THE MESENCEPHALIC LOCOMOTOR REGION IN THE DECEREBRATE CAT: PROJECTIONS AND THE ROLE OF NORADRENALINE

by

JOHN D. STEEVES, B. Sc.

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY

UNIVERSITY OF MANITOBA

WINNIPEG, MANITOBA

CANADA, R3E OW3

MAY 1979

THE MESENCEPHALIC LOCOMOTOR REGION IN THE DECEREBRATE CAT: PROJECTIONS AND THE ROLE OF NORADRENALINE

BY

JOHN D. STEEVES

A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

"As to what nervous mechanism it is which, present in the decerebrate preparation and absent from the spinal, contributes so importantly to reflex standing and to the extensor phase of the step, and tends to convert alternating reflexes into tonic postures by suppressing refractory phase, a main portion of it clearly lies between the levels of anterior collinculus and hinder edge of pons."

(Sherrington, 1910, p. 116)

Abstract

Studies in decerebrate cats have demonstrated that repetitive stimulation of a discrete area below the inferior collinculus, the mesencephalic locomotor region (MLR), gives rise to locomotion, even in the deafferented state. However, very little is known about the axonal projections of the neurons within the radius of effective current spread from the MLR stimulating electrode. Previous work has shown that acute low spinal, deafferented cats are able to perform stepping movements with the hindlimbs in response to an i.v. injection of L-3, 4-dihydroxyphenylalaine (L-DOPA), a precursor of noradrenaline (NA), and there is evidence for the direct activation of NA neurons which are found in close proximity to the MLR.

The present study examined the axonal projections of neurons corresponding to the MLR, the cross-sectional areas of the spinal cord that are essential for MLR evoked locomotion, and the possible role of NA in the control of locomotion. By employing the method of autoradiography the fiber pathways originating from the MLR were delineated. No direct projections were found to the spinal cord but projections were found to the gigantocellular and magnocellular reticular formation of the pons and medulla from which locomotion can also be evoked in decerebrate cats. Projections were also seen to other areas of the brainstem such as the subthalamic nucleus, dorsal, lateral, and posterior nuclei of the hypothalamus, intralaminar nuclei of the thalamus, ventral tegmental area of Tsai, superior colliculus,

pontine reticular nuclei, and periaqueductual gray region. Stimulation of some of these areas will also evoke locomotion in decerebrate cats.

By selectively disrupting restricted areas of the spinal cord at the high cervical level in a MLR evoked locomoting cat, it was found that the only cross-sectional area of the cord which could not be disrupted was the ventrolateral funiculus. One of the main descending pathways coursing through the ventrolateral funiculus is the reticulospinal pathway originating from the gigantocellular and magnocellular reticular formation. Therefore, it would appear that one of the essential and perhaps common pathways for the activation of spinal stepping mechanisms is the pathway originating in the gigantocellular and magnocellular reticular formation, which descends through the spinal cord within the ventrolateral funiculus. Nevertheless, the initiation of locomotion by supraspinal structures is probably mediated by several descending systems, any one of which is not sufficient by itself to activate spinal stepping mechanisms.

To determine the importance of a descending NA system in the initiation and maintenance of locomotion in the decerebrate cat, specific pharmacological interventions were employed. An 80% depletion of NA by the neurotoxin 6-hydroxydopamine (6-OHDA) did not alter locomotion in the decerebrate cat. Nor did the tyrosine hydroxylase inhibitor, α-methyltyrosine (AMT) which reduced NA levels by 80%, and also blocked de novo NA synthesis. Finally, it has been reported that the α-adrenergic blocking agent, phenoxybenzamine, (POB) inhibits MLR evoked locomotion.

Yet the administration of POB in the present study did not effect MLR initiated locomotion. Therefore, a descending NA system does not appear to be essential for the initiation of locomotion by supraspinal structures.

ACKNOWLEDGEMENTS

There have been many people who have been instrumental in the completion of this dissertation. First of all, I would like to express my gratitude to my parents, Jack and Doreen, for their patience and guidance as I stumbled through my youth, insisting on doing things the "hard" way.

I am also deeply indebted to Dr. Larry M. Jordan for supervision of my graduate student training, and more importantly for his understanding and friendship. It has been a privilege to observe his creative and logical approach to science. His enthusiastic assistance in the design and execution of my research has proven to be invaluable.

