THE ACTIVITY AND FUNCTION OF RENSHAW CELLS

AND Ia INHIBITORY INTERNEURONS IN THE CONTROL OF

LOCOMOTION IN THE POSTMAMMILLARY CAT

by

Carol A. Pratt, B.S., M.S.

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Carol A. Pratt
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Supervising Professor: Larry M. Jordan, Ph.D.

It is now well established that the feline spinal
cord can produce co-ordinated rhythmic locomotor activity
in flexor and extensor motoneurons in the absence of
phasic descending and afferent signals (Grillner and
Zangger, 1974; for review see Wetzel and Stuart, 1976).
Various models of spinal locomotion generators have been
proposed despite a relative paucity of information
regarding the activity of identified spinal neurons during
locomotion. The only well identified spinal interneurons
known at present are the inhibitory interneurons, Renshaw
cells (RCs) and Ia inhibitory interneurons (IaINs).
These interneurons have been shown to be rhythmically
active during "fictive" locomotion which occurs in the
absence of rhythmic input to the spinal cord, and it has

been suggested that RCs and IaINs could be components of the spinal locomotion generator (Miller and Scott, 1977). Two reports (Severin, et al. 1968; Bergmans, et al. 1969) have suggested that RCs are inhibited during locomotion, however, and although RCs have been found to be rhythmically active during fictive locomotion, the possibility still exists that segmental afferents originating in moving limbs may depress the activity of RCs during locomotion. The purpose of the present study was to resolve the issue of whether RCs are functional during locomotion and to determine the possible roles of RCs and IaINs in the control of the rhythmic behavior of motoneurons during locomotion.

The evidence obtained in the present study indicates that RCs are not inhibited during locomotion evoked by stimulation of the mesencephalic locomotor region in precollicular-postmammillary non-paralyzed (controlled treadmill locomotion) and paralyzed (fictive locomotion) cats. Motoneuron activity recorded in ventral root filaments was significantly inhibited by antidromic stimulation of an L7 ventral root (recurrent inhibition) during controlled treadmill locomotion when segmental

afferentation was intact. Significant recurrent
inhibition of IaINs and RCs was also produced by
antidromic ventral root stimulation during fictive
locomotion. A positive linear relationship existed
between the amount of recurrent inhibition of motoneurons
and IaINs and the normal frequency of cell firing prior
to ventral root stimulation.

The patterns and phase relationships of motoneuron,
RC and IaIN activity during fictive locomotion were
determined by normalizing each step cycle and computing
the period of activity and the frequency histogram for
each cell. Flexor and extensor RCs were active throughout
the flexion and extension phase of the step cycle,
respectively, with their periods of maximal activity
occurring at the end of their active periods. They
usually stopped firing just after the cessation of activity
in flexor and extensor motoneurons. The pattern of
quadriceps coupled IaIN activity was more variable, but
in general, the IaINs were mainly active from late flexion
to late extension with their period of maximal activity
occurring during early to middle extension.

The data suggest that IaINs mediate reciprocal
inhibition during fictive locomotion, thereby contributing

to the hyperpolarization of antagonist motoneurons during their inactive phase of the step cycle. The recurrent inhibition of IaINs may limit the depth of reciprocal inhibition but does not appear to interfere with the appropriate timing of reciprocal inhibition. RCs also probably function to keep motoneuron discharge within moderate frequencies during their active phase. The increased frequency of RCs at the end of their active period indicates that the recurrent inhibition of motoneurons and IaINs may be maximal at these times which could assist in terminating the activity in agonist motoneurons and the reciprocal inhibition of antagonist motoneurons at the end of each phase of the step cycle. In addition, RCs may transmit information about the activity of motoneurons to supraspinal structures via the ventral spinocerebellar tract.

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INTRODUCTION

A. Evidence for and discussion of spinal locomotion
generators

The pioneering work of Sherrington (1910), Phillipson
(1905) and Graham Brown (1911, 1914a, b) at the turn of
the century stimulated years of debate over the relative
importance of central versus peripheral nervous system
control of locomotion. Phillipson (1905) argued that the
locomotor rhythm was a manifestation of a sequence of
interlocking reflexes. Sherrington (1910) demonstrated
that non-specific stimulation of the spinal cord caudal
to the site of transection could evoke walking movements
in a deafferented limb. Graham Brown (1911) dramatically
made a case for the central programming of the locomotor
rhythm when he showed that cutting the spinal cord of a
decerebrate cat produced stepping movements in
bilaterally deafferented hindlimbs. Graham Brown's work
(1914b) further indicated that the spinal circuits that
are responsible for producing locomotor rhythms are an
intrinsic part of the central nervous system from before
birth, since fetal kittens removed from the uterus of a

decerebrate cat displayed co-ordinated four-limb locomotor movements when placed in a saline bath. Thus, the basic locomotor rhythms are not learned phenomena. The evidence that has accumulated over the last eight decades overwhelmingly supports the proposition that neuronal circuits exist in the spinal cord that are capable of producing co-ordinated rhythmic activity in flexor and extensor muscles in the absence of rhythmic supraspinal and segmental afferent input (Shurrager and Dykman, 1951; Grillner and Zangger, 1974; for reviews see Grillner, 1975 and Wetzel and Stuart, 1976).

Definitive evidence that the capability for generating locomotor rhythmicity is an intrinsic property of the spinal cord was provided by Grillner and Zangger (1974), who showed that a chronic spinal (Th13) kitten could walk on a treadmill in response to simultaneous, low frequency (5Hz) stimulation of the L7 dorsal roots even after the hindlimbs had been bilaterally deafferented (L3 - S4). The evoked locomotion was similar to that seen in intact cats (Engberg and Lundberg, 1969; Rasmussen, et al. 1978) as evidenced by the normal alternating EMG

activity recorded from muscles which flex and extend the knee. The same results were obtained in experiments in which cats were curarized and locomotion was monitored by recording from peripheral nerve filaments (Grillner and Zangger, 1974), thereby excluding the possibility of a contribution from ventral root afferents (Coggeshall, et al. 1974). Grillner and Zangger's work (1974) was especially significant because it demonstrated conclusively that the spinal cord can generate rhythmic motoneuron activity in response to tonic stimulation when it is isolated from any phasic input. These findings have been confirmed by others (Jankowska, et al. 1967a, b; Sjostrom and Zangger, 1975; Edgerton, et al. 1976) who have observed rhythmic motoneuron activity in acute spinal, paralyzed cats in which locomotion was induced by intravenous (i.v.) administration of dihydroxyphenylalanine (DOPA) and Nialamide.

The fact that a non-specific mechanical (Brown, 1911), electrical (Sherrington, 1910; Grillner and Zangger, 1974; Baev, 1977b) or chemical (Jankowska, et al. 1967a, b; Budakova, 1973; Sjostrom and Zangger, 1975, Edgerton, et al. 1976) signal can cause the isolated spinal cord to produce locomotor activity in hindlimb muscle nerves, indicated that the specificity

of intralimb muscle activation during locomotion is programmed by a spinal pattern generator which can be activated by descending, ascending or afferent pathways (Grillner, 1976). The available evidence suggests that a continuum of movement capability exists whereby the sophistication and adaptability of the motor output will be directly related to the magnitude of the directional and feedback information made available to the spinal locomotion generator via supraspinal and afferent pathways. At the base of the continuum is a substrate pattern of highly co-ordinated alternating extensor and flexor muscle activity (Grillner and Zangger, 1975) that is manifested in modes of locomotion that are very similar to those seen in intact cats (Engberg and Lundberg, 1969; Rasmussen, et al. 1978), albeit of an automatic type. The spinal locomotion generator appears to be highly specific and to contain the programming necessary to orchestrate the complexities and subtleties of the timing of hindlimb muscle group activation during locomotion. Evidence for this was provided by Grillner and Zangger (1975) who found that the unioue EMG pattern of semitendinosis activity during walking persisted even after bilateral deafferentation of the hindlimbs.

Supraspinal and afferent input appear to be primarily involved in setting the energy level of the spinal generator which affects the vigor and gait of locomotion (Shik, Severin and Orlovsky, 1966; Grillner, 1976; Wetzel and Stuart, 1976) while the program governing intralimb muscle activation is determined by the circuitry of the spinal rhythm generator (Orlovsky, 1972; Grillner and Zangger, 1974, 1975). It is generally assumed that the spinal locomotion generator consists of a network of interneurons that communicate with motoneurons of a single limb and most likely with generators of other limbs. This assumption is supported by the fact that stimuli which provoke stepping were not found to alter alpha motoneuron excitability directly, but rather seemed to unveil a spinal neuronal network that appears to be uniquely associated with locomotion (Grillner and Shik, 1973). Brainstem stimulation in decerebrate cats and DOPA in acute spinal cats evoke stepping movements in the hindlimbs; in both preparations there is a depression in the short latency inhibitory pathway from ipsilateral cutaneous and high threshold muscle afferents to extensor motoneurons while long latency reflex effects to ipsilateral flexor motoneurons are enhanced. Jankowska, et al. (1967a, b)

proposed that the effects of DOPA on spinal reflexes could be explained by the release of spinal interneuronal networks from tonic inhibition and suggested that these networks could, in fact, function as locomotion generators. This view has received support from others (Grillner and Shik, 1973; Fu, et al. 1975).

The neuronal network described by Jankowska, et al. (1969a, b) is similar to the model originally proposed by Graham Brown (1914a) which consisted of separate pools of interneurons (half-centers) each controlling the activity in either flexor or extensor motoneurons at a particular joint and linked by mutual inhibition. Various modified versions of this reciprocal half-center hypothesis have also been proposed (Cleveland, et al. 1972; Grillner, 1973, 1975; Pearson, 1976; Shik and Orlovsky, 1976; Miller and Scott, 1977). Other spinal locomotion generator models that have been proposed with varying degrees of support include a closed chain or ring of neurons, an open chain of reflexes and asymetric models based upon the activity of pacemaker neurons (c.f. Grillner, 1975; Shik and Orlovsky, 1976; Edgerton, et al. 1976).

There is evidence that the generator for each muscle group for a particular limb is autonomous (Shik, Orlovsky

and Severin, 1966; Edgerton, et al. 1976), but also that
the pattern generators associated with the hindlimbs can be
coupled to other rhythm generators residing within the
spinal cord, such as those associated with the forelimbs
and respiration (Viala, et al. 1979). A tight coupling
between hindlimb locomotor activity and the rhythm of
respiration was observed in curarized, high spinal (C-2)
rabbits treated with DOPA and Nialamide. The entrainment
between the two rhythms was abolished by an additional
transection at T12. It was postulated that discrete
bursting generators exist in the cervico-thoracic spinal
cord and in the lumbar cord. The lumbar oscillator, which
operates at a faster tempo than its more rostral counterparts,
was presumed to set the dominant rhythm in the intact spinal
cord.

B.   Experimental cat locomoting preparations

The various concepts of mammalian spinal generators that
have emerged are of limited usefulness because they have been
based more on theory and knowledge of pathways that have been
studied in non-moving animals than on empirical knowledge.
Very little is known at present about the activity of

identified spinal neurons during locomotion. Extrapolation from data generated during static conditions to dynamic conditions is limited in view of the apparent ability of the spinal generator to modulate afferent input during locomotion, most dramatically illustrated by the reflexive resetting of gait (Duysens, 1977). The complexity and diversity of heretofore described neuronal elements and pathways has made possible a large number of overlapping and even conflicting notions of motor control and pattern generation which can only be resolved by studying the actual performance of the neuronal elements during normal or at least dynamically varying conditions.

Of great importance to the study of the neural control of movement has been the development of cat preparations which permit intra- and/or extra-cellular recordings of a single cell during locomotion. Hindlimb walking can be observed in chronic spinal cats with low thoracic or upper lumbar spinal cord lesions (Sherrington, 1910; Shurrager and Dykman, 1951; Grillner, 1973; Grillner and Shik, 1973; and Grillner and Zangger, 1974); in acute spinal cats after i.v. administration of DOPA (Grillner, 1969a, b, 1975; Budakova, 1971, 1973) or Clonidine (Forssberg and Grillner,

1973); and in high decerebrate cats in response to continuous electrical stimulation of the brainstem (Shik, et al. 1966a, b; Severin, et al. 1967a, b, 1968; Kulagin and Shik, 1970; Severin, 1970; Feldman and Orlovsky, 1975; Grillner and Zangger, 1975; Jordan and Steeves, 1976; McCrea and Jordan, 1976b; Menzies, et al. 1978; Jordan, et al. 1979). The latter preparation was developed by Shik, Severin and Orlovsky (1966) who reported that continuous stimulation (25 - 50 Hz) of a discrete area 6 - 7 mm below the inferior colliculus, the mesencephalic locomotor region (MLR), evoked co-ordinated four limb stepping in cats with a precollicular - postmammillary brainstem transection which had been placed on a treadmill (controlled locomotion). A more rostral transection (from the rostral border of the superior colliculi to the caudal edge of the optic chiasm) produces a preparation that will walk spontaneously or in response to low frequency stimulation of the dorsal roots (Orlovsky, 1969; Budakova, 1971, McCrea and Jordan, 1976a, b).

The speed of locomotion displayed by these preparations while on a treadmill is dependent upon the speed of the treadmill belt and is associated with patterns of EMG activity for the prime movers of each limb that are similar

to those seen in intact walking cats. The decrease in the duration of the step cycle that occurs at faster speeds of walking is accomplished by a reduction in the duration of the stance phase while the swing phase remains relatively constant (Kulagin and Shik, 1970; Grillner, 1973); this is also consistent with adjustments seen in intact cats (Engberg and Lundberg, 1969; Rasmussen, et al. 1978). Evidence that the observed stepping behavior is the product of the activiation of a spinal locomotor generator is provided by the fact that in normal cats, and in these walking preparations, the EMG activity in hindlimb extensors precedes foot contact (Kulagin and Shik, 1970; Grillner, 1973). Thus, the initiation of extensor motoneuron activity is programmed centrally and is not reflex in origin.

Preparations in which the animal is actually walking, either on a treadmill or in the air, are restricted to extracellular recordings, most notably electromyograms (EMGs), electroneurograms (ENGs) and recordings from ventral or dorsal roots. Microelectrode recordings of single units in the spinal cord are extremely difficult and intracellular recordings impossible because of the movements of the spinal cord. For these reasons, the development of

the "fictive" locomotion preparation in which rhythmic motoneuron activity typical of locomotion can be recorded from peripheral nerves or cut ventral root filaments in paralyzed animals has greatly facilitated the advancement of our knowledge of the activity of spinal neurons during locomotion.

The fictive locomotion preparation provides a recording environment with sufficient stability to permit intracellular recordings in the presence of rhythmic neuronal discharge. Fictive locomotion has been observed in acute spinal cats (Jankowska, et al. 1967b; Sjostrom and Zangger, 1975; Edgerton, et al. 1976; Andersson, et al. 1978; Schomburg and Behrends, 1978a, b) and rabbits (Viala, et al. 1979) injected with DOPA and Nialamide; chronic spinal cats in which locomotion was induced by stimulation of the dorsal columns or cut dorsal roots (Grillner and Zangger, 1974); in thalamic cats with (Baev, 1977a) and without (McCrea and Jordan, 1976a, b; Perret, 1976; Baev, 1977c, d) noradrenaline agonists; in rabbits with intact neuraxis under light anaesthesia (Viala and Buser, 1965, 1969); in decorticate rabbits with bilaterally deafferented hindlimbs (Viala and Viala, 1976); in rabbits with a precollicular

decerebration (Viala and Buser, 1965); in decorticate cats

(Perret, 1973); and in mesencephalic cats with the hindlimbs

bilaterally de-efferented (Feldman and Orlovsky, 1975).

Stimulation of the MLR was reported not to be sufficient to

produce fictive locomotor activity in paralyzed cats (Shik,

Orlovsky and Severin, 1966) or in cats with deafferented

hindlimbs (Shik, et al. 1966a, b) unless the forelimbs were

rhythmically active. It has recently been shown by Jordan

and coworkers (1979), however, that rhythmic motoneuron

activity similar to that seen before paralyzation can be

evoked in paralyzed cats by stimulation of the MLR in the

absence of any rhythmic forelimb movement. Fictive locomotor

activity in flexor and extensor motoneurons, as evidenced

by recording the activity of motor axons in cut ventral root

filaments (Jordan, et al. 1979) or by intracellular

recordings of alpha motoneurons (McCrea and Jordan, 1976b;

Menzies, et al. 1978) was evoked by MLR stimulation. Dosages

of the paralyzing drug, gallamine triethidide, were used that

were sufficient to block alpha and gamma motoneurons

(Carli, et al. 1967). The fact that fictive locomotion

occurs is consistent with other available evidence which

indicates that cyclic afferent input is not essential for the

production of rhythmic motoneuron activity.

