Nervous System.** Yale University Press, New Haven

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

Shik, M.L. (1983) Action of the brainstem locomotor region on spinal stepping generators via propriospinal pathways. In: **Spinal Cord Reconstruction**, C. C. Kao, R. P. Bunge and P. J. Reier, eds., pp. 421-434, Raven Press, New York

Shik, M.L., Severin, F.V., and Orlovsky, G.N. (1967) Structures of the brain stem responsible for evoked locomotion. Fiziol. Zh. (SSSR) 53: 1125-1132

Shik, M.L., and Yagodnitsyn, A.S. (1977) The pontobulbar "locomotor strip". Neurophysiol. 9: 72-74

Shik, M.L., and Yagodnitsyn, A.S. (1978) Unit responses in the "locomotor strip" of the cat hindbrain to microstimulation. Neurophysiol. 10: 373-379

Shimamura, M., and Kogure, I. (1983) Discharge patterns of reticulospinal neurons corresponding with quadrupedal leg movements in thalamic cats. Brain Res. 230: 27-34

Shimamura, M., Kogure, I., and Fuwa, T. (1984a) Supraspinal descending control on forelimb stepping in thalamic cats. Neurosci. Lett., Suppl. 17: S55

Shimamura, M., Kogure, I., and Fuwa, T. (1984b) Role of joint afferents in relation to the initiation of forelimb stepping in thalamic cats. Brain Research 297: 225-234

Shimamura, M., Kogure, I., and Wada, S. (1982) Reticular neuron activities associated with locomotion in thalamic cats. Brain Res. 231: 51-62

Sholomenko, G.N., and Steeves, J.D. (1987) Effects of selective spinal cord lesions on hind limb locomotion in birds. Exp. Neurol. 95: 403-418

Skinner, R.D., Garcia-Rill, E., Griffin, S., Nelson, R., and Fitzgerald, J.A. (1984) Interstitial nucleus of Cajal (INC) projections to the region of Probst's tract. Brain Res. Bull. 13: 613-621

Skinner, R.D., and Willis, W.D. (1970) Spinal cord potentials produced by ventral cord volleys in the cat. Exp. Neurol. 27: 305-317

Skinner, R.D., Willis, W.D., and Hancock, M.B. (1970) Actions of ventral cord pathways on spinal neurons. Exp. Neurol. 27: 318-333

Steeves, J.D., and Jordan, L.M. (1980) Localization of a descending pathway in the spinal cord which is necessary for controlled treadmill locomotion. Neurosci. Lett. 20: 283- 288

Steeves, J.D., and Jordan, L.M. (1984) Autoradiographic demonstration of the projections from the mesencephalic locomotor region. Brain Research 307: 263-276

Steeves, J.D., Sholomenko, G.N., and Webster, D.M.S (1987) Stimulation of the pontomedullary reticular formation initiates locomotion in decerebrate birds. Brain Res. 401: 205-212

Stewart, W.A., and King, R.B. (1963) Fiber projections from the nucleus caudalis of the spinal trigeminal nucleus. J. Comp. Neurol. 121: 271-286

Tebecis, A.K. (1973) Transmitters and reticulospinal neurons. Exp. Neurol. 40: 297-308

Torvik, A. (1956) Afferent connections to the sensory trigeminal nuclei, the nucleus of the solitary tract and adjacent structures. J. Comp. Neurol. 106: 51-141

Walberg, F., Dietrichs, E., and Norby, T. (1984) The medullary projection from the mesencephalic trigeminal nucleus. An experimental study with comments on the intrinsic trigeminal interconnections. Exp. Brain Res. 56: 377-383

Williams, B.J., Livingston, C.A., and Leonard, R.B. (1984) Spinal cord pathways involved in initiation of swimming in the stingray, Dasyatis sabina: spinal cord stimulation and lesions. J. Neurophysiol. 51: 578-591

Yamaguchi, T. (1986) Descending pathways eliciting forelimb stepping in the lateral funiculus: experimental studies with stimulation and lesions of the cervical cord in decerebrate cats. Brain Res. 379: 125-136

Yamaguchi, T. (1987) Monopodal fictive locomotion evoked by cervical cord stimulation in decerebrate cats. Neurosci. Lett. 74: 69-74

Yokota, T., and Nishikawa, N. (1980) Reappraisal of somatotopic tactile representation within trigeminal subnucleus caudalis. J. Neurophysiol. 43: 700-712

Zemlan, F.P., Kow, L.-M., and Pfaff, D.W. (1983) Effects of interruption of bulbospinal pathways on lordosis, posture, and locomotion. Exp. Neurol. 81: 177-194