Special thanks are also due to Dave McCrea, John Menzies, Carol Albert, Brian Schmidt, and Cathy Elliott. As fellow students in Dr. Jordan's laboratory, they have advised and encouraged me through the good and bad times of the past few years. When not engaged in discussions of scientific merit, I was most appreciative of Dave for joining me in Monty Python sketches, John for his Porky Pig impersonation, Carol for our 20/20 hindsight discussions of the "sporting" world, Brian for his belief that a lobotomy would cure me, and Cathy for her delicious cakes and cookies.

Others who have contributed in diverse and distinctive ways to the research in this dissertation are: Dr. Ralph Jell, Dr. Ed Kroeger, Kathy Kowbel, Susan Pylypas, Diana Salter, and Barbara Skovgaard. I would also like to thank Carol Kunz for the excellent typing of this dissertation. The helpful comments and constructive criticisms of

my examining committee were also appreciated. I am grateful to the staff and students of the Department of Physiology, who have enriched my life in one way or another.

Finally, I dedicate this dissertation to my wife, Claudia, who offers more love and understanding than I deserve. Thank you Claudia for showing me the sunny side of life and allowing me to share your beauty.

Table of Contents

Introduction

Locomotion is defined, simply, as movement from one place to another. The basic underlying mechanisms of vertebrate locomotion are the coordinated limb movements, which are controlled by the alternate contractions of antagonistic limb muscles. However, very little is known about the nature of the neuronal networks which control the muscles.

Graham Brown (1911) discovered that stepping could be evoked in the bilaterally deafferented hindlimbs of acute, decerebrate, unanesthetized cats by transection of the low thoracic spinal cord. Graham Brown concluded that the lumbosacral cord contained local centers (stepping generator) capable of coordinating muscle activity within a limb and between limbs, which did not require peripheral input for the initiation or maintenance of the stepping movements. More recently, Grillner and Zangger (1974) have confirmed that the transected and deafferented lumbosacral cord can generate rhythmic alternating stepping movements. After spinalizing kittens at the thoracolumbar level of the cord they cut the dorsal roots (L_3 - S_4) of both hindlimbs. Stimulation of the dorsal columns in the caudal portion of the transected cord evoked rhythmic alternating activity in knee flexors and extensors. Similar results were obtained when recording from peripheral nerve filaments in curarized preparations. The stimulus for the lumbosacral stepping generator could be either activation of descending fivers or antidromically stimulating ascending fibers which, via collaterals, activate the generator.

Several other studies, using pharmacological agents, suggest that the neuronal organization subserving rhythmic alternating movements is intrinsic to the spinal cord. Budakova (1973) showed that acute spinal cats, which exnibited no locomotor activity prior to treatment, commenced stepping on a treadmill after intravenous (i.v.) infusion of L - 3, 4 - dihydroxyphenylalanine (L-DOPA). L-DOPA is thought to act through the release of noradrenaline (NA) from descending fibers, which then acts on the NA receptors of spinal neurons (Anden, et al, 1964, 1966b). Forssberg and Grillner (1973) have also shown that i.v. infusion of Clonidine, which is thought to directly stimulate central - adrenergic receptors (Anden et al, 1970), enables acute spinal cats to walk on a treadmill. Finally, after bilateral deafferentation or curarization of acute spinal cats, rhythmic alternating activity in peripheral nerves to antagonistic hindlimb muscles can still be obtained after i.v. L-DOPA or Clonidine (Grillner and Zangger, 1974). The results of these studies are interesting based on the findings of earlier fluorescence histochemical work which showed that there are no intraspinal neurons containing NA, and all the noradrenergic fibers visualized within the spinal cord originate in the brainstem (Carlsson, et al, 1964; Dahlstrom and Fuxe, 1965). Furthermore, the possibility that this locomotion is due to effects mediated by dopamine (DA) is not considered likely, since spinal cord DA has never been biochemically measured in appreciable amounts (Anden, 1965; Rawe et al 1977a).

It may be inferred from these studies that stimulation of noradrenergic receptors or release of NA from descending noradrenergic fibers in the spinal cord activates intrinsic neuronal mechanisms which give rise to the rhythmic alternating locomotor movements. Indeed, Anden et al (1966a) and Jankowska et al (1967 a, b) have shown that intrinsic spinal neuronal

circuits which could give rise to locomotion are activated after an i.v. injection of L-DOPA.