C.   Activity of lumbosacral neurons during locomotion

The first published studies in which the activity of

spinal neurons was recorded in walking cats appeared in

1972 (Arshavsky, et al. 1972a, b; Orlovsky and Feldman),

and only a few studies have been reported since that

time (Feldman and Orlovsky, 1975; Edgerton, et al. 1976;

McCrea and Jordan, 1976a). Unfortunately, the yield from

some of these experiments has been handicapped by the fact

that the neurons studied were not always identified

conclusively, nor have the firing patterns and

interrelationships of various neurons been analyzed in

sufficient detail in all cases.

1.   Unidentified lumbosacral neurons

Surveys of lumbosacral neurons have been conducted in

decerebrate cats where locomotion was induced by MLR

stimulation (Orlovsky and Feldman, 1972) and in acute spinal,

curarized cats treated with DOPA and Nialamide (Edgerton, et

al. 1976) in which the firing patterns of lumbosacral

neurons during locomotion were examined in the hopes of

elaborating populations of interneurons that might function

as components of the spinal locomotor generator. In neither

case, however, were the neurons clearly identified as

interneurons, except for Ia inhibitory interneurons (IaINs),

and the possibility exists that many of the cells studied

were motoneurons or tract cells. Despite these restrictions,

some valuable information can be obtained from these studies.

Orlovsky and Feldman (1972) found 30 neurons that were

spontaneously active at rest but which became rhythmically

active during controlled treadmill locomotion. Of these 30

neurons, 37% were active during the support phase, 26% during

transfer, 37% were active during the transition between

flexion and extension and 13% displayed two bursts of

activity during the step cycle. No geographical specificity

of these subpopulations of neurons could be discerned. In

another series of experiments in which the hindlimbs were

bilaterally deafferented, 63% of the neurons encountered

were phasically active during locomotion. The phasic activity

of many of these cells began before the onset of stepping

movements and continued after stepping had terminated,

thereby suggesting the influence of central programming.

It is possible, however, that the modulation of these

neurons was coupled to that in single α or γ motoneurons,

but since only EMG and limb movement recordings were available, this could not be determined.

Lumbosacral neurons that were rhythmically active during fictive locomotion in the absence of any phasic input have been observed in acute spinal cats (Edgerton, et al. 1976). The majority of neurons studied were activated by stimulation of peripheral nerves at latencies in excess of 15 ms and from cutaneous nerves. Comparisons of the firing patterns of these neurons to the efferent activity recorded in nerve filaments revealed some interesting details that may provide some insight into their function. Phase relationships were examined by comparing the timing between midpoints of the neuron and efferent bursts in normalized step cycles. It was found that the midpoints of neuron activity coincided with that of the flexor or extensor burst in a majority of cells. In some cases, however, such strict coupling with flexors or extensors was not apparent as the midpoint was situated in the transition phases between flexion and extension. These findings are in agreement with those of Orlovsky and Feldman (1972). Three distributions were also seen in patterns of firing as some fired at a steady rate throughout the burst of activity while others exhibited peaks

at either the beginning or the end of the burst. The frequency of discharge in neurons was seen to be directly proportional to efferent activity in some neurons while in others an inverse relationship was observed. These patterns of activity may reflect functional relationships, i.e., as excitatory or inhibitory interneurons to motoneurons, respectively.

In nonparalyzed acute spinal cats treated with DOPA and Nialamide, interneurons were found in the dorsolateral part of Rexed's layer VII whose response to stimulation of the flexion reflex afferents (FRAs) paralleled that seen in motoneurons and primary afferents (Jankowska, et al. 1967b). Three populations of interneurons were found among the 47 recorded neurons; 31 were excited by the ipsilateral FRAs and inhibited by the contralateral FRAs; 12 responded in the opposite manner; and 4 interneurons were activated from volleys in both the ipsilateral and contralateral FRAs. A close correlation was observed between the spontaneous oscillations in motoneuron membrane potential and the discharge of these interneurons. Interneurons that were activated from the ipsilateral FRAs fired coincidentally with the occurrence of excitatory postsynaptic potentials (EPSPs) in flexor

motoneurons; EPSPs in extensor motoneurons were coupled to bursts of activity in interneurons excited from the contralateral FRAs. There appeared to be good evidence, therefore, that these interneurons were responsible for mediating the long latency, long lasting actions of FRAs on motoneurons and primary afferents after DOPA. The reciprocal organization of the excitatory and inhibitory input to these interneurons from the two hindlimbs was interpreted to suggest that they might be organized in a half-center oscillating arrangement similar to that first proposed by Graham Brown (1914a).

2. Ventral and dorsal spinocerebellar tract cells

Only four identified spinal neurons have been studied in walking cats, these being ventral and dorsal spinocerebellar tract cells (VSCT and DSCT cells), IaINs and Renshaw cells. DSCT cells were shown to be rhythmically active during controlled treadmill locomotion and to fire in phase with muscles from which they received Group I input (Arshavsky, et al. 1972a). After deafferentation of the ipsilateral hindlimb, although all four limbs still engaged in MLR-evoked locomotion, no modulation of DSCT cells was observed. VSCT cells, on the other hand, were found to be rhythmically active in mesencephalic and thalamic cats walking on a treadmill even after deafferentation of the hindlimbs and cerebellectomy

(Arshavsky, et al. 1972b). The activity of these cells
was coupled to hindlimb activity, and it was suggested that
VSCT cells transmit information regarding the activity of
the hindlimb spinal generator to the cerebellum (Arshavsky,
et al. 1972b). This proposal is compatible with Lundberg's
(1971) suggestion that VSCT cells may function as sensors
of the excitatory input and inhibitory output of the
inhibitory interneurons that impinge on alpha motoneurons,
this feedback information then being relayed to supraspinal
structures engaged in the descending control of interneurons
in reflex pathways.

3.   Ia inhibitory interneurons

(a) Physiology and anatomy

Activation of primary muscle spindle afferents results
in the monosynaptic excitation of motoneurons innervating
the muscles containing the parent annulospiral endings and a
short latency inhibition of antagonist motoneurons (Lloyd,
1941). The pathway mediating this classical stretch reflex
(Liddell and Sherrington, 1924) is one of the most rigorously
studied spinal reflex pathways. Although originally thought
to be "direct" (Lloyd, 1941), it was subsequently shown by
Eccles, et al. (1956) that the intraspinal delay associated

with reciprocal inhibition indicated a disynaptic linkage.
It was proposed that an interneuron from the intermediate
zone was interposed in the pathway between Ia afferents
and antagonist motoneurons. Based on physiological
experiments, Hultborn, et al. (1971b) determined that the
cells mediating reciprocal inhibition, the IaINs, were, in
fact, located dorsomedial to the motoneuron nuclei in the
ventral part of Rexed's lamina VII. In an elegant series
of experiments, Jankowska and coworkers (Jankowska and Roberts,
1972a, b; Jankowska and Lindstrom, 1972) definitively
determined the electrophysiological and morphological
characteristics of IaINs. Direct evidence was provided that
interneurons in lamina VII that met the accepted criteria
for identification as IaINs (Hultborn, et al. 1971b) do
mediate reciprocal inhibition since they were shown to
produce monosynaptic inhibitory postsynaptic potentials
(IPSPs) in motoneurons (Jankowska and Roberts, 1972b) that
are reported to be mediated by the inhibitory neurotransmitter
glycine (Lodge, et al. 1977).

(b) Input: concept of alpha-gamma-linked reciprocal
inhibition

It is apparent that IaINs do not function simply to

convert Ia afferent excitation into inhibition, since it has been shown that they receive a diversified and extensive array of inputs from segmental and descending systems (Hultborn, 1972; Hultborn and Udo, 1972; Hultborn, et al. 1976b, c). Stimulation of ipsilateral FRAs produced the same pattern of effects in IaINs as had been observed in flexor and extensor motoneurons, i.e., IaINs monosynaptically coupled to Ia afferents from flexor nerves received a greater net excitation than did extensor coupled IaINs while opposite results were obtained with stimulation from the contralateral FRAs (Hultborn, et al. 1976b). The reciprocal organization of the effects of FRA stimulation on IaINs was not as definitive as that which had been observed on motoneurons (Eccles and Lundberg, 1959; Jankowska, et al. 1976a).

A convergence of descending systems upon IaINs was suggested by the evidence that the transmission in the Ia inhibitory pathway was facilitated by volleys in the corticospinal (Lundberg and Voorhoeve, 1962), vestibulospinal (Grillner, et al. 1968) and rubrospinal (Hongo, et al. 1969) tracts (CST, VST, RuST, respectively). These results were essentially confirmed by Hultborn, et al. (1976c) who recorded from IaINs and observed the following pattern of

descending input: polysynaptic excitation, sometimes mixed with inhibition, in flexor and extensor motoneurons from the CST; stimulation of the VST produced mono- and disynaptic EPSPs in extensor coupled IaINs and disynaptic IPSPs in flexor coupled IaINs; flexor and extensor coupled IaINs received mainly polysynaptic excitation from the RuST, but occassional monosynaptic EPSPs and disynaptic IPSPs were also seen. Conclusive data on the effects of the reticulospinal pathway (RST) on IaINs is not yet available; this pathway has been shown to monosynaptically excite flexor motoneurons (Grillner and Lund, 1968).

It is particularly interesting that the pattern of effects elicited from many segmental and descending pathways upon IaINs closely parallels that observed by the same neuronal pathways upon α and γ motoneurons that are monosynaptically linked to the same Ia afferents (Granit, 1955; Grillner, 1969). The observed similarity of convergence onto α and γ motoneurons prompted Granit (1955) to suggest that α and γ motoneurons are normally activated in unison, the idea being that this coactivation would provide α motoneurons with both "direct" and "indirect" (via the gamma loop) routes of excitation. Based upon the evidence that IaINs shared

this convergence, Hongo, et al. (1969) expanded Granit's

concept to include the concomitant reciprocal inhibition

of antagonists and coined the phrase "α - γ - linked reciprocal

inhibition". By coupling the excitation of agonist α and

γ motoneurons with the reciprocal inhibition of antagonist

motoneurons, Hongo, et al. (1969) suggested that this

pathway would effectively subserve movements involving reciprocal

innervation of flexor and extensor muscles. Such movements

would be further facilitated by the mutual inhibition which

was shown to exist between IaINs coupled to antagonist

motoneurons (Hultborn, et al. 1976a). Hypothetically, as

the activity of an extensor Ia afferent increased, extensor

motoneurons would be excited along with extensor coupled

IaINs, resulting in an inhibition of flexor motoneurons

and flexor coupled IaINs projecting to extensor motoneurons.

Extensor activity would thus be supported by direct

excitation as well as by disinhibition. Supraspinal control

of the Ia inhibitory pathway, on the other hand, would allow

the degree of reciprocal inhibition to be more independent

of the level of Ia excitation. Either a decrease in excitation

or an increase in inhibition of the IaIN by descending

pathways could be useful in supporting movements requiring

a coactivation of flexor and extensor muscles.

(c) Activity during locomotion

It is not surprising that the Ia inhibitory pathway has been considered to be an important component in the spinal control of locomotion. Fu, et al. (1975) reported that IaINs were strongly facilitated by the FRA after injections of DOPA in acute spinal cats, a preparation which reportedly releases a spinal neuronal pathway involved in locomotion (Jankowska, et al. 1967b; Grillner and Shik, 1973). It was proposed by Fu and coworkers (1975) that IaINs mediate the late, long lasting inhibition produced by FRA stimulation after DOPA and that these interneurons were part of a spinal locomotion generator. This concept was strongly reinforced by the demonstration that IaINs are rhythmically active during locomotion in the absence of phasic afferent input (Feldman and Orlovsky, 1975; Edgerton, et al. 1976).

Feldman and Orlovsky (1975) were the first to report the rhythmic behavior of IaINs during locomotion. Twenty-three quadriceps (Q) coupled and 2 posterior biceps-semitendinosis (Pb-St) coupled IaINs were recorded extracellularly during controlled treadmill locomotion. All IaINs were rhythmically

active with their periods of maximal firing occurring in phase with the muscles from which they received their Ia input (homonymous muscles). IaINs fired spontaneously prior to locomotion at frequencies of 5 - 50 Hz, which is in agreement with other data (Hultborn, et al. 1971b), but during locomotion their frequency of firing rose to 50 - 250 Hz ($\bar{X}$ = 150 Hz). Some IaINs were completely silent when antagonist motoneurons were active while others just fired at reduced rates. The IaINs continued to display rhythmic activity even after hindlimb de-efferentation, cerebellectomy and lesioning of the associated peripheral nerve, thus proving that the rhythmicity was not produced by the γ - loop, cerebellum or pre-synaptic inhibition, respectively. These findings were confirmed by Edgerton, et al. (1976) who recorded the activity of 5 IaINs during fictive locomotion in acute spinal cats treated with DOPA and Nialamide. The available evidence thus indicates that the modulation of IaINs that occurs during locomotion is centrally programmed.

4. Renshaw cells

(a) Physiology and pharmacology of Renshaw cell responses to antidromic stimulation of motor axons

Another inhibitory interneuron which has received considerable attention and which has been thought to be involved in the control of motoneuron activity is the Renshaw cell. Antridomic stimulation of cut ventral roots or deafferented peripheral motor nerves was shown by Renshaw (1941) to either inhibit or facilitate monosynaptic reflex activity in ventral root filaments. Subsequent microelectrode recordings in the ventral horn revealed the presence of interneurons that discharged repetitively at high frequencies in response to single shocks applied to a cut ventral root at minimum latencies of .6 - .7 msec (Renshaw, 1946). Although Renshaw acknowledged existing anatomical evidence of recurrent colatterals from motor axons in the ventral horn (Cajal, 1909), he refrained from concluding that antidromic activation of these interneurons was responsible for the observed motoneuron inhibition. Later Eccles, et al. (1954) provided definitive electrophysiological evidence that antidromic stimulation of motor nerves produced a hyperpolarization of the motoneuron by recording the "recurrent IPSP" (RIPSP) with an intracellular electrode. The onset of the RIPSP occurred after an intraspinal delay of about 1.5 msec, indicating a

disynaptic pathway, and coincided with the discharge of interneurons which were monosynaptically activated by antidromic stimulation of ventral roots or motor nerves. The multiple spike, high frequency burst of these interneurons, which he named Renshaw cells, was identical to that which had been described by Renshaw (1946).

When the proximal portion of an entire ventral root is stimulated, a characteristic discharge is evoked in Renshaw cells which consists of 10 - 20 action potentials and which usually lasts 30 - 50 msec (Renshaw, 1946; Eccles, et al. 1954). The frequency of Renshaw cell firing during the first 20 msec or so can be as high as 1500 Hz; a progressive decline in the frequency occurs throughout the remainder of the burst until action potentials are separated by about 20 msec (Renshaw, 1946; Eccles, et al. 1954, 1956; Curtis and Eccles, 1958; Curtis and Ryall, 1966c). Following this discharge, there is a depression in spontaneous (Curtis and Ryall, 1966c) and evoked Renshaw cell activity (Renshaw, 1946; Eccles, et al. 1954, 1961b). Curtis and Ryall (1966c) reported that there was also a third phase in the typical antidromically-evoked Renshaw cell response during which Renshaw cells fired at frequencies (60 Hz) in excess of spontaneous firing rates.

This "late discharge" has only been observed in spinal, barbituate cats (Curtis and Ryall, 1966c; Ryall, 1970) and only when Renshaw cells were spontaneously active at rates greater than 5 - 10 Hz. Thus, it seemed to be related to the general level of depolarization (Curtis and Ryall, 1966c) and may be a function of the anaesthetic.