Since proprioceptive and exteroceptive afferent input are not necessary for the initiation and maintenance of stepping in the spinal animal, then what are the central nervous system (CNS) mechanisms responsible for the activation of the spinal stepping generator? The use of decerebrate animal preparations has aided investigators in their attempts to answer this question.

In an early pioneering study, Sherrington (1910) reported that acute intercollicular decerebrated cats were capable of standing and reflex walking. The ability of decerebrate cats to stand, as opposed to acute spinal cats which are unable to stand, was suggested to be due to the rigidity present within the limb extensors of the decerebrate preparation. The reflex walking evoked in Sherrington's decerebrate preparations was more effective than that witnessed in the high spinal (decapitate) preparations. He attributed the difference to an unknown nervous mechanism situated between the levels of the superior colliculi and the caudal edge of the pons.

Subsequently, several investigators (Hinsey et al, 1930; Bard and Macht, 1958; Villablanca, 1966) have studied the locomotor capabilities of decerebrate cats. The extent and effectiveness of locomotion depends on the level of transection (see Figure 1), whether the preparation is chronic or acute, and on the amount of background decerebrate rigidity (cf. Wetzel and Stuart, 1976). The capability for spontaneous locomotion is retained in premammillary preparations, where the level of transection extends along a plane

from the rostral border of the superior colliculi dorsally, to the anterior margin of the mammillary bodies ventrally (Hinsey et al, 1930). Such preparations are capable of spontaneous righting from a prone position, standing, and aimless walking after a survival period of only 1 day. If, however, the plane of transection is altered so that ventrally the transection extends to the caudal border of the mammillary bodies (postmammillary preparation) then the cat is unable to exhibit any spontaneous righting, standing, or walking until approximately 2 weeks after the transection (Bard and Macht, 1958; Villablanca, 1966). These experiments indicated the importance of structures within the brainstem which are necessary for the expression of locomotion. Many studies have been undertaken to elucidate these essential brainstem structures.

Waller (1940) discovered that stimulation of the subthalamic region in lightly anaesthetized animals evoked locomotion. More recently, acute premammillary preparations have been made to walk on a treadmill by stimulation of this subthalamic locomotor region (SLR) (Orlovsky, 1969a). The profound differences in locomotor ability between an acute premammillary and an acute postmammillary cat might be explained by the presence of the SLR in the former but not the latter preparation. Bilateral destruction of the SLR, in otherwise intact animals, inhibits voluntary locomotion for approximately two weeks; nevertheless during the interim period, stimulation of the mesencephalic locomotor region (MLR) enables the cat to perform coordinated locomotion (Sirota and Shik, 1973).

Therefore, the discovery of Shik et al (1966a) is of particular interest. They decerebrated cats at the postmammillary level and suspended the animals over a treadmill. As mentioned above, such postmammillary cats are unable to spontaneously step in an acute state. However, when they stimulated a discrete area below the inferior colliculus, the MLR, with a weak repetitive stimulation ($\sim$60 Hz) the cats began to walk. Increasing the strength of stimulation and/or the treadmill speed resulted in faster stepping and sometimes a conversion from an "out of phase" bilateral step mode characteristic of walking, to an "in phase" bilateral step mode characteristic of galloping. They concluded that the locomotion evoked in the postmammillary cat is identical to that of an intact cat walking in a straight line. A subsequent study showed that an acute postmammillary cat, stimulated in the MLR, can walk unassisted across a floor providing there are no obstacles in its path (Sirota and Shik, 1973).

The locomotion evoked in postmammillary preparations by stimulation of the MLR is not dependent on direct peripheral afferent input. Grillner and Zangger (1975) deafferented both hindlimbs of such a preparation and showed that the locomotor pattern evoked by MLR stimulation was unchanged from that observed in a cat having an intact himdlimb afferent input. However, it is possible that propriospinal influences arising in the cervical cord facilitate locomotion in the hindlimbs, and mask the effects of bilateral hindlimb deafferentation. This possibility seems unlikely, since lumbar ventral root filament activity is identical both before and after paralysis in MLR evoked locomotion (Jordan et al, 1979).

In intact cats, under chronic conditions, MLR stimulation also induces a cat to walk (Sirota and Shik, 1973). The locomotion induced by MLR stimulation is greatly improved after bilateral lesions of the SLR or the centrum medianum-parafascicular nucleus complex of the thalamus (Sirota and Shik, 1973). The interactions between these areas are poorly understood and will require further investigations before the identity of their relationship can be established. Bilateral lesions of the MLR in the intact cat result in an inability of the animal to run. The hindlimb movements also become uncoordinated after such lesions.

The first suggestion of the anatomical identity of the MLR was also proposed by Shik et al (1967). On the basis of histological verification of the sites of the MLR stimulating electrodes, they stated the MLR corresponds to the caudal division of the cuneiform nucleus (CNF). A fundamental question that arises from these findings is whether it is the neurons of the cuneiform nucleus or axons passing through this region, but originating elsewhere, which are responsible for evoking locomotion due to MLR stimulation.

Several studies have provided indirect evidence that it is the neuronal cell bodies, situated within the effective radius of current spread from the MLR stimulating electrode, that are responsible for initiating locomotion in the postmammillary cat. Bilateral lesions of the red nuclei (Shik et al, 1968), or removal of the superior and inferior colliculi bilaterally (Shik et al, 1967) does not alter MLR evoked locomotion. Therefore, they concluded that the synaptic activation of rubrospinal and tectospinal neurons is not necessary

for locomotion evoked by MLR stimulation. Also, if the ventral border of the transection is moved approximately 3 mm caudal to that in the postmammillary preparation (i.e. behind the exit of the 3rd cranial nerve) then MLR stimulation becomes ineffective (Shik et al, 1967). Such a small shift would not have such a decisive effect if the locomotion, evoked by MLR stimulation, depended on direct excitation of axons descending from more rostral areas.

Another important question is whether the MLR activates the spinal stepping generator directly or indirectly. Orlovsky (1969b) did not find any monosynaptic connections to the spinal cord from the MLR. However, monosynaptic responses were evoked in reticulospinal neurons of the dorsal medial pons and medulla by stimulation of the MLR (Orlovsky, 1970a). This finding prompted an extensive investigation into the activities of single neurons of the reticulospinal, vestibulospinal, and rubrospinal tracts, which are well known fast-conducting, descending pathways.

Orlovsky (1970b, 1972b,c) found that vestibulospinal neurons fire rhythmically during locomotion and are maximally fired during the stance (extensor) phase of the hindlimb step cycle, whereas the reticulospinal and rubrospinal neurons are rhythmically active during the swing (flexor) phase of the step cycle. He also noted that stimulation of each of these descending pathways during locomotion resulted in activation of the same appropriate hindlimb motoneurons, as would accur during stimulation when the cat was at rest (orlovsky, 1972a). However, the synchrony between the activity in a descending pathway and that in a corresponding motoneuron does not mean that these descending pathways are responsible for the initiation and control of stepping movements.

Orlovsky noted that the rhythmic modulation of activity in these descending pathways ceases if one of the hindlimbs is temporarily arrested. Also, stimulation of the descending pathways during locomotion does not affect the frequency of stepping or the duration of the stance and swing phases of the step cycle. It has been suggested that these fast-conducting descending pathways only serve a modulatory role in the control of locomotion, and their rhythmic activity is dependent on ascending influences from the spinal cord directly or indirectly via the cerebellum (cf. Shik and Orlovsky, 1976).

In acute premammillary cats, removal of the cerebellum results in the disappearance of rhythmic discharges in reticulospinal, vestibulospinal and rubrospinal neurons during locomotion evoked by SLR or MLR stimulation (Orlovsky, 1970c, 1972b, c). Also, the fact that locomotion can be evoked in a decerebellate premammillary or postmammillary cat provides further evidence against the involvement of these fast-conducting descending pathways and the cerebellum in the initiation of locomotion.

It has been suggested that MLR stimulation evokes locomotion via the excitation of the descending noradrenergic system (cf. Grillner, 1975). Since stimulation of the MLR enables a postmammillary cat to walk, it is reasonable to hypothesize that MLR stimulation "releases" the intrinsic spinal stepping generator in a manner similar to that observed in acute spinal cats after i.v. infusions of L-DOPA or Clonidine. An investigation by Grillner and Shik (1973) revealed that stimulation of the MLR produced changes in spinal cord reflexes and polysynaptic pathways which could be the underlying mechanism for locomotion.