As would be predicted from Dale's Law (1914), the activation of Renshaw cells by motor axon collaterals has been shown to be cholinergic (Eccles, et al. 1954, 1956; Curtis and Eccles, 1958; Ueki, et al. 1961; Curtis and Ryall, 1966a, b, c). Eccles, et al. (1954) were the first to study the pharmacology of the motoneuron-Renshaw cell synapse. Although intravenous (i.v.) injections of anticholinergic drugs, namely dihydro-β-erythroidine (DHBE) and atropine, consistently depressed the Renshaw cell response to ventral root stimulation, and anti-cholinesterases such as eserine increased Renshaw cell excitability, intraarterial injections of acetylcholine (ACh) facilitated only half of the Renshaw cells tested. In later studies (Curtis and Eccles, 1958; Curtis and Ryall, 1966a) in which ACh was applied iontophoretically to Renshaw cells, it was shown that ACh consistently excited Renshaw cells and facilitated their response to ventral root

stimulation. The discrepancy between the effects of intraarterial and iontophoretic administration of ACh prompted Curtis and Eccles (1958) to suggest that at least two diffussional barriers were involved. One, the blood brain barrier, was thought to prevent the access of the drug to the central nervous system. Secondly, a barrier around the motoneuron-Renshaw cell synapse which prevented the ACh released from axon terminals from diffusing away from the active site, was proposed to explain the repetitive response of the Rensaw to a single stimulus.

The actions of both DHBE and atropine were restricted to a reduction of the latter part of the Renshaw cell burst; the first few spikes were not affected even with large doses. While eserine resulted in a prolongation of Renshaw cell discharge, it also had no effect on the high freauency early discharge.

The actions of ACh in the peripheral nervous system have been shown to be mediated by two populations of receptors, nicotinic and muscarinic, as described by Dale (1914). Nicotinic receptors are blocked by DHBE (Unna, et al. 1944) while atropine selectively blocks muscarinic receptors (Dale, 1914). ·Thus, the demonstration by Eccles,

et al. (1954) that both DHBE and atropine inhibit Renshaw cell responsiveness suggested that these two types of ACh receptors were also present in the central nervous system. To test this possibility, Curtis and Ryall, (1966a) iontophoretically applied a number of nicotinic and muscarinic compounds to Renshaw cells and found that all excited Renshaw cells. Renshaw cell discharge was most abruptly altered by ACh, but nicotine produced the most vigorous Renshaw cell firing. In general, the nicotinic compounds were found to be more potent in exciting Renshaw cells than the muscarinic compounds, but, in agreement with others (Eccles, et al. 1956; Curtis and Eccles, 1958), less potent than synaptic activation resulting from antidromic stimulation. A comparison of the ability of iontophoretically applied DHBE and atropine to block the excitatory action of ACh on Renshaw cells indicated that while Renshaw cells appeared to possess both types of receptors, the effects of ACh were predominantly mediated via nicotinic receptors (Curtis and Ryall, 1966b). After DHBE, the rapid, short lasting excitation of Renshaw cells by ACh was converted to a weaker, longer lasting excitation which had a much slower onset. The remaining excitation, assumed to be mediated by

muscarinic receptors, was removed by subsequent administration of atropine. These results are consistent with the finding that mecamylamine, a nicotinic blocker, also reduced the number of spikes in the early phase of the Renshaw cell response to ventral root stimulation (Ueki, et al. 1961).

According to Curtis and Ryall (1966c) the three phases of Renshaw cell response to antidromic stimulation (a high frequency initial discharge, a pause and a late response) could be explained in terms of the differential actions of muscarinic and nicotinic receptors. DHBE was found to reduce the number of spikes appearing in the initial phase, although, again, it had no effect on the first few spikes. Atropine inhibited the late Renshaw cell discharge but had no effect on the initial high frequency phase of firing. Thus, it was suggested that the initial high frequency response was a result of the interaction of ACh with nicotinic receptors while the late response was mediated by muscarinic receptors. The fact that cholinesterase inhibitors were found to have no effect on the first few spikes was interpreted to suggest that the high frequency rates were the result of an above threshold concentration of ACh in the synaptic cleft and that the frequency of Renshaw cell firing

during that period was limited only by their refractory period. A depression in the sensitivity of the Renshaw cell to iontophoretic ACh administration was observed during the pause, and it was suggested that this phase was due to a nonspecific desensitization to ACh.

The number of spikes that are produced within the initial Renshaw cell response to ventral root stimulation increases as the stimulus intensity increases up to a maximum (Renshaw, 1946; Eccles, et al. 1961b). There is a considerable convergence of motor axon collaterals onto a single Renshaw cell (Renshaw, 1946; Eccles, et al. 1954, 1961b; Ryall, 1970). Stimulation of an entire ventral root will result in the simultaneous activation of all of the motor axons contained in that root and a massive convergence onto individual Renshaw cells. Such an activation pattern is, of course, highly unphysiological since during normal movement motoneurons are recruited sequentially, and the firing of motoneurons within a given pool is asynchronous rather than synchronous (Gilson and Mills, 1941). As one would expect, the pattern of Renshaw cell discharge that is evoked by stimulation of a cut ventral root is very different than that which is observed when the cell is activiated by

the more normal orthodromic route (via the dorsal roots)
or is spontaneously active. The evidence also indicates
that the pharmacological activation of Renshaw cells may
vary with the mode of synaptic excitation (Ryall and Haase,
1975).

(b) Physiology and pharmacology of Renshaw cell responses
to orthodromic stimulation

Renshaw cells normally discharge spontaneously,but in
contrast to antidromic stimulation, single spikes rather
than high frequency bursts are typically seen (Curtis and
Eccles, 1958; Curtis and Ryall, 1966b). This spontaneous
firing, which is likely due to the leakage of quanta of
ACh from motor axon terminals (Curtis and Eccles, 1958), is
inhibited by atropine but not by DHBE (Curtis and Ryall,
1966b; Ryall and Haase, 1975). Renshaw cell discharges
evoked in response to physiologically more normal orthodromic
stimulation are also much different than those evoked by
antidromic stimulation. The high frequency initial phase
and the late, long lasting excitation were never observed
when Renshaw cells were activated by dorsal root stimulation
even when high stimulation intensities were used (Curtis
and Ryall, 1966c). A depression in the spontaneous firing
of Renshaw cells lasting up to 500 msec was observed after

the Renshaw cell response to dorsal root stimulation which
was similar but longer than the pause seen in the
antidromically-evoked Renshaw cell response (Curtis and
Ryall, 1966c). Neither i.v. injections of atropine (Curtis
and Ryall, 1966c) nor intravenously or iontophoretically
applied DHBE had any significant effects on these
orthodromically-evoked Renshaw cell discharges (Curtis,
et al. 1961; Curtis and Ryall, 1966c). In chloralose,anesthetized,
decerebrate cats, Renshaw cells responded to stretch of the
triceps surae tendon by producing a "burst-like series of
spikes" of moderate frequency (about 50 Hz) (Hellweg, et al.
1974). This response, assumed to be mediated by axon
collaterals as a consequence of alpha motoneuron activation
by Ia afferents, was completely inhibited by mecamylamine.
Conversely, in barbituate decerebrate or spinal cats,
Renshaw cell response to stretch of the Achilles' tendon
was abolished by atropine but not mecamylamine (Ryall and
Haase, 1975).

The available evidence thus suggests that stimulation
of an entire cut ventral root results in an excessive,
unphysiological liberation of ACh onto Renshaw cells. The
high frequency, triple phased Renshaw cell discharge that is

subsequently evoked is probably due to an inefficient
removal of the overly abundant ACh which is made available
because of the tremendous convergence upon Renshaw cells.
Indeed, when a small ventral root filament rather than a
whole ventral root was antidromically stimulated, Renshaw
cells produced a monosynaptic spike instead of a high
frequency burst (Ross, et al. 1975). Renshaw cells
followed ventral root filament stimulation one for one
within the physiological range of motoneuron firing.
Spontaneous Renshaw cell firing appears to be due to the
interaction of ACh with muscarinic receptors. The limited
and conflicting results on the pharmacology of the
orthodromic activation of Renshaw cells (Hellweg, et al.
1975; Ryall and Haase, 1975) forces the important question
of whether synaptic events are normally mediated by nicotinic,
muscarinic or perhaps some other yet unidentified cholinergic
receptor (Curtis and Ryall, 1966b) to be left unanswered
until additional data is available.

(c) Synaptic input to Renshaw cells

1. Antidromic activation

The existence of a diffuse branching of axon collaterals
from alpha motoneurons into the gray matter of the ventral

horn was established by Cajal (1909) through the use of
Golgi impregnation techniques. These observations have
been confirmed by other Golgi studies (Scheibel and Scheibel,
1971) and more recently by intracellular injections of
horseradish peroxidase into triceps surae alpha motoneurons
(Cullheim, 1977). A maximum of 5 collaterals per axon was
observed in the latter study. The 19 motoneurons impaled
and stained gave off a total of 734 collaterals, 78% of
which terminated in the ventromedial part of the anterior
horn (the Renshaw cell area) while 21% interestingly
terminated within the homonymous motoneuron nuclei. The
evidence indicated that the latter projections terminated
upon the parent motor axon or motoneuron dendrites; as of
the present, the function of the collateral contacts onto
homonymous motoneurons is unknown.

The latency (.6 - .7 msec) of Renshaw cell discharge
evoked by the stimulation of motor axons, the demonstration
of the existence of recurrent axon collaterals and their
projections to regions known to contain Renshaw cells
(Eccles, et al. 1954; Willis and Willis, 1966; Erulkar,
et al. 1968) all support the hypothesis that the excitation
of Renshaw cells consequent to stimulation of motor axons is

the result of synaptic contact between motor axon collaterals
and Renshaw cells. Although afferents have been histologically
demonstrated to be present in ventral roots (Coggeshall,
et al. 1974), the fact that chronic transection of ventral
roots has been shown not to depress either the ability of
Renshaw cells to respond to ventral root stimulation or the
recurrent inhibition of motoneurons (Willis, et al. 1967)
rules out the possibility that Renshaw cells are activated
via ventral root afferents. The majority of evidence indicates
that Renshaw cells are driven by axon collaterals from alpha
motoneurons exclusively (Eccles, et al. 1954; Ellaway, 1968,
1971), but there is limited evidence suggesting that Renshaw
cells also receive collaterals from fusimotor motoneurons
(Kato and Fukushima, 1974).

2.   Orthodromic activation

Several lines of evidence indicate that orthodromic
activation of Renshaw cells also occurs as a consequence of
motoneuron firing. High levels of anaesthesia were shown to
depress the reflex activity of motoneurons and to abolish
Renshaw cell responses to orthodromic stimulation of muscle
nerves (Eccles, et al. 1954). A latency of 0.8 msec was found
to exist between the onset of a monosynaptic reflex recorded

in a ventral root and the initiation of Renshaw cell
activity recorded via a microelectrode (Haase and Vogel,
1971a). DHBE depressed the Renshaw cell response without
affecting spontaneous or reflex motoneuron activity. Ross,
et al. (1972) performed a quantitative analysis of the
relationship between Renshaw cell discharge and the number
of synchronously active motoneurons and found that the
number of spikes in an orthodromically-evoked Renshaw cell
burst was linearly related to the height of the associated
monosynaptic reflex. Renshaw cell discharge was also
observed to be proportional to the size of monosynaptic
reflexes evoked by vibration of peripheral nerves in
de-efferented, decerebrate cats (Pompeiano, et al. 1974);
in agreement with Haase and Vogel (1971a), a minimum latency
of .62 msec separated the onset of the monsynaptic reflex and
the first appearance of Renshaw cell activity.

It appears from the work of Haase and Vogel (1971a),
Ross, et al. (1972) and Pompeiano, et al. (1974, 1975b, c)
that stimulation of Ia afferents results in a disynaptic
activation of Renshaw cells which is secondary to the
excitation of alpha motoneurons. Similar results have been
obtained in studies in which Renshaw cell discharges have

been evoked as a result of dorsal root stimulation. Curtis and Ryall (1966c) reported that a few Renshaw cells responded to stimulation of low threshold muscle afferents at minimum latencies which exceeded a monosynaptic reflex by about 1 msec. Ross, et al. (1972) found that most Renshaw cells did not respond to low threshold stimulation of dorsal roots, and that the 20% which did respond were activated disynaptically. Ryall and Piercey (1971) stimulated individual muscle nerves in de-efferented cats and found that low threshold stimulation of only the biceps-semitendinosis nerve evoked short latency ($\leq$ 2msec) Renshaw cell discharges, the shortest latency observed being 1.3 msec.

There is evidence available which suggests that segmental afferents other than Ia afferents may be able to excite Renshaw cells via routes that do not include motoneurons. Ipsilateral stimulation of cutaneous and high threshold muscle afferents (FRAs) has been shown to evoke Renshaw cell discharges at latencies ranging from 3 to 20 msec (Eccles, et al. 1954; Curtis and Ryall, 1966c; Ryall and Piercey, 1971; Piercey and Goldfarb, 1974). These longer latency Renshaw cell discharges may be produced by polysynaptic reflex activity in motoneurons or perhaps by direct

excitation of Renshaw cells by polysynaptic FRA pathways. The latter possibility is supported by the demonstration that the disynaptic Renshaw cell discharges evoked by low threshold stimulation of the dorsal roots were depressed by DHBE (Curtis and Ryall, 1966c) while the longer latency discharges evoked by high threshold stimulation were not affected by either DHBE (Curtis et al. 1961; Curtis and Ryall, 1966c) or atropine (Curtis and Ryall, 1966c). In addition, while Piercey and Goldfarb (1974) found that high threshold stimulation of ipsilateral hindlimb nerves produced Renshaw cell discharges that were closely correlated to the polysynaptic reflex activity evoked in motoneurons, they also saw definite examples of Renshaw cell discharges that occurred only after cessation of motoneuron activity. These orthodromically-evoked Renshaw cell bursts were insensitive to doses of mecamylamine that effectively depressed Renshaw cell responses to antidromic stimulation. Thus, the evidence indicates that the orthodromic activation of Renshaw cells from segmental afferents is mediated by pathways involving motoneurons as well as those in which a motoneuron is not interposed; stimulation of Ia afferents and FRAs appears to excite Renshaw cells via motor axon collaterals while some

FRA excitation of Renshaw cells is via apparently non-cholinergic synapses.

Stimulation of segmental afferents has also been shown to inhibit Renshaw cell activity. A depression in spontaneous (Curtis and Ryall, 1966c; Ryall and Piercey, 1971; Piercey and Goldfarb, 1974) and antidromically-evoked Renshaw cell discharge, as well as the recurrent inhibition of motoneurons (Bergmans, et al. 1969), have been observed after stimulation of ipsilateral FRAs. These results have been interpreted to represent a means of supporting the flexion reflex by disinhibiting extensor motoneurons (Haase, et al. 1975). Natural stimuli such as touch and pressure applied to ipsilateral and contralateral hindlimbs and the tail at both noxious and innocuous intensities were reported by Wilson, et al. (1964) to significantly inhibit Renshaw cell response to antidromic stimulation of ventral roots. This inhibition appeared to be largely mediated by cutaneous fibers. High threshold stimulation of contralateral muscle and cutaneous nerves also resulted in a predominant inhibition of Renshaw cells that was usually less potent but of longer duration than that produced by natural stimulation. Occassionally, a weak excitation of Renshaw cells was

observed after stimulation of contralateral nerves. The
inhibition of Renshaw cells resulting from stimulation of
contralateral nerves was thought to be mediated by Group
II and III fibers in the muscle nerves and alpha fibers in
the cutaneous nerves. Stimulation of Group II afferents has
been shown to reduce the amount of recurrent inhibition
produced in motoneurons by ventral root stimulation which
had been maximal when muscle nerves were stimulated at Group
I strength (Fromm, et al. 1977). Similarly, Pompeiano,
et al. (1975a) observed that the discharge rate of Renshaw
cells was decreased when the intensity of electrical
stimulation of the gastrocnemius-soleus (G-S) nerve was
increased from Group I to Group II strength.

The profile of segmental orthodromic input to Renshaw
cells may be summarized as follows: a disynaptic activation
from stimulation of Ia afferents that is mediated via motor
axon collaterals; excitation from ipsilateral FRAs that is
probably mediated both through alpha motoneurons and direct
polysynaptic pathways to Renshaw cells; predominant
inhibition from contralateral FRA stimulation; inhibition
from noxious and non-noxious natural stimulation of all
areas of the body and inhibition from ipsilateral Group II

fibers. It should be noted that recent electrophysiological data (Kirkwood and Sears, 1974; Stauffer, et al. 1976) indicate that Group II fibers should not be included in the group of afferents referred to as the FRAs as originally defined by Holmquist and Lundberg (1961).