Stimulation at a strength that evoked walking, prior to curarization, induced a depression of inhibitory short-latency reflex effects to α- motorneurons from cutaneous and high threshold muscular afferents without changing the direct excitability of the α-motorneurons. At the same time, long-lasting discharges having a long central delay could be evoked from peripheral nerves during MLR stimulation. There is a strong resemblance between these discharges and those evoked in the spinal cat after an i.v. infusion of L-DOPA, a precursor of NA which is able to cross the blood-brain barrier (Jankowska et al, 1967a, b). This could come about if the MLR was a source of descending NA-containing fibers terminating in the spinal cord. Alternatively, stimulation of the MLR might cause indirect activation of NA- containing neurons located in brainstem areas outside the confines of the MLR. In order to test the former suggestion, Steeves et al (1975) looked for the presence of catecholamine (CA)- containing cell bodies within the confines of the functionally effective MLR. They found CA-containing neurons, of the nucleus locus coeruleus, within 50μm of the electrode tip position. On the basis of quantitative evaluations of the spread of current from an electrode tip through CNS tissue (Stoney et al, 1968; Wise, 1972; Bagshaw and Evans, 1976), the MLR stimulus (20-190μA, 30 Hz) necessary to evoke locomotion would directly activate those CA- containing cells. Subsequent studies (Kuypers and Maisky, 1975, 1977) have demonstrated that the nucleus locus coeruleus projects fibers to the spinal cord. Together these studies suggest that MLR stimulation results in direct activation of descending NA fibers and subsequently their spinal terminals, thereby activating

the spinal stepping generator which gives rise to locomotion.

However, it is unlikely that this descending NA system alone is responsible for the initiation of locomotion since selective destruction of lumbosacral NA terminals by intraspinal injection of, the CA- specific neurotoxin, 6-hydroxydopamine (6-OHDA) does not alter MLR evoked locomotion in post-mammillary cats (Jordan and Steeves, 1976).

Most recently, the autoradiographic and horseradish peroxidase techniques for tracing neuronal pathways have demonstrated the existence of many new brainstem descending pathways (cf. Kuypers and Maisky, 1975, 1977; Basbaum et al, 1978; Castiglioni et al, 1978). Possibly some of these projections are related to the initiation of MLR evoked locomotion. As a final introductory note, a group of investigators have recently shown that it is possible to evoke locomotion in a post-mammillary cat by weak stimulation of a number of areas in the reticular formation, throughout the pons and medulla (Mori et al, 1977, 1978; Shik and Yagodnitsyn, 1977). As yet, they have been unable to determine whether these locomotor areas are axonal tracts arising from cells originating in the MLR, or are "columns" of inter-connected cells (polysynaptic pathways).

Therefore, our present understanding of the role of supraspinal structures in locomotor control is very incomplete. We know that weak stimulation of a circumscribed area beneath the inferior colliculus, the MLR, initiates a complex behavior, locomotion, in a post-mammillary cat. We do not know for certain if the MLR projects fibers directly to the spinal cord, or whether the activation of the spinal stepping generator is via a descending polysynaptic pathway. There is uncertainty

surrounding the importance of a descending NA system in the initiation and maintenance of locomotion. Finally, there is only limited information available as to how the MLR interacts anatomically or physiologically with other CNS areas implicated in motor performance.

The proposed research attempts to answer some of the questions outlined above. With the use of autoradiographic methods, the axonal projections of neurons activated by MLR stimulation will be determined. This experimental procedure (see methods) will enable us to determine whether the MLR projects directly to the spinal cord, as well as revealing what other areas of the brainstem receive projections from neurons corresponding to the MLR.

The basis of the autoradiographic method relies on the incorporation of a tritium labelled amino acid into protein by cell bodies in close proximity to the injection site, the MLR, and its subsequent movement, via axoplasmic transport, towards the axon terminals (cf. Cowan and cuenod, 1975). Ultrastructural studies indicate that the sites of protein synthesis for neurons, the ribosomes, are concentrated within the cell body and large dendrites, where they are found as elements of rough endoplasmic reticulum (or Nissl bodies) (Droz, 1967). Ribosomes have never been observed within the axons of neurons (Palay and Palade, 1955). Heuser and Miledi (1970) have shown that labelled leucine and glutamate, whether applied externally or injected intra-axonally, are not incorporated into protein by the squid giant axon. Finally, Cowan et al (1972) have injected labelled amino acids into the corpus callosum, a known axonal pathway connecting the two cerebral hemispheres, which does not contain any neuronal cell bodies. They did not find any

radioactive label in either hemisphere, indicating that axons are unable to incorporate and transport labelled amino acids. Therefore, by using autoradiography, an injection into the MLR will only label axons arising from cell bodies within the MLR. The labelled amino acid will not be incorporated or transported by fibers passing through the MLR. Although it has been shown that axon terminals are capable of taking up labelled amino acids, only a small amount of protein synthesis accurs in the terminals (Cotman and Taylor, 1971). Also, Cowan et al (1972) have found no morphological evidence for a significant retrograde transport of tritiated amino acids. Thus, any label found overlying a CNS structure will have been transported in an orthograde direction from the cell bodies of origin at the injection site.