3.   Supraspinal control of recurrent inhibition

The first report that supraspinal centers could affect the recurrent inhibitory pathway to motoneurons was published by Granit, et al. (1960). It was found that the recurrent inhibition of monosynaptic discharges of G-S motor axons in a ventral root filament could be reduced or enhanced by stimulation of the anterior lobe of the cerebellum. Haase and Van Der Meulin (1961) showed that stimulation of either the cerebellum or the ventromedial area of the reticular formation could either facilitate or inhibit Renshaw cell discharges. The fact that facilitation from brainstem stimulation was only observed when Renshaw cells were driven orthodromically and not antidromically suggested an interneuronal rather than direct control of Renshaw cells. It is also apparent that supraspinal control of Renshaw cells is not solely mediated indirectly by action on motoneurons since it has been shown that stimulation of the

nucleus interpositus (Haase and Vogel, 1971b) and the contralateral capsula interna and red nucleus (Meyer-Lohmann, et al. 1978) can have independent and opposite effects on monosynaptic reflexes and Renshaw cell discharge. Thus, it appears that the brain is capable of modulating Renshaw cell activity both through the descending control of motoneurons and by interneuronal pathways impinging on Renshaw cells.

(d) Projections of Renshaw cells

1. Alpha motoneurons

Antidromic stimulation of a motor nerve produces a maximum amount of recurrent inhibition (phrase coined by Brooks and Wilson (1959) to describe the inhibitory effects of Renshaw cells) in motoneurons innervating the same muscle (homonymous motoneurons) while a lesser degree of recurrent inhibition is evoked in synergist motoneurons (Renshaw, 1941; Eccles, et al. 1954, 1961a; Wilson, et al. 1960). Recurrent inhibition was also seen to extend to motoneurons that were functionally unrelated to one another, i.e., from flexors to extensors and vice versa (Renshaw, 1941; Eccles, et al. 1954, 1961a; Granit, et al. 1957; Brooks and Wilson, 1959; Wilson, et al. 1960). The recurrent inhibition of

alpha motoneurons has been shown to be strictly ipsilateral (Renshaw, 1946; Eccles, et al. 1954; Willis and Willis, 1966), which is consistent with the anatomical data of Jankowska and Lindstrom (1971).

Recurrent inhibition is known to be most effective among motoneurons that are located close to one another, usually within one segment of the spinal cord (Renshaw, 1941; Eccles, et al. 1954, 1961a; Wilson, et al. 1960; Hultborn, et al. 1971c), which conforms with data on the axonal projections of Renshaw cells to motoneuron nuclei (Jankowska and Roberts, 1972). The demonstration by Eccles, et al. (1961a) that the size of the RIPSPs recorded in motoneurons was directly related to the closeness of the motoneuron pool which had been antidromically stimulated supported the suggestion of Eccles, et al. (1954) that the distribution of recurrent inhibition was related to the proximity of motoneuron pools. The lack of any functionally meaningful pattern led to the postulation that recurrent inhibition represented a negative feedback system to motoneurons that would nonspecifically suppress intense motoneuron discharge (Renshaw, 1941; Eccles, et al. 1954).

The fact that recurrent inhibition seems to be most

effective among motoneurons that are proximal to one another
does not preclude the possibility that the distribution of
recurrent inhibition is based upon functional relationships
since the spinal organization of motoneuron nuclei somewhat
reflects functional relationships (Romanes, 1951).
Although it had been reported by others (Renshaw, 1941;
Eccles, et al. 1954, 1961a; Wilson, et al. 1960) that
recurrent inhibition was consistently observed among
motoneurons of diverse function, Hultborn, et al. (1971c)
recognized the significant fact that recurrent inhibition
was never seen between motoneurons innervating muscles which
were strict antagonists at the same joint. Thus, a
functional distribution of the recurrent inhibition of
motoneurons was revealed in which "motor nuclei to muscles
which are linked in Ia synergism are mutually connected by
recurrent inhibition, regardless of their location in the
spinal cord" (Hultborn, et al. 1971c).

During the stretch reflex, Ia afferents most powerfully
excite homonymous motoneurons while heteronymous motoneurons
(to synergists) are only weakly excited (Lloyd, 1946; Eccles,
et al. 1957a). The recurrent inhibition among synergists
may function to focus or localize the excitation to

homonymous motoneurons, thereby facilitating movements requiring precision (Brooks and Wilson, 1959; Hultborn, et al. 1971b). This suggestion was forwarded by Brooks and Wilson (1959) based upon the demonstration that after DHBE administration (i.v.) reflex activity which had been localized in nerves to homonymous muscles spread to the nerve of the heteronymous muscle as well.

There is also evidence available that indicates that motoneurons which respond tonically to muscle stretch are more susceptible to recurrent inhibition than those which respond phasically (Granit, et al. 1957, 1961; Kuno, 1959; Granit and Rutledge, 1960; Eccles, et al. 1961a). Tonically responding motoneurons tend to have smaller somas (Eccles, et al. 1957a; Kernell, 1966; Burke, 1967), higher input resistances (Kernell, 1966; Burke, 1967), slower axonal conduction velocities (Eccles, et al., 1958; Kuno, 1969; Burke, 1967); smaller diameter axons (Hursh, 1939; Eccles, et al. 1958, 1961; Henneman, 1965a), smaller axonal spikes (Granit, et al. 1957; Henneman, 1965a), longer after-hyperpolarizations (Eccles, et al. 1957, 1958, 1961a; Kuno, 1959; Burke, 1967) to discharge at lower frequencies (Granit, et al. 1957; Eccles, et al. 1958; Kernell, 1965),

and tend to innervate motor units with slower contraction times (Eccles, et al. 1958; Kuno, 1959; Burke, 1967) than phasic motoneurons. Motoneurons that discharged tonically during muscle stretch were effectively inhibited by antidromic stimulation of cut ventral roots while phasic motoneurons that responded to stretch with a brief high frequency burst were much more resistant to recurrent inhibition (Granit, et al. 1957; but see Granit and Rutledge, 1960). Antidromic stimulation of the nerve to the red, slowly contracting soleus muscle evoked larger RIPSPs in homonymous motoneurons than those seen in gastrocnemius motoneurons which innervate a pale, fast contracting muscle (Kuno, 1959). Eccles, et al. (1961a) recorded intracellularly from alpha motoneurons while stimulating various hindlimb nerves in deafferented cats and found that the size of the RIPSP was directly proportional to the duration of the after-hyperpolarization, which was inversely related to conduction velocity.

More effective recurrent inhibition of tonically firing motoneurons than of phasic motoneurons would be consistent with electrophysiological data which indicates that the input resistance of a cell is inversely related to cell size

(Kernell, 1966). Thus, if all motoneurons receive a similar convergence of Renshaw cell synapses, a larger aggregate RIPSP would be generated in the smaller motoneurons (Henneman, 1965a). Granit, et al. (1957) proposed that the more powerful recurrent inhibition of tonically active motoneurons, which are involved in the maintenance of posture, serves to stabilize their discharge during the stretch reflex. The afferent limb of the stretch reflex was viewed to be controlled by γ motoneurons (which were resistant to recurrent inhibition in their studies) and the output controlled by Renshaw cells. Others (Eccles, et al. 1961a) have suggested that the pattern of recurrent inhibition may be of importance in quieting tonic motoneurons so as to facilitate phasic motoneuron activity during rapid movements.

Henneman, et al. (1965b), however, have presented evidence indicating that phasically active motoneurons are the most susceptible to recurrent inhibition. Motoneuron activity in ventral root filaments was evoked in response to several reflexes which were associated with mono - and polysynaptic pathways. In the majority of cases, smaller motoneurons (as judged by the height of the axonal spike)

were recruited before larger motoneurons while the order

of inhibition was the reverse. The explanation that has

been offered (Henneman, 1977) is that since smaller

motoneurons are recruited first, by the time larger

motoneurons have been recruited, the excitatory drive to

the smaller motoneurons will be excessive and will render

them less susceptible to inhibition. This theory is compatible

with the concept of surplus excitation that was introduced

by Granit and Rutledge (1960) to describe excitatory drive

to neurons that was in excess of that needed to produce

maximal activity. Surplus excitation was seen to effectively

oppose the effects of recurrent inhibition. Despite the

fact that these authors consistently observed that tonically

firing motoneurons were most susceptible to recurrent

inhibition, they state, "We have often seen high-threshold,

rapidly firing cells silenced before low-threshold tonic

ones run on a greater surplus of excitation".

There is also evidence that phasic motoneurons may

provide a more powerful input to Renshaw cells than do tonic

motoneurons, as was first suggested by Granit, et al. (1957).

Renshaw cells which were disynaptically excited by

stimulation of the G-S nerve responded to ramp stretch of

the G-S tendon with a burst of spikes (Hellweg, et al. 1974).

The Renshaw cell bursts were more closely related to the activity of phasic motoneurons than to that of the smaller tonic spikes seen in ventral root filaments. Vibration of the G-S muscle was also shown to be a more powerful excitant of G-S coupled Renshaw cells than static stretch of the same muscles (Pompeiano, et al. 1975a). It was suggested that during static stretch tonic motoneurons are primarily activated asynchronously while vibration causes a more synchronous activation of motoneurons and a recruitment of large phasic motoneurons. Pompeiano, et al. (1975b) postulated that Renshaw cells are primarily driven by phasic motoneurons whose higher frequency of discharge results in a more potent inhibition of tonic motoneurons.

2. Gamma motoneurons

Gamma motoneurons are also inhibited by antidromic stimulation of motor axons, but the distribution and amount of recurrent inhibition is more variable than that seen in alpha motoneurons (Brown, et al. 1968; Ellaway, 1968, 1971; Kato and Fukushima, 1974). The recurrent inhibition of motoneurons is thought to be primarily mediated by alpha motoneuron axon collaterals, although there is some evidence that Renshaw cells may be activated by collaterals from gamma

motoneurons as well (Kato and Fukushima, 1974).

3.   Ventral spinocerebellar tract cells

A small number (5 of 200) of VSCT cells were also shown
to be inhibited by Renshaw cells (Lindstrom and Schomburg,
1973). The cells receiving recurrent inhibition also
received monosynaptic excitation from Ia afferents running
in the muscle nerves which produced the recurrent inhibition.
A further similarity between these VSCT cells, IaINs and
motoneurons is that all populations of cells are inhibited
by IaINs (Gustafsson and Lindstrom, 1970, 1973; Lundberg
and Weight, 1971; Hultborn, et al. 1976a). The similarity
of convergence onto alpha motoneurons, IaINs and some VSCT
cells suggests that subpopulations of VSCT cells exist,
based upon their input, which transmit highly specific
information to the cerebellum (Lindstrom and Schomburg, 1973).
The group of VSCT cells which receives input identical to that
of alpha motoneurons and IaINs may be involved in transmitting
information specifically dealing with the balance of Ia
excitation and inhibition in motoneurons (Lundberg, 1971;
Gustafsson and Lindstrom, 1973; Lindstrom, 1973).

4.   Renshaw cells

Ryall, et al. (1970) found that antidromic stimulation

of hindlimb motor nerves in deafferented cats could inhibit
the spontaneous or ACh-evoked discharge of Renshaw cells
which had not been excited by the antidromic volley.
Renshaw cells which were excited by antidromic stimulation
of a peripheral nerve could also be inhibited by a
conditioning volley in another peripheral nerve, an
observation which was confirmed by Hultborn, et al. (1971d).
The latency of the inhibition had a mean of 2.2 msec. There
was thus a close correlation between the time course of
the early Renshaw cell response and the inhibition of Renshaw
cells produced by antidromic stimulation. It was postulated,
therefore, that the observed inhibition was due to a mutual
inhibition among Renshaw cells. The fact that antidromic
stimulation of motor nerves was still effective in inhibiting
Renshaw cells which were activated by the iontophoretic
application of ACh, independently of motoneurons, further
supported the notion that the inhibition was directed to
Renshaw cells and not to motoneurons.

The duration of the Renshaw cell - Renshaw cell
inhibition ($\leq$ 500 msec.) was similar to the duration of the
pause (Ryall, 1970) seen in antidromically evoked Renshaw
cell responses (Curtis and Ryall, 1966c; Ryall, 1970). The

duration of the pause was found to be proportional to the
intensity of the early discharge (Ryall, 1970). Because
the duration of the pause and the time course of the
inhibition among Renshaw cells appeared correlated with
the early discharge, and because DHBE attenuated the early
response while prolonging the pause, it was concluded that
the mutual inhibition among Renshaw cells was responsible
for the pause (Ryall, 1970). Renshaw cells within a
motoneuron pool could be inhibited by antidromic stimulation
of homonymous motor nerves as well as nerves to other muscle
groups (Ryall, et al. 1971). Mutual inhibition of Renshaw
cells was strictly ipsilateral and could be seen among
Renshaw cells located at least 5 mm away from one another
(Ryall, et al. 1971). The most effective Renshaw cell -
Renshaw cell inhibition was produced by antidromic
stimulation of the ventral root of the adjacent segment
(Ryall, 1970).

5.   Ia inhibitory interneurons

It was originally shown by Renshaw (1941) that
antidromic stimulation of motor axons could result in either
an inhibition or facilitation of motoneurons. Wilson and
coworkers (Wilson, 1959; Wilson, Talbot and Diecke, 1960;

Wilson and Burgess, 1962) were the first to focus
attention on the facilitatory effects of antidromic
stimulation. In high spinal, unanaesthetized cats with the
hindlimb deafferented ipsilaterally, Wilson (1959) found
that antidromic stimulation of hindlimb motor nerves could
produce a 20 - 50% increase in the amplitude of monosynaptic
reflexes recorded in nerves to plantaris and tibialis
anterior. The period of facilitation, which lasted 50 - 100
msec and was similar to the duration of RIPSPs in motoneurons
reported by others (Eccles, et al. 1954, 1961a), was
attenuated by DHBE. The latency for the onset of "recurrent
facilitation" was found to be about 3 msec, which is about
1.2 msec longer than that for recurrent inhibition;
recurrent inhibition appeared to be disynaptic and
recurrent facilitation trisynaptic.

It was hypothesized that recurrent facilitation could
be due to either activation by axon collaterals of interneurons
that excited motoneurons or to recurrent inhibition of
interneurons that tonically inhibited motoneurons. The
latter possibility would lead to an excitation of
motoneurons by way of disinhibition rather than the addition
of excitation. These theories were tested by examining the

effects of lowering the resting membrane potential of motoneurons on recurrent facilitatory potentials (RFPs) (Wilson and Burgess, 1962). Increasing the membrane potential of the motoneuron would increase the amplitude of a RFP that was due to an EPSP but would reverse a RFP that was due to the removal of background inhibition. Wilson and Burgess (1962) found that hyperpolarizing current injected into the motoneuron converted the RFP to an IPSP. Thus, it was suggested that the recurrent facilitation of motoneurons was due to the recurrent inhibition of tonically active inhibitory interneurons.

Several investigators had reported observing unidentified tonically active interneurons that were inhibited by antidromic ventral root stimulation (Frank and Fuortes, 1956; Hunt and Kuno, 1959; Wilson and Burgess, 1962). Hultborn, et al. (1971a) tested whether the interneurons that are inhibited during recurrent facilitation are the interneurons that mediate the Ia reciprocal inhibition of antagonist motoneurons. These authors found that IPSPs evoked in motoneurons by stimulating the Ia afferents in muscle nerves to antagonists could be depressed by a conditioning stimulus applied to cut ventral roots. In a subsequent study,

Hultborn, et al. (1971b) searched for and found a population of interneurons that were monosynaptically excited by Ia afferents and disynaptically inhibited by antidromic stimulation of ventral roots. These cells (IaINs) were located in the ventral horn dorsomedial to motor nuclei in Rexed's lamina VII and tended to be closer to the entry zone of the Ia afferents that excited them than to the antagonist motoneurons which they inhibited. Recurrent inhibition of IaINs had an average latency of 1.3 msec and a duration of 50 - 80 msec. The spontaneous activity of the IaINs was sensitive to the level of the anaesthesia but was usually in the range of 20 - 60 Hz, although higher frequencies were also seen.