A second aspect of this thesis will attempt to isolate the spinal funicular trajectories of descending supraspinal signals necessary for MLR initiated locomotion in the cat. In acute spinal animals, the best cross-sectional areas of the cord to stimulate, so as to evoke locomotion, are the dorsal columns or the dorsolateral funiculus (Sherrington, 1910; Grillner and Zangger, 1974). Under chronic conditions, if a small portion of the ventromedial funiculus is left intact at the low thoracic cord level, then the rest of the cross-sectional cord can be cut and the cat will still be able to walk (Afelt, 1974; Windle et al, 1958). The conclusion that the walking by these chronic cats is due to the few intact fibers in the ventromedial funiculus is suspect, since complete transected spinal cats and dogs are known to engage in stepping movements and sometimes even walk (Hart, 1971; Shurrager and Dykman, 1951). Therefore, it would be interesting to study which cross-

sectional areas of the cord are functionally necessary for MLR evoked locomotion.

The third and final part of this thesis involves a series of experiments designed to investigate the role of descending NA systems in the initiation and control of locomotion. As outlined above, there is reason to believe that the descending NA system is not necessary for the activation of the spinal stepping generator, even though there has been agreat deal of compelling indirect evidence favoring its involvement.

The first series of experiments involves a continuation of previous work in this laboratory using the specific CA neurotoxin 6-OHDA (Jordan and Steeves, 1976). It was Tranzer and Thoenen (1968) who discovered that 6-OHDA caused an actual destruction of the terminal endings of sympathetic neurons. Subsequently it has been shown that 6-OHDA selectively destroys CNS CA - containing neurons, which results in a marked reduction of CA levels (See Kostrzewa and Jacobowitz, 1974). Therefore, 6-OHDA will be injected into a lateral ventricle of a cat to maximally destroy all CNS CA cells and fibers. Then, the animal will be decerebrated and tested to see whether locomotion can be evoked by MLR stimulation. The percentage reduction of NA by 6-OHDA will be determined by the Schellenberger and Gordon (1971) method for assaying monoamines. To assess the selectivity of 6-OHDA, 5-hydroxytryptamine (5-HT) will also be measured.

The second series of experiments will involve the injection of α-methyltyrosine (AMT) prior to MLR stimulation. Spector et al (1965) have shown that AMT reduces tissue NA levels by the inhibition of

tyrosine hydroxylase, an enzyme involved in the formation of NA (see figure 2). Tyrosine hydroxylase is the rate-limiting step in NA biosynthesis, converting dietary tyrosine to L-DOPA (Nagatsu et al, 1964). Once tyrosine hydroxylase is inhibited, tissue NA levels fall at a rate determined by the normal turnover of NA in the tissue (Udenfriend et al, 1966). In the cat, maximal depletion of CNS NA by AMT occurs after 24 hr (Rawe et al, 1977b). However, maximal inhibition of tyrosine hydroxylase activity in brain occurs after only 4 hr. (Udenfriend et al, 1966). Therefore, to assure that NA will not be present in the CNS, this study will utilize two injections of AMT. The first injection will be given 24 hr. prior to the experiment, to ensure maximal depletion of NA stores; the second will be given 4 hr. prior to the experiment, to prevent the de novo synthesis of NA during the MLR stimulation trials.

Finally, one argument for the involvement of a descending NA pathway in the initiation of locomotion has been that the α-adrenergic blocking agent, phenoxybenzamine (POB) (cf. Marley and Stephenson, 1972), inhibits MLR initiated locomotion (see Grillner, 1973, 1975). Due to the lack of experimental details in Grillner's reports, and the apparent discrepancy between the effects of POB and intraspinal 6-OHDA, it would be interesting to re-examine the effects of POB on MLR evoked locomotion.