Hultborn, et al. (1971c) then conducted a detailed systematic analysis of the distribution of the recurrent inhibition of motoneurons and IaINs by comparing the ability of antidromic stimulation of various hindlimb nerves to depress Ia IPSPs in motoneurons. Maximal RIPSPs in motoneurons were produced by antidromic stimulation of the nerves to homonymous and synergist muscles. The most pronounced depression of Ia IPSPs was produced by antidromic stimulation of muscle nerves to antagonists, i.e., the nerves

containing the Ia afferents producing the inhibition.
Thus, the antidromic stimulation of the nerve to Q evoked
RIPSPs in Q motoneurons and synergists and depressed IPSPs
in Pb-St motoneurons which had been evoked by stimulation
of the Ia afferents in the Q nerve. The latter effect
was due to recurrent inhibition of Q-coupled IaINs.

During normal movements individual motoneurons would
be subjected to both recurrent and reciprocal inhibition,
although the temporal relationship of their occurrence is
not known. Cleveland, et al. (1972) compared the effect
of each type of inhibition alone with the inhibitory effect
of the two combined on monosynaptic reflexes recorded from
muscle nerves. Summation of reciprocal and recurrent
inhibition led to a greater depression of the monosynaptic
reflex than either form of inhibition produced alone, but
the combined inhibition was less than the sum of the
inhibitory effects of each alone. The authors concluded
that this occlusion indicated a convergence of reciprocal
and recurrent inhibitory effects onto motoneurons and that
they were uniformly distributed throughout the motoneuron
pool. The fact that recurrent inhibition was evoked in
motoneurons when the ventral root containing agonist motor

axons was stimulated but disinhibition prevailed with
stimulation of the adjacent ventral root, suggested that
Renshaw cells of a given motoneuron pool inhibit only IaINs
projecting to antagonist motoneuron pools. Cleveland,
et al. (1972) presented a schematic summarizing the
interconnections between antagonistic systems of alpha
motoneurons, IaINs and Renshaw cells which is reproduced
in modified form in Figure 1.

Of particular significance to Hultborn and coworkers
(1971c) was the fact that motoneurons and IaINs that received
the same monosynaptic Ia input were recurrently inhibited
by the same population of Renshaw cells thus suggesting a
"functional unity between the recurrent control of
motoneurons and Ia inhibitory interneurons". It was
postulated that the recurrent inhibition of IaINs may be of
particular importance in movements involving a high degree
of gamma drive in which the excitation of agonist motoneurons
would be coupled to increased inhibition of antagonist
motoneurons. Recurrent inhibition of IaINs would offset
the progressive accumulation of inhibition focused on
antagonists. Support for this hypothesis was obtained from
experiments in unanaesthetized decerebrate cats in which

levels of reciprocal inhibition of Pb-St motoneurons were
analyzed during increasing degrees of muscle stretch of the
Q muscle (Hultborn and Lundberg, 1972). The reciprocal
inhibition of test monosynaptic reflexes in Pb-St motoneurons
increased linearly with increased static stretch up to a
point after which it plateaued. This data was interpreted
as confirmation of the theory that the recurrent inhibition
of IaINs functions to maintain a constant level of reciprocal
inhibition of antagonists in the face of intense Ia drive to
agonists. During static stretch, the recurrent inhibition
of IaINs projecting to antagonist motoneurons could serve
to stabilize the amount of reciprocal inhibition. This
mechanism may be useful in maintaining the excitability level
of antagonist motoneurons or in allowing a cocontraction of
flexors and extensors about a joint.

Based on the evidence presented by Hellweg, et al.
(1974) that Renshaw cells responded phasically with elevated
discharge rates to ramp stretch of a homonymous muscle,
Benecke and coworkers (1975) wondered whether the recurrent
inhibition of IaINs would be augmented during the dynamic
phase of ramp stretch as well. It was found that during the
dynamic phase of ramp stretch of the triceps surae muscle,

IaINs, which received Ia excitation from the stretched muscle, displayed an increase in discharge frequency which was interrupted by a period of inhibition. The depression of IaIN discharge occurred coincidentally with the discharge of triceps surae-coupled Renshaw cells. Furthermore, after DHBE, IaINs which had displayed a pause in their discharge during ramp stretch now exhibited increased firing rates throughout the dynamic and static phases of muscle stretch. Thus, it was concluded that Renshaw cells are capable of modulating IaIN activity during dynamic movements.

In another series of experiments, Benecke, et al. (1975) presented an example of orthodromically induced recurrent facilitation. A monosynaptic reflex evoked in a ventral root by electrical stimulation of the peroneal nerve in decerebrate cats was conditioned by stretch of the triceps surae muscle. Extracellular recordings were obtained of triceps surae-coupled IaINs and Renshaw cells. The data revealed that stretch of the triceps surae resulted in a facilitation of the peroneal monosynaptic reflex concomitant with an increase in Renshaw cell activity and a decrease in IaIN discharge. The evidence

indicated that during phasic stretch reflexes IaINs could be effectively inhibited by Renshaw cells, and that this was linked to a facilitation of antagonist motoneurons. It was suggested by Benecke, et al. (1975) that the gamma loop would be too slow to exert a pronounced effect during rapid alternating movements, and that in these situations, recurrent inhibition of IaINs could facilitate the switching of activity between antagonistic pools of motoneurons.

(e) Renshaw cell morphology

The definitive morphological identification of Renshaw cells was performed by Jankowska and Lindstrom (1971). It was found that Renshaw cells, which had been stained with intracellular injections of Procion Yellow, had somas ranging from 10 - 15u in diameter, axons which projected at least 400u and dendrites extending up to 150u. As had been reported by others (Renshaw, 1946; Eccles, et al. 1954; Willis and Willis, 1966) Renshaw cells were found to be located ventromedial to the motor nuclei in Rexed's lamina VII. In a subsequent electrophysiological study, Jankowska and Smith (1973) established that Renshaw cell axons terminated in motoneuron nuclei as well as in more dorsal areas of the spinal cord up to 12 mm away.

Motoneurons receiving axon terminals from Renshaw cells were usually located within 1 mm of the Renshaw cell soma.

The work of Jankowska and Lindstrom (1971) and Jankowska and Smith (1973) provided morphological confirmation of the physiological evidence that Renshaw cells can affect cells 2 - 3 segments away (Hultborn, 1971c; Ryall, et al. 1971; Cleveland, et al. 1972). Renshaw cell axons most likely terminate on all of their target cells within 2 - 4 mm from their soma (Jankowska and Smith, 1973). The longer axonal projections, which can extend up to 12 mm, probably terminate on other IaINs and other Renshaw cells. Projections to α and γ motoneurons appear to be primarily restricted to motoneurons within 1 or 2 spinal segments (Eccles, et al. 1954, 1961a), although exceptions can be seen (Hultborn, et al. 1971c).

(f)   Renshaw cell pharmacology

Strychnine has been shown to depress the recurrent inhibition of alpha motoneurons (Eccles, et al. 1954; Brooks and Wilson, 1959; Larson, 1969), gamma motoneurons (Ellaway, 1971), IaINs (Belcher, et al. 1976) and other Renshaw cells (Curtis, et al. 1976). Strychnine does not affect Renshaw cell discharge (Eccles, et al. 1954; Larson,

1969) or the resting membrane potential of motoneurons
(Curtis, 1962; Fuortes and Nelson, 1963). It was further
shown that glycine hyperpolarizes alpha motoneurons
(Curtis, et al. 1967) and blocks the ACh induced or
spontaneous firing of Renshaw cells (Ryall, et al. 1972);
the inhibitory effects of glycine were blocked by strychnine
in each case. Based upon the work of Curtis, et al. (1971)
indicating that strychnine is a specific antagonist of
glycine, it has been concluded that the inhibitory
neurotransmitter released by Renshaw cells is glycine
(Curtis, et al. 1976).

(g) Renshaw cell activity during locomotion

Although many possible functions for recurrent
inhibition have been proposed, ranging from regulation of
motoneuron discharge to control of reciprocal inhibition of
antagonist motoneurons, an important role for recurrent
inhibition in the control of locomotion was largely
discounted for some years due to reports that suggested
that Renshaw cells might be depressed during locomotion.
In fact, it has been argued that an inhibition of Renshaw
cells would be necessary during walking to allow "alpha-
gamma-linked reciprocal inhibition" (Hongo, et al. 1969)

to function (Hultborn, 1972, 1976; Feldman and Orlovsky, 1975).

Severin, et al. (1968) reported that recurrent inhibition is diminished during controlled treadmill locomotion in decerebrate cats. In their study, motoneuron activity in an L7 ventral root filament was elicited by passive static and cyclic muscle stretch and during locomotion evoked by electrical stimulation of the mesencephalic locomotor region (MLR). Antidromic stimulation of the L7 ventral root produced a reduction of firing during static and cyclic stretch, but "no noticeable effect" on the discharge frequencies of the same motoneurons during locomotion. This data led to the conclusion that recurrent inhibition was not an effective determinant of motoneuron discharge frequency during locomotion. The authors suggested that the decrease in efficiency of recurrent inhibition could be attributed to either an inhibition of Renshaw cells during locomotion, or that motoneurons active during locomotion operated under "surplus excitation" (Granit and Rutledge, 1960) which rendered the motoneurons resistant to the hyperpolarizing effects of Renshaw cells. Recurrent inhibition of a motoneuron should occur only if

the motoneuron investigated is a synergist of the motor

axons antidromically stimulated. An antagonist motoneuron

would be expected to discharge at higher frequencies during

antidromic stimulation as a result of recurrent inhibition

of the Ia inhibitory interneurons impinging on these

motoneurons. Evidence for recurrent facilitation during

locomotion was presented by Severin, et al. (1968)

indicating that the recurrent inhibition of IaINs was still

functional during locomotion.

Bergmans, et al. (1969) found that both RIPSPs in

alpha motoneurons and Renshaw cell discharges evoked by

ventral root stimulation were diminished when FRAs were

conditioned in acute spinal cats treated with DOPA. This

preparation is thought to activate a spinal neuronal network

that is associated with locomotion (Jankowska, et al. 1967a).

Fu, et al. (1975), however, reported that the recurrent

inhibition of IaINs persisted in acute spinal cats after

DOPA administration. Lastly, Feldman and Orlovsky (1975)

observed that 2 of 4 IaINs studied were significantly less

affected by ventral root stimulation during fictive

locomotion than during resting states.

More recently, however, direct microelectrode

recording of Renshaw cells in fictively locomoting cats
by McCrea and Jordan (1976a) have demonstrated conclusively
that Renshaw cells are not inhibited during fictive walking.
Renshaw cells were not only found to be active during
locomotion, but their activity was, in fact, modulated with
their bursts of activity occurring in phase with agonist
motoneurons. Evidence that the recurrent inhibitory pathway
to motoneurons was still functional during locomotion was
found by the demonstration that no significant change in
the amplitude of RIPSPs evoked in a motoneuron could be
observed among the various phases of locomotion (McCrea
and Jordan, 1976b).

Phasic Renshaw cell activity has also been observed
in paralyzed decerebrate cats in which fictive scratching
was evoked by tactile stimulation of the ear (Delyagina
and Feldman, 1978). Again, flexor and extensor-coupled
Renshaw cells were active coincidentally with periods of
flexor and extensor muscle activity, respectively. The
modulation of the Renshaw cells appeared to be "set by a
spinal generator" since their rhythmic activity persisted
after spinalyzation.

Based on the evidence that both IaINs (Feldman and

Orlovsky, 1975; Edgerton, et al. 1976) and Renshaw cells
(McCrea and Jordan, 1976a) have been shown to be
rhythmically active during locomotion in the absence of
any cyclic afferent input, it has been proposed that
antagonistic systems of alpha motoneurons, IaINs and Renshaw
cells constitute the spinal locomotion generator (Miller
and Scott, 1977). This model is essentially identical to
the system illustrated by Cleveland, et al. (1972) and
shown in Figure 1. An assumption of this model is that
the periodic recurrent inhibition of IaINs results in a
cyclic disinhibition of alpha motoneurons that is responsible
for membrane depolarization. Recurrent cyclic inhibition has
been shown to generate the oscillatory neuronal firing
pattern subserving swimming movements in the leech (Stent,
et al. 1978), and Renshaw cells were proposed to be
instrumental in controlling the limb movements in Urodela
(Szekely, 1965).

Our understanding of the neuronal mechanisms underlying
the rhythmic depolarization and hyperpolarization of alpha
motoneurons seen during locomotion in the cat is severely
handicapped by a lack of information regarding the phase
relationship between interneuron firing and the oscillation
of motoneuron membrane potential. The attribution of function

to various populations of interneurons is primarily
speculation since so little is known about the details of
the activity patterns of these units during locomotion.
For instance, if Renshaw cells function to turn off
homonymous alpha and gamma motoneurons while inhibiting
IaINs and disinhibiting antagonists, it would be expected
that their period of peak discharge should coincide with a
reduction of activity in alpha motoneurons and IaINs.
Unfortunately, this kind of information is not available.

5.   Alpha motoneurons

(a)   Extracellular recordings

Severin, et al. (1967) were the first to examine the
behavior of single motoneurons during locomotion by recording
the activity of alpha motoneurons from the proximal ends of
cut L7 or S1 ventral root filaments during controlled
treadmill locomotion. While this procedure has been
employed by others to document the occurrence of locomotor
activity (McCrea and Jordan, 1976a, b; Jordan, et al. 1978;
Menzies, et al. 1978, Pratt, et al. 1979), only a few
studies have utilized ventral root recordings to examine the
firing characteristics of alpha motoneurons during locomotion
(Severin, et al. 1967, 1968; Zajac and Young, 1976).

The most complete analysis to date was performed by
Severin and coworkers (1968). Ventral root efferents were
functionally identified by their response to passive flexion
and extension of the various hindlimb joints, a procedure
which succeeded in isolating extensor motoneurons more easily
than flexors. In a total of 23 experiments, recordings were
obtained from 85 motoneurons to ankle extensors and 9
motoneurons to ankle flexors. During controlled locomotion,
these motoneurons exhibited discrete bursts of activity
that were coupled to the support and transfer phases of the
step cycle, respectively. The burst of extensor motoneuron
activity usually consumed about 1/3 or 1/2 of the total
duration of the support phase. Although this was not
analyzed, it appeared that the flexor motoneurons were
active for relatively shorter periods during the transfer
phase of the step cycle (Severin, et al. 1968, Figure 5).
The average burst duration for extensor motoneurons was
120 msec and consisted of an average of 5 action potentials.
The range of motoneuron firing frequency during locomotion
was 15 - 94 Hz for extensor motoneurons and 35 - 70 Hz for
flexor motoneurons. These values are consistent with those
observed by Zajac and Young (1976).

It was reported by both Severin, et al. (1967) and Zajac and Young (1976) that the frequency of motoneuron firing was not synchronized with the frequency of MLR stimulation. The discharge pattern within the burst was similar for flexor and extensor motoneurons; the initial spikes often occurred at a very short latency while the interspike interval (ISI) in the remainder of the burst was longer and relatively constant. Zajac and Young (1976) found that all flexor and 86% of the extensor motoneurons displayed an initial doublet or triplet at the onset of each burst and suggested that this firing pattern represented a method for maximizing tension production in associated motor units.

The ISI was not altered by changes in the strength of MLR stimulation or in the speed of the treadmill belt. When the strength of brainstem stimulation was increased, an augmentation of total muscle EMG was observed in the absence of any change in the activity of single motoneurons. If there were more than one efferent in a given filament, the recruitment of additional units could be observed as the stimulation strength was increased. Thus, the increase in muscle force that accompanied higher strengths of MLR

stimulation resulted from the recruitment of additional

motor units rather than an increase in the firing of any

given motoneuron. An increase in the speed of the treadmill

belt while the strength of MLR stimulation remained constant

resulted in a shortening of the support phase and a concomitant

decrease in the duration of the extensor motoneuron burst.

The frequency of motoneuron firing did not change.

(b) Intracellular recording

Edgerton, et al. (1976) were the first to obtain

intracellular recordings of alpha motoneurons in paralyzed,

acute spinal cats treated with Nialamide and DOPA and to

monitor the rhythmic membrane potential oscillations that

occurred during fictive locomotion. Alternating periods of

depolarization and hyperpolarization were observed with the

depolarized period occurring in phase with bursts of activity

corresponding flexor or extensor efferents. Some alpha

motoneurons produced spikes during the depolarized phase

while others displayed membrane potenial oscillations in

the absence of any spikes. These observations have been

confirmed by others (Andersson, et al. 1978; Schomburg and

Behrends, 1978a, b). This evidence suggests that the spinal

locomotor generator engages a wide population of motoneurons

and induces rhythmicity before and independently of raising some motoneurons above threshold for generating spikes and activating motor units. These findings are consistent with the fact that lumbosacral interneurons have been observed to be rhythmically active before any rhythmic muscle activity is detectable (Feldman and Orlovsky, 1975). The property of rhythmicity may be conferred onto motoneurons solely by the spinal generator while the strength of input to any given motoneuron, which is probably a function of extra-generator input, i.e., supraspinal influences and afferentation, may determine the amount of motoneuron discharge.

It has recently been shown that the response to non-noxious stimuli is dependent upon the phase of the step cycle in which it is presented (Forrsberg, et al. 1977). Intracellular recordings of alpha motoneurons in fictively locomoting acute spinal cats revealed that maximal responses (EPSPs) to low threshold stimulation of muscle nerves (Schomburg and Behrends, 1978a), cutaneous nerves (Schomburg and Behrends, 1978b) and the dorsum of the paw (Andersson, et al. 1978) occurred during the periods of peak depolarization in flexor and extensor motoneurons. Smaller amplitude

responses and even conversion to an IPSP were observed

during the hyperpolarized phase (Schomburg and Behrends,

1978b). Differences in the amplitude of the evoked EPSPs

that were observed during the depolarized and hyperpolarized

phases of the step cycle were not accompanied by changes in

conductance (Andersson, et al. 1978), nor could they be

completely explained by changes in motoneuron excitability

(Forrsberg, et al. 1977).

These data have been interpreted by some investigators

to suggest that the observed phase modulation of reflexes

in motoneurons may be due to either a sharing of the last

order interneurons used by reflex and locomotion pathways

or to a phasic depression of reflex interneurons by

locomotion coupled interneurons (Andersson, et al. 1978).

Such speculation is consistent with the fact that Ia

inhibitory interneurons have been shown to be responsive

to Group I strength stimulation only during their active

phase of the step cycle (Feldman and Orlovsky, 1975).

Certain periods within the step cycle have also been shown

to be more labile than others. Reflex responses in

motoneurons (Forrsberg, et al. 1978) and resetting of the

locomotion rhythm (Duysens, 1977) could be evoked in response

to cutaneous stimuli presented throughout the step cycle

unless the stimulus occurred at the turning points in the step cycle, i.e., during transition from flexion to extension or the reverse. Taken together, these data would suggest that the spinal locomotion generator phasically modulates the pathways to extensor and flexor motoneurons during locomotion such that they are basically in on or off states. The receptivity of motoneurons to non-noxious stimuli when they are out of phase would be depressed to promote fluid, continuous stepping and to reduce the occurrence of unwanted perturbations.

6. Gamma motoneurons

Gamma motoneurons have been shown to be coactivated with alpha motoneurons during controlled treadmill locomotion in mesencephalic cats (Severin, et al. 1967b; Severin, 1970) and during spontaneous walking in decorticate cats (Perret and Buser, 1972; Perret and Berthoz, 1973). Of particular significance was the demonstration by Sjostrom and Zangger (1974) that alpha and gamma motoneurons were active in parallel during fictive locomotion in acute spinal, DOPA treated cats. The evidence thus suggests that the coactivation of alpha and gamma motoneurons is centrally programmed within the locomotion generator and argues

against a role of the gamma loop in controlling the sequence of alpha motoneuron activation. This postulate is further supported by the fact that procaine blockage of gamma motoneuron input to extensors resulted in diminished EMG amplitudes but did not alter the rhythm of stepping during controlled locomotion (Severin, 1970).

In summary, alpha and gamma motoneurons, VSCT cells, IaINs, and Renshaw cells have been shown to be rhythmically active during locomotion in the absence of cyclic afferent input. It is noteworthy that all of these cells also receive recurrent inhibition from Renshaw cells. The physiological significance of this correlation is unknown.

D. Research Plan

The present study addresses questions regarding the activity and function of Renshaw cells and IaINs and attempts to determine what role, if any, these interneurons have in the generation and control of the rhythmic motoneuron membrane potential oscillation that underlies the locomotor rhythm.

Although it has been shown that Renshaw cells are not inhibited during fictive locomotion (McCrea and Jordan, 1976a), the possibility still exists that Renshaw cells

may be inhibited by supraspinal structures or segmental afferents that have been shown to inhibit Renshaw cells in non-walking decerebrate cats (Haase and Van Der Meulin, 1961; Wilson, et al. 1964; Curtis and Ryall, 1966c; Haase and Vogel, 1971; Ryall and Piercey, 1971; Piercey and Goldfarb, 1974; Fromm, et al. 1977) and in acute spinal cats treated with DOPA (Bergmans, et al. 1969). Recurrent inhibition produced by antidromic ventral root stimulation during two different modes of rhythmic motoneuron activity, cyclic passive limb movement and controlled treadmill locomotion, were compared to determine whether the recurrent inhibitory pathway to alpha motoneurons is functional when the spinal generator is operating in the presence of cyclic afferent input, an element of normal locomotion which is lacking in the fictively locomoting preparation.

The efficacy of recurrent inhibition of IaINs during locomotion was assessed by recording from IaINs in fictively walking cats and comparing their normal rates of discharge with those observed during 1 sec. trains of antidromic stimulation of a ventral root. If recurrent inhibition of motoneurons or IaINs is a critical component of the spinal generation of locomotion, it would be expected that

antidromic stimulation of an entire ventral root and subsequent activation of a massive population of Renshaw cells could alter the normal locomotor rhythm. Records of motoneuron activity in ventral root filaments were analyzed before, during and after trains of ventral root stimulation to determine whether any evidence of rhythm resetting could be observed.

The excitability of the Ia system to agonist and antagonist motoneurons during fictive locomotion was tested by evoking Ia mediated EPSPs and IPSPs in alpha motoneurons, and comparing the amplitudes of the postsynaptic potentials that occurred during the various phases of the step cycle. Analysis of this data provided some insight as to the possibility of cyclic inhibition of IaINs as well as whether motoneuron membrane depolarization results from the removal of inhibition or the addition of excitation.

A review of the data available on the activity of neuronal units that are thought to participate in the control of locomotion at the segmental level makes evident that while much has been learned in the last 12 years, much of the information is fragmentary and does not permit the determination of the interrelationships among units.

Such analysis is critical to understanding the contribution
of various units to the control of locomotion. Miller and
Scott (1977) have proposed that the spinal locomotion
generator consists of antagonistic systems of Renshaw
cells, IaINs and motoneurons, but there is little evidence
that the firing patterns of these cells during locomotion
conform to those predicted by the model. To date, only
three studies have been published on the activity of either
IaINs or Renshaw cells during locomotion. It is known that
both IaINs and Renshaw cells discharge basically in phase
with agonist motoneurons, but the details of the phase
relationships and firing patterns of these cells are not
known. It would seem to be a reasonable assumption that
an analysis of the interrelationships among neuronal
activity patterns that occur during locomotion should
reveal information about neuronal function. In fact, such
an analysis is a prerequisite to understanding the
interactions among various populations of spinal neurons
and how they may be involved in the generation of locomotion.
Therefore, the activity of Renshaw cells, IaINs and
identified motoneurons were recorded in the same preparation
which allowed each cell's discharge pattern to be compared to

a common ventral root filament. By normalyzing each step

cycle, an analysis of the phase relationships among neurons

considered as possible elements of a spinal generator was

possible, and it could be determined whether they were

consistent with proposed models. For instance, if Renshaw

cells function to turn off agonist motoneurons and IaINs

projecting to antagonist motoneurons, periods of maximal

Renshaw cell discharge frequency would be expected to just

precede cessation of firing in agonist motoneurons and to

occur concurrently with depressions in IaIN discharge.

Frequency histograms of Renshaw cell and IaIN

discharge during locomotion were also compared with

membrane potential changes in identified motoneurons

measured during locomotion and plotted in a normalized step

cycle. This analysis allowed a determination of the

correlation between motoneuron membrane hyperpolarization

and inhibitory interneuron discharge frequency.

The pattern of neuronal discharge that occurred within

a burst was also analyzed in hopes of revealing information

regarding the synaptic input to these cells during fictive

locomotion. It was of interest to determine whether IaINs

turn off completely at some point in the step cycle or

simply show a decrease in discharge frequency; in the latter case, the relationship between the minimal firing rates seen during locomotion and the spontaneous discharge rates of the cell were determined. Another question asked was how the pattern of Renshaw cell firing that occurred during fictive locomotion compares with that obtained by antidromic stimulation of motor axons and what that might suggest about the pharmacology of Renshaw cell activation during locomotion. The literature (Severin, et al. 1967b, 1968; Zajac and Young, 1976) indicates that motoneurons fire at a constant rate except for the first 2 - 3 spikes during controlled treadmill locomotion. It was of interest in the present study to compare motoneuron firing patterns observed during treadmill and fictive locomotion to determine whether motoneuron firing is primarily a function of membrane characteristics or segmental afferentation.

Not only is the identity of the last order neurons which impinge on motoneurons and render them rhythmic during locomotion not known, but it is also unknown whether the spinal generator circuits to flexor and extensor motoneurons are the same. Recent evidence indicates that the spinal locomotion generator must contain a source of excitatory

input to motoneurons, since the removal of inhibition alone did not appear to be an adequate explanation of the genesis of motoneuron rhythmic depolarization (Menzies, et al. 1978; Pratt, et al. 1978). Spinal circuits specific for flexor and extensor motoneurons have been suggested to operate during fictive scratching in cats (Berkinblit, et al. 1978b). In contrast to flexor motoneurons, extensor motoneurons are known to receive tonic monosynaptic and polysynaptic excitation via the vestibulospinal tract (Grillner, et al. 1970)., and it was thought to be important to determine whether a similar bias in excitatory input to flexor and extensor motoneurons existed during locomotion. This was analyzed by comparing flexor and extensor motoneuron membrane potential oscillations that occurred relative to the resting membrane potential during fictive locomotion which were averaged over several step cycles and plotted in normalized form.

M E T H O D S

A.   Anaesthesia and surgical procedures

All experiments were performed on adult cats of either sex weighing between 2.0 and 5.0 kg. Two different locomoting preparations were employed for these studies. The effect of recurrent inhibition on motoneuron discharge rates and patterns during locomotion was studied in 10 cats which walked on a treadmill in response to stimulation of the MLR (controlled treadmill locomotion). The remaining experiments involved a fictive locomotion preparation and were performed on a total of 40 cats. These preparations will hereafter, be referred to as the treadmill and fictive locomotion preparations, respectively.

Halothane anaesthesia, carried in a mixture of nitrous oxide (70%) and oxygen (30%) was administered via a Dragger halothane vaporizer throughout all surgery. Anaesthesia was induced by directing a flow of 4% halothane to an airtight box (45 x 25 x 27 cm) which held the animal. After induction, the cat was removed from the box and anaesthesia maintained with 2 - 3% halothane delivered by a face mask and later by a tracheal catheter.

A heating pad placed under the cat while it was lying on the surgical table was used to keep the cat's temperature between 36° and 38° C during the subsequent surgery. The right and left common carotid arteries were ligated. A cannula consisting of a silastic catheter (Dow unsterile silastic), filled with a lactated Ringer's solution containing 16% heparin was inserted into the right carotid artery proximal to the ligation. A Stratham pressure transducer (Model P23AC) was interposed between the arterial catheter and a Grass polygraph which provided a continuous blood pressure recording. The right femoral vein was also cannulated for i.v. administration of drugs and fluids throughout the experiment. A tracheostomy was performed and a metal T catheter inserted into the trachea which was used to maintain anaesthesia and, in some experiments, to permit artificial respiration of animals which had been paralyzed.

Lactated Ringer was administered intravenously at a slow drip rate (about 6/sec.) to replace loss of body fluids which occurred during the course of the surgery. A midline incision extending from about T10 to the base of the tail was made through the skin and fascia of the back. The

spinalis dorsi muscles were removed bilaterally. Muscle bleeding was curtailed by cautery. An incision was made in the fascia between L7 and S1 to permit access to the L7 lamina. Using rongeurs and bone cutters, the lamina and dorsal portions of the L4 - L7 vertebrae (L3 - L7 in the treadmill locomotion preparation) were removed. The spinal canal was widened to an extent that would permit sufficient access to the spinal roots during subsequent dissection while being careful to avoid rupture of the radicular arteries. Bone wax and cautery were used to control any bleeding that was encountered. Gauze pads soaked in Ringer's solution were used to cover the exposed section of the spinal cord to prevent excessive drying of the spinal dura.

Following completion of the laminectomy, the animal was transferred to a spinal frame with its head securely positioned in a stereotaxic head holder. Although the configuration of the spinal frame varied depending on the type of preparation being used, the stereotaxic head holders associated with each frame were essentially identical and allowed all decerebrations to be performed in a uniform manner. An extensive bilateral craniotomy was performed, and all bone edges sealed with bone wax to minimize blood

loss as well as the possibility of introducing air into the cranial venous sinuses and subsequent formation of air embolisms. A blunt spatula was used to remove the cerebral cortices and then to transect the brainstem along a plane extending from the rostral border of the superior colliculi dorsally to the caudal edge of the mammillary bodies ventrally (a postmammillary or mesencephalic preparation). The level of the transection is schematically represented by the dashed line in Figure 2. Anaesthesia was discontinued immediately after completion of the decerebration. Brain tissue rostral to the site of transection was removed. The base of the cranium and the exposed surfaces of the brainstem caudal to the lesion were covered with adsorbable hemostat (Surgical R). The saggital sinus was ligated and cut rostral to the tie. The skull cavity was then covered with a 3% agar solution to prevent drying of the remaining brain tissue.

Acute postmammillary cats do not walk spontaneously (Hinsey, et al. 1930; Grillner and Shik, 1973), but will engage in co-ordinated locomotion when the MLR, located 6.0 - 7.0 mm below the dorsal surface of the inferior colliculus is repetitively stimulated with low intensity

current (Shik, et al. 1966a). The MLR is reported to
be a discrete area about 3 mm in diameter located 1 - 2
mm caudal to the rostral border of the inferior colliculus
(Shik, et al. 1967). This area, which is located between
the ventral border of the cuneiform nucleus and the dorsal
edge of the brachium conjunctivum, is illustrated in
Figure 2.

The animal was allowed to recover from the effects of
the anaesthesia for at least 1 hour before MLR stimulation
was initiated. The tip of an insulated monopolar
stimulating electrode (Kopf SNE 300; exposed tip = 0.25 mm;
diameter = 0.1 mm) was placed on the dorsal surface of
the brain at a point 4 mm lateral to the midline and 1.5 mm
caudal to the division between the superior and inferior
colliculi. The electrode was then lowered to a depth of
4.0 mm which is well above the dorsal border of the MLR
(Shik, et al. 1966a; Grillner and Shik, 1973). Square
wave pulses of .5 msec duration were delivered by a
constant current generator at a rate of 30 Hz through the
MLR electrode as it was lowered at .5 mm increments.
The electrode was lowered in such a fashion until a depth
was reached that consistently produced a co-ordinated

four-limb walking gait at the lowest current strength possible. Sites which generated such locomotion were normally found between 5.5 and 6.5 mm below the surface at current strengths ranging from 50 to 100 uA.

B.   Treadmill locomotion preparation

1.   Experimental arrangement

The apparatus and experimental arrangement used for the experiments involving controlled treadmill locomotion are schematically illustrated in Figure 3. The animal was supported over the treadmill by the stereotaxic head holder and the spinal frame, the heights of which were adjusted to allow free movement of the limbs and their contact with the treadmill belt. Metal clamps associated with the frame which were placed over the iliac crests and the L_3 spinous process immobilized the $L_3 - S_1$ vertebral spine and the pelvic girdle. The skin surrounding the laminectomy was tied to the frame to form a pool which was filled with warm mineral oil and heated by an infra-red lamp to maintain the pool's temperature between 36° and 38° C.

The left L7 ventral root was cut and its central end placed on platinum biopolar electrodes for electrical stimulation. A thin filament isolated from the ventral root was positioned on platinum bipolar recording electrodes. Action potentials in the filament were fed through a preamplifier to the display of a dual beam oscilloscope and an audio-monitor as well as to a 4 channel FM tape recorder (Hewlett-Packard 3960) for subsequent filming and analysis.

Initial efforts to produce activity in the ventral root filament involved passive flexion and extension of the left hind limb around the hip, knee and ankle joints. Motoneurons that were activated by limb displacement were identified according to function, i.e., as knee extensors or flexors, on the basis of their response to stretch of a specific muscle group at a particular joint. Motoneuron activity during locomotion was monitored to determine whether it was phasically active and whether its peak activity occurred during the stance or swing phase of the step cycle. Filaments were repeatedly partitioned until as few units as possible were present so as to optimize

analysis. Once motoneuron activity was generated and could be reliably reproduced, passive alternate flexion and extension around the appropriate joint by the experimentor at a rate approximating that limb's involvement in a walking step cycle (one displacement per second) was then employed to produce cyclic activity in the filament.

2. Procedures for data analysis

Trains of square wave pulses of 1 msec duration were randomly delivered to the L7 ventral root during periods of passive cyclic limb movement and locomotion. Train durations of 2, 3 or 4 seconds were used so as to encompass more than one step cycle during locomotion within the stimulation period. Various stimulation parameters were employed in an attempt to discern possible relationships between recurrent inhibition or recurrent facilitation during rhythmic activity and either frequency, duration or strength of antidromic stimulation. Current strengths of 30, 50, 75, 150 and 300 uA were used at frequencies of either 10, 20, 30 or 70 Hz in various trials.

The mean firing rate (F) of a motoneuron was computed using a method employed by Fromm, et al. (1977) in which the number of spikes within 1 second intervals were counted

for 3 seconds before stimulation (Fi), each one-second

interval of antidromic stimulation (Fs) and for 1 second

post-stimulation (Fp). These values were then divided by

the number of one-second bins comprising the measure to

obtain an average expressed in spikes/second. A t test

for paired observations was used to test significance of

the difference between Fi and Fs for each motoneuron; an

alpha level of .05 or greater was necessary for significance.

An alternate procedure of determining motoneuron

discharge frequency which was utilized by Severin, et al.

(1968) involves counting the number of spikes within a

burst and calculating the frequency on the basis of the

observed interspike interval. This method was not

feasible in the present study because of the substantial

recurrent inhibition produced during ventral root stimulation

which often eliminated all but an occasional spike during

the period of antidromic stimulation.

It was also of interest to determine whether ventral

root stimulation was capable of altering the locomotor

rhythm in addition to its effects on motoneuron discharge.

To examine this possibility, records were selected that

showed evidence of consistent locomotor activity before

and after the period of antidromic stimulation. Means
and standard errors of normal (occurring during locomotion
prior to antidromic stimulation) burst durations (BD_n)
and interburst intervals (IBI_n) were computed and were
used to predict where the first burst of motoneuron activity
should occur following cessation of antidromic stimulation.
If the difference between the actual and the predicted
burst occurrence exceeded the associated standard error,
it was concluded that ventral root stimulation had produced
a re-setting of the locomotor rhythm.

In cases where some motoneuron activity persisted
during the period of antidromic stimulation, the individual
interburst intervals (IBI_s) were examined with particular
consideration given to those in which ventral root
stimulation either began or ceased. Since a reduction in
the duration of the burst would result in an increase in
the IBI if the subsequent burst occurred where predicted,
it was necessary for the IBI_s to be significantly different
(difference greater than the standard error) from that
which would have been observed had the preceeding burst
been of normal duration to conclude that the normal
locomotor rhythm had been altered.

Relationships among the various motoneuron discharge and antidromic stimulation parameters were examined through multiple linear regression and correlation analysis. Since previous investigators (Granit, et al. 1960) had reported maximum efficiency of recurrent inhibition on tonic reflex activity at frequencies of antidromic ventral root stimulation ranging from 30 - 40/sec., it seemed appropriate to examine the existence of a similar relationship during rhythmic patterns of motoneuron discharge. Thus, the data were pooled on the basis of antidromic stimulation frequencies and a factorial analysis of variance was performed on the percent reduction in motoneuron discharge frequency produced during cyclic passive limb movement and locomotion.

The relationship between initial motoneuron firing frequency (F_i) and efficiency of recurrent inhibition was analyzed by linear regression and correlation analysis. Efficiency of recurrent inhibition was expressed as a percentage ($F_i - F_s/F_i \times 100$) and in absolute terms as ΔF ($F_i - F_s$). An analysis of covariance was also performed in which F_i was the covariate and F_s the dependent variable. This allowed a comparison between the efficiency of recurrent inhibition during cyclic passive limb movement

and locomotion once the effect of initial firing frequency had been removed.

C. Fictive locomotion preparation

1. Nerve cuffs

The purpose of these experiments was to obtain microelectrode recordings from identified motoneurons, IaINs and Renshaw cells during fictive locomotion. It was essential that each neuron be identified in terms of its connections with various muscles or muscle groups (a functional identification). All three populations of neurons receive their most pronounced excitation from homonymous muscle nerves and a weaker excitation from muscle nerves to synergist muscles. Spinal neurons, especially IaINs and motoneurons, could thus be functionally identified by recording their post-synaptic responses to orthodromic stimulation of various hindlimb muscle nerves. Nerve cuffs placed around intact selected hindlimb nerves permitted an assortment of nerves to flexors and extensors at the hip, knee and ankle joints to be stimulated throughout the course of the experiment. Each nerve cuff consisted of a 5 - 7 mm length of silastic tubing (Dow,

unsterile silastic, inside diameters ranging from .030 to .062 mm). Two 40 gauge enamel-insulated silver wires were inserted into each cuff with a small hypodermic needle. Both wires were perpendicular to the long axis of the nerve cuff and had an interelectrode distance of 2 - 3 mm. The section of wire inside the cuff was gently pressed against the inside wall of the cuff to ensure ample room for the nerve; the wire electrodes usually covered about 180° of the inside diameter of the cuff. The insulation was scraped off of the wire inside the cuff and the terminal 2 - 3 mm of both electrodes. The cuff was cut longitudinally along the surface opposite the electrode wires. Nerves were dissected free of surrounding tissue and gently placed inside the cuff. The leads were connected to a Grass stimulator and the integrity of the nerve-electrode connection tested by eliciting a muscle twitch at low stimulus strengths. The nerve cuff was then secured by placing a tie about the outer circumference of the cuff being careful not to put pressure on the nerve. A coating of soft elastomer (Silastic, 382 Medical Grade) was put about the cuff to seal either end of the cuff and to increase the stability of the connection. The electrode leads were

tied to the skin adjacent to the incision leaving sufficient slack subcutaneously to prevent them from being dislodged during limb movement. Nerve cuff function was tested again prior to closing the wound. One cutaneous nerve, sural (Sur), and the following muscle nerves of the left hindlimb were routinely cuffed for stimulation: quadriceps (Q), sartorious (Sart), gracilis (Gr), semitendinosus (St), posterior biceps (PB), semimembranosus (Sm), lateral gastrocnemius (LG) and tibialis anterior (TA).

2. Mechanical support

Following placement of the nerve cuffs and the laminectomy, the cat was transferred to a shielded room and placed in a modified Transvertex spinal frame and stereotaxic head holder. The animal was suspended in the frame, with legs pendant, by metal clamps on the ilium and the L3 vertebral spine. L-shaped metal clamps which pressed against opposing longitudinal surfaces of the vertebral canal and sturdy pointed metal pins which ran from the frame at right angles to just below the iliac crests, also served to immobilize the spinal canal from L3 to S1 and the pelvic girdle. The skin of the back was tied to form a paraffin-

filled pool which was maintained at 36° - 38° C by a heating lamp.

3.   Spinal cord dissection

Under a dissecting microscope, a longitudinal cut was made in the spinal dura along the exposed section of the spinal cord. The dura was reflected so that the dorsal and ventral roots were exposed. The left L5 to S1 ventral roots were cut at their point of exit from the spinal canal and placed on platinum bipolar stimulating electrodes. A fine filament, containing as few motor axons as possible, was isolated from either the L6 or L7 ventral root and mounted on platinum bipolar recording electrodes. This preparation will be referred to as preparation I.

In some experiments, the left L5 - L7 dorsal roots were cut while all of the ventral roots were left intact except for a small filament separated from either the L6 or L7 ventral root. These experiments, referred to as preparation II, were designed to selectively record from motoneurons and Renshaw cells; the activation of Renshaw cells by antidromic stimulation of individual muscle nerves allows a more conclusive functional identification than is sometimes possible with orthodromic stimulation.

4. Potentiometer recordings

Movements of the hindlimb in a rostro-caudal plane
were measured by connecting the distal end of a length of
cord (20 - 25 mm) which was tied about the cat's ankle,
to a potentiometer. When the cat performed walking
movements, the potentiometer signal, which resembled
a sinusoid, was recorded on magnetic tape. Since in most
experiments the left L5 - S1 ventral roots were severed,
potentiometer recordings were usually obtained only for
the right hindlimb. The excursions of both hindlimbs were
monitored in preparation II.

5. Fictive locomotion

Because the administration of drugs that potentiate
the effects of noradrenaline will evoke stepping in acute
spinal cats, and since similar patterns of reflex activity
in alpha (Jankowska, et al. 1967a; Grillner, 1973; Grillner
and Shik, 1973; Grillner and Zangger, 1974) and gamma
(Grillner, 1969a, b, 1973) motoneurons have been observed
in all of the locomotion preparations, it has been
suggested that a specific descending noradrenergic system
activates the spinal locomotion generator (Jankowska, et al.
1967b; Forrsberg and Grillner, 1973; Grillner, 1973;

Grillner and Shik, 1973). It has been shown by Jordan and Steeves (1976), however, that cats with significant ($>$ 91% of control values) depletions in spinal cord levels of noradrenaline displayed apparently normal locomotor activity during controlled treadmill and unrestrained locomotion. This evidence suggests that noradrenaline is not essential for activating the spinal machinery responsible for producing locomotion and raises questions concerning the specificity of action of precursors and agonists of noradrenaline in the spinal cord.

Although single unit recordings of spinal neurons can be obtained during fictive locomotion in acute spinal cats treated with DOPA, the fact that the periods of locomotion occur spontaneously limit the usefulness of this model in studying the activity of spinal neurons during locomotion. Neither the comparison of a given cell's activity before and during locomotion nor an analysis of a cell's behavior as the locomotion generator is turned on is possible unless the experimentor can control the periods of locomotion. The mesencephalic preparation developed by Shik and coworkers (1966a) is a more attractive model for investigating spinal neuronal elements that may be involved

in the control of locomotion for two reasons. Firstly,
locomotion is not dependent upon systemically applied drugs
which have a broad spectrum of action peripherally and
centrally, the specificity of which has not been established.
The second advantage of the preparation arises from the
fact that locomotion can be turned on and off at will,
thereby allowing greater control and flexibility of the
experimental paradigm.

Animals were decerebrated in the manner described
previously (Section A). After recovery from the effects
of anaesthesia, locomotion was induced by stimulation of
the MLR. While the cat stepped in the air, locomotion was
monitored by recording the activity of rhythmic motor axons
in the ventral root filament, as well as by the potentiometer
recordings of hindlimb movement. Both sets of signals were
stored on magnetic tape. Several procedures were employed
to identify the motor axon(s) in the ventral root filament.
In some cases, short latency reflex activity could be
evoked in the filament in response to stimulation of the
hindlimb muscle nerves. Attempts were also made to evoke
reflex activity in the filament by muscle stretch or
palpation. Inspection of ventral root filament and

potentiometer recordings allowed identification of the

filament as being either a flexor or an extensor, and it

was usually possible to determine the joint to which the

motor axon was coupled.

After consistent locomotion had been attained at the

lowest stimulus intensity possible and the filament

identified, the animal was paralyzed with a dose of gallamine

triethiodide (Flaxedil) (2.0 - 3.0 mg/kg), and artificial

respiration was initiated. End tidal CO_2 was monitored

and maintained within 4 - 6% by adjustment of tidal volume

and respiratory rate. Paralysis was judged to be complete

when stimulation of hindlimb nerves failed to produce

an observable muscle contraction. The MLR was then

stimulated and rhythmic ventral root activity (fictive

locomotion) recorded. Two examples of locomotor activity

evoked in L7 ventral root filaments before and after the

administration of Flaxedil in two different experiments are

illustrated in Figure 4. The stimulus artifact in the top and

bottom traces in A indicate the period of MLR stimulation.

In B, MLR stimulation started at the beginning of the trace.

In both A and B, it can be seen that rhythmic motoneuron

activity, which is similar to that observed before Flaxedil

was given, can be evoked after paralyzation. The middle
trace in A is the potentiometer recording of the right
hindlimb; flexion is indicated by an upward deflection.
It can be seen that the motor axons in the left L7 ventral
root filament in A innervate an extensor muscle.

6.   Microelectrode recordings

Intra- and extracellular recordings from motoneurons
and interneurons in the lumbar enlargement were obtained
using glass micropipettes filled with either 3M NaCl,
KCl or KAc. Fiber-filled glass capillary blanks (A.M.
Systems, Inc., G-C-120) were pulled on a Narashige electrode
puller and beveled to obtain tip diameters $\leq$ 1 micron.
The microelectrodes had resistances ranging from 5 - 20
megohms. A Transvertex mechanical microdrive, which was
driven by remote control, was used to lower the micro-
electrode into the spinal cord. A pair of fine straight
forceps were used to make a hole in the pia at the
recording site so as to minimize the possibility of breaking
the tip when penetrating the cord. A meter on the remote
control unit was always zeroed while the microelectrode
was flush against the dorsal surface of the spinal cord
before the descent was started. The meter indicated the

dorso-ventral position of the microelectrode in the cord and thus allowed a rough assessment of the location of neurons which were encountered.

In a majority of the experiments, recording sites were selected between the rostral and caudal borders of the L5 spinal segment. The motoneuron pools located in this area (Q, Sart, Gr and adductor femoris) are coupled to muscles acting at the hip and knee (Romanes, 1951). It was decided to sample from neurons coupled to hip and knee muscles since they tend to perform more reliably during locomotion (Engberg and Lundberg, 1969) and have been implicated to have more profound effects on the locomotor rhythm (Orlovsky, 1972a; Grillner and Shik, 1973) than those associated with the ankle and the digits.

7. Experimental arrangement and paradigm

Figure 5 schematically illustrates the stimulating and recording arrangement used in experiments involving preparation I. The circle in the upper right hand corner of the diagram represents the face of a Tektronix 565 dual beam oscilloscope. One beam had a 2 channel amplifier and the other beam a 4 channel amplifier. A Grass (S88) stimulator in series with a stimulus isolation unit (Grass

SIU5) and constant current unit were used to stimulate the MLR. MLR stimulation evoked rhythmic motoneuron activity in a ventral root filament which was recorded on platinum bipolar electrodes (W.P.I. Miniature Probe), amplified (W.P.I. DAM 5A differential preamplifier), sent to an audio monitor and displayed at a slow sweep speed on the oscilloscope. A silver ball-tipped electrode placed on the surface of the spinal cord near the dorsal root entry zone recorded the arrival of the stimulus volley delivered to the peripheral nerves and allowed measurement of the intraspinal delay in neuron response. These cord dorsum potentials were amplified (W.P.I. DAM 5A differential preamplifier) and displayed at fast sweep speed on the oscilloscope; the line prior to the onset of the cord dorsum represents the stimulus artifact.

Signals which were recorded from single spinal neurons by the microelectrode were led into a high impedance voltage follower circuit (W.P.I. model M4A electrometer) and thence to an audio monitor and both AC and DC coupled vertical amplifiers (Tektronix 5A22N differential amplifiers in the Tektronix D10 oscilloscope) and displayed on two oscilloscopes (Tektronix D10 and 565).

A digitimer (Medical Systems, Corp., Model 4030) was used
to synchronize the stimulators (connected to the leg
nerves and ventral roots) and the sweep of the
oscilloscopes.

Data was photographed (Grass C4N Kymograph camera
and Stoelting, Co., Model RCM-3 reflexor) using Kodak
2495 RAR film which was later developed (Kodak D-19) and
analyzed. The AC and DC signals from the microelectrode,
the ventral root filament recording and a trigger pulse
(A gate) emitted from the oscilloscope (Tektronix 565)
were stored on a tape recorder (Hewlett Packard 3690)
with 4 FM channels for subsequent reproduction either by
filming or with an ultraviolet oscillograph recorder
(SE Labs, 3006/DL, 4 channel, -3db at 9 KHz; Kodak
Linagraph Direct Print Paper, #1895.

(a) Identification of spinal neurons

Spinal neurons were identified by their characteristic
responses to stimulation of peripheral leg nerves and/or
ventral roots. In preparation I, the following procedures
were used to identify IaINs, Renshaw cells and motoneurons.
Motoneurons were recorded intracellularly and identified
by their single action potential evoked in response to

ventral root stimulation (Brock, et al. 1952). Motoneurons
were functionally identified by recording their post-
synaptic potentials evoked by low threshold stimulation of
various hindlimb muscle nerves. Stimulus strengths were
used that excited mainly the large, low threshold Ia
afferents but which were too low to activate higher threshold
afferents. Graded electrical stimulation has been shown to
be an effective method of selectively activating the Ia
afferents (c.f. Watt, et al. 1976) particularly in the
nerves to thigh muscles (McIntyre, 1974). The presence of
a late component in the cord dorsum potential was used to
indicate that Ib and higher threshold afferents were being
stimulated (Eccles, et al. 1957b). Motoneurons were
classified as belonging to a muscle (homonymous) if
stimulation of the muscle nerve at Ia strength evoked a
maximal monosynaptic EPSP; as a synergist if a smaller
mono- or disynaptic EPSP was evoked and as a antagonist
if a disynaptic IPSP was evoked (Eccles, et al. 1957a).
Intraspinal delays less than 1.0 msec were judged as being
conclusively monosynaptic while those between 1.0 and 1.5
msec were classified as being possibly monosynaptic (Watt,
et al. 1976). IaINs were identified according to the three

main criteria established by Hultborn, et al. (1971b):
1) monosynaptic excitation from Ia afferents from the
homonymous muscle; 2) recurrent inhibition from antidromic
stimulation of the ipsilateral ventral root associated
with the spinal segment in which the IaIN is located; and
3) ability to follow stimulation of the nerve to the
homonymous muscle at frequencies of at least 300 Hz.
The functional identification of IaINs was determined in
a similar manner as that used with motoneurons. Renshaw
cells were identified by their characteristic high frequency
discharge in response to a single stimulus delivered to
the ventral root (Renshaw, 1946). Renshaw cells were
coupled to individual motoneuron pools on the basis of their
response (number of impulses per burst) to orthodromic
stimulation of leg nerves (Eccles, et al. 1961b).

In preparation II, motoneurons and Renshaw cells were
identified by antidromic stimulation of muscle nerves.
With this method, both cell types, especially Renshaw
cells, could be definitely identified as being coupled to
particular motoneuron pools.

8. Procedures for data analysis

(a) Recurrent inhibition of IaINs

The amount of recurrent inhibition of IaINs that occurred during locomotion in response to 1 sec. trains of antidromic ventral root stimulation was determined by the same procedure used to analyze the recurrent inhibition of motoneurons in the treadmill locomotion preparation. The recurrent inhibition obtained during locomotion was compared to that produced in control, non-locomoting conditions. The discharge frequencies exhibited by IaINs during the various phases of the step cycle were also analyzed during trials in which the ventral roots were not stimulated to determine whether the frequency of IaIN firing dropped below spontaneous firing rates during their inactive phase of the step cycle. This analysis may provide information regarding the magnitude of recurrent inhibition of IaINs that occurs normally.

(b) EPSPs and IPSPs in motoneurons

Ia mediated EPSPs and IPSPs were evoked in motoneurons during fictive locomotion by low threshold stimulation of hindlimb nerves. The amplitudes of these postsynaptic potentials were measured from the DC trace and correlated with the membrane potential measured at the onset of the postsynaptic potential. A correlation and regression

analysis was performed on the data to determine the relationship between the two parameters.

(c) Phase relationships among IaIN, Renshaw cell and motoneuron activity during locomotion

A substantial part of this study involved efforts to relate the activity of populations of neurons thought to be associated with a spinal generator that occurred during fictive locomotion in hopes of being able to draw some conclusions about the possible functions or interactions they might have on one another. To allow a comparison of how one cell behaved during a step cycle to the behavior of other cells, the data was normalized so that each step cycle became a unit. This procedure also facilitated averaging the activity of any given cell over a number of step cycles of varying duration. Other investigators (Orlovsky and Feldman, 1972; Edgerton, et al. 1976; Berkinblit, et al. 1978a, b; Delyagina and Feldman, 1978) have used similar techniques to normalize data on rhythmically active spinal neurons, but this is the first time that the procedure has been used to compare the activity patterns of different populations of spinal neurons.

The normalizing procedures used and the parameters that

were measured are illustrated in Figure 6. The data

presented in Figures 6 and 7 will be discussed in detail

in later sections of this thesis and are presented here

only to illustrate the analysis techniques employed.

Figure 6 shows the rhythmic activity of two TA motoneurons

which have been recorded simultaneously during fictive

locomotion. The top trace shows the rhythmic discharges

of a TA motoneuron recorded in an L7 ventral root filament.

The bottom trace is the intracellular record (DC trace)

of a TA motoneuron which displayed typical membrane potential

oscillations with bursts of action potentials superimposed

on the depolarized phases during fictive locomotion. MLR

stimulation was initiated at the beginning of the records

(extreme left). Motoneuron activity in the ventral root

filament is used to normalize each step cycle, as shown in

the top trace in Figure 6. The total duration of the step

cycle (T) is defined as the interval between successive

onsets of the motoneuron burst. The intervals denoted as

X and Y represent the time from the onset of the step

cycle to the onset and termination of spinal neuronal

activity, respectively. X thus indicates the time within

the normalized step cycle that the neuron being recorded

turns on and Y the point at which it switches off. The X and Y points define the activity periods for each neuron which are then plotted in a normalized step cycle as shown in Figure 7.

Each step cycle was divided into 10 equal intervals (bins) and the frequency of neuron discharge determined for each bin. A frequency histogram, representing the average of 10 consecutive step cycles, can then be plotted within a normalized step cycle as shown in Figure 7. Analysis of the data in this manner provides useful information as to the period of peak discharge frequency during the step cycle for an individual neuron. Normalization of neuronal activity relative to specific motoneurons recorded from ventral root filaments allowed a comparison of the activity patterns of cells recorded throughout a given experiment and of neurons coupled to individual motoneuron pools across experiments.

In cases where intracellular recordings of motoneurons were obtained during fictive locomotion, relationships between interneuronal discharge and motoneuron membrane potential changes were also determined as shown in Figure 7. The membrane potential was measured from the DC trace at the midpoint of each bin within the normalized step cycle as

shown in the bottom trace in Figure 6, averaged over 10

step cycles and plotted in normalized form as shown in the

top panel in Figure 7.

(d) Membrane potential oscillations in flexor and extensor

motoneurons

The measurement of the motoneuron membrane potential

during the step cycle was measured relative to the resting

membrane potential which was taken as the value of the

membrane potential measured at rest before the initiation

of MLR stimulation. Membrane potential oscillations in

flexor and extensor motoneurons were plotted relative to

the resting level in normalized form and compared to

determine whether there was any evidence of different

synaptic inputs to flexor and extensor motoneurons during

locomotion.

(e) Discharge patterns of IaINs, Renshaw cells and

motoneurons

The intraburst pattern of neuronal firing that occurred

during fictive locomotion was also determined for IaINs,

Renshaw cells and motoneurons to obtain more information

about the function of and synaptic input to these cells.

The pattern of discharge for the interneurons was determined

by analysis of the frequency histograms of their discharge plotted in normalized step cycles. Particular attention was paid to the time within the step cycle that the IaINs and Renshaw cells displayed their maximal firing rates since they would be assumed to correlate with periods of maximal inhibitory effect. By determining the phase relationships of these periods of peak discharge among IaINs, Renshaw cells and motoneurons, it was possible to see whether an increase or decrease in firing in one population of neurons could be attributed to the activity in another set of neurons.

The intraburst pattern of discharge of motoneurons was determined by measuring the interspike intervals (ISIs). Measurements were made from extracellular records (ventral root filaments) of motoneuron activity during controlled treadmill locomotion and from intracellular records of motoneurons during fictive locomotion. A factorial analysis was performed to determine significance of difference among ISIs and between the discharge patterns observed during treadmill and fictive locomotion.

9.   Abbreviations

Ach	Acetylcholine
CST	Corticospinal tract
DOPA	Dihydroxphenylalanine
DSCT	Dorsal spinocerebellar tract
E	Extensor
E_M	Membrane potential
EMG	Electromyogram
ENG	Electroneurogram
EPSP	Excitatory postsynaptic potential
E_R	Resting membrane potential
F	Flexor
ΔF	Change in frequency of discharge from control (Fi) to during antidromic ventral root stimulation (Fs); $\Delta F = Fi - Fs$
Fi	Frequency of discharge in spikes/sec during activity without antidromic ventral root stimulation
FRA	Flexion reflex afferents. This group of afferents includes cutaneous and high threshold muscle and joint afferents.
Fs	Frequency of discharge in spikes/sec during period of antidromic ventral root stimulation
G	Gastrocnemius

Gr	Gracilis
Hz	Hertz (frequency per second)
IaIN	Ia inhibitory interneuron
IPSP	Inhibitory postsynaptic potential
ISI	Interspike interval
i.v.	Intravenous
LG	Lateral gastrocnemius
MLR	Mesencephalic locomotor region
MN	Motoneuron
msec	Millisecond
PB	Posterior biceps
Q	Quadriceps
RC	Renshaw cell
RIPSP	Recurrent inhibitory postsynaptic potential
RST	Reticulospinal tract
RuST	Rubrospinal tract
S	Soleus
Sart	Sartorious
Sm	Semimembranosus
St	Semitendinosus
Sur	Sural
TA	Tibialis anterior

VSCT   Ventral spinocerebellar tract

VST   Vestibulospinal tract

R E S U L T S

A.   Effects of antidromic ventral root stimulation during
     controlled treadmill locomotion and cyclic passive
     limb movement

1.   Recurrent inhibition of motoneurons

The mean discharge frequencies for the 19 motoneurons
studied before (Fi) and during (Fs) antidromic stimulation
of the L7 ventral root during both cyclic passive limb
movement and locomotion are presented in Table 1. These
data were obtained from 4 successful walking preparations.
For each cell within each test condition, the difference
between Fi and Fs was tested for significance using a
paired t test, and the results of these tests are indicated
in Table 1 by asterisk(s). Table 2 presents a summary of
the effects of antidromic stimulation of the L7 ventral
root on 19 motoneurons tested during both cyclic passive
limb movement and locomotion. During cyclic passive limb
movement, the mean initial frequency of motoneuron discharge
(Fi) was 10.8 spikes/sec which was reduced to 3.2 spikes/sec
during antidromic stimulation (Fs). The net amount of
recurrent inhibition (ΔF) was 7.6 spikes/sec which

represents a 72.3% reduction in motoneuron discharge. Both Fi (12.4 spikes/sec) and ΔF (8.4 spikes/sec) were greater during locomotion than during cyclic passive limb movement. The reduction in motoneuron discharge during cyclic passive limb movement (72.3%) was very similar to the value obtained during locomotion (68.5%) and indicates that the efficacy of recurrent inhibition is not significantly diminished during locomotion.

T-tests for paired observations were used to determine whether the difference between Fi and Fs for each motoneuron was significant. The results of these tests are presented in the bottom row of Table 2. Motoneuron activity was significantly reduced by L7 ventral root stimulation in 15 of 19 motoneurons during cyclic passive limb movement and in 13 of 19 motoneurons during locomotion. A x^2 analysis confirmed that there was no significant difference (p = .48) between the proportion of motoneurons whose discharge rates were significantly lowered during cyclic passive limb movement as compared to those reduced during locomotion.

The response of three motoneurons to antidromic stimulation of the L7 ventral root during cyclic passive limb movement and locomotion is illustrated in Figure 10.

Records A and B are from one filament while records C and D are from a different filament. The larger amplitude spike in records A and B (motoneuron #14) is a knee extensor· the smaller amplitude spike is an ankle extensor that was activated during cyclic passive limb movement (10A) but not during locomotion (10B) and therefore, was not analyzed. In the trial shown in 10A, the initial frequency of discharge (Fi) for the knee extensor during cyclic passive limb movement was 13.7 spikes/sec. This motoneuron was completely inhibited throughout the 3 second period of antidromic stimulation. During locomotion, the Fi for this motoneuron was 18.3 spikes/sec and the Fs was 0.6 spikes/sec, a reduction of 96.7%.

Two identifiable motoneurons are displayed in Figures 8C and 8D , both of which were ankle extensors. The larger amplitude spike (motoneuron #9, was reduced by 96.3% (from 8 to 0.3 spikes/sec) during cyclic passive limb movement and just 14.2% during walking (from 12 to 10.3 spikes/sec). The smaller amplitude spike (motoneuron #10) showed a similar response to antidromic stimulation during cyclic passive limb movement as it was reduced from an Fi of 10 spikes/sec to an Fs of 0.3 spikes/sec, a decrease of 97%.

Recurrent inhibition was much more effective in reducing the activity of the smaller spike during locomotion, however, since it was completely inhibited from an initial firing rate of 8.3 spikes/sec.

Figure 10 highlights the fact that the amount of recurrent inhibition observed in motoneurons varies substantially and indicates that it cannot be predicted by the size of the action potential recorded in the filament or by the mode of activation. In 5 instances, (motoneurons 5, 10, 12, 13 and 16), there was a greater relative reduction during locomotion than during cyclic passive limb movement. This was not completely due to higher discharge rates during locomotion as compared to cyclic passive limb movement since motoneurons 13 and 16 were active at lower rates during locomotion. Likewise, in some cases such as motoneuron 14, (Figure 10A, B), the larger amplitude spike is very susceptible to recurrent inhibition while the smaller amplitude spike displays a greater resistance to inhibition by Renshaw cells. In records 10 C and D just the opposite is true, as the smaller spike is completely inhibited while the larger spike is only marginally affected (a 14.2% decrease). This finding contradicts evidence presented by

Henneman, et al. (1965b) that motoneurons are recruited and inhibited in an orderly sequence based upon the size of the motoneuron (assumed to correlate with the size of the action potential recorded in a ventral root filament) such that larger motoneurons are the last to be recruited and the first to be inhibited or to fall out of activation.

A multiple linear regression and correlation analysis was performed on 8 experimental parameters including Fi, Fs, ΔF and relative reduction in motoneuron firing during antidromic ventral root stimulation (Fi-Fs/Fi) during cyclic passive limb movement and controlled treadmill locomotion. The means and standard deviations for these 8 parameters are listed in Table 3. Table 4 contains the correlation coefficients (r) and regression coefficients (b) along with their associated tests of significance for the comparisons that were of primary interest. A t test was used to test whether the correlation between any two variables was significantly different from zero, while an analysis of variance was used to test for linearity. The regression coefficients are presented for only those comparisons which were found to have a significant linear relationship.

A significant ($p \leq .05$) linear relationship existed
between Fi and Fs during both cyclic passive limb movement
and locomotion ($r = .524$ and $.465$ respectively) which is
in agreement with data reported on tonically firing
motoneurons (Granit, et al. 1960; Granit and Renkin, 1961).
The regression lines describing the relationship between
Fi and Fs during cyclic passive limb movement and treadmill
locomotion are presented in Figure 9 along with the
regression coefficients and correlation coefficients.
There was no significant difference between the two
regression lines. If there were no recurrent inhibition,
Fi would equal Fs and the regression line would pass through
the origin with a slope of one. A regression line with
an intercept other than zero but with a regression coefficient
of one would represent a situation in which recurrent inhibition
decreased motoneuron discharge by a constant amount
independent of Fi. To test the significance of recurrent
inhibition, the population regression line generated from
an analysis of covariance was compared to a theoretical
line of slope $b = 1$ and the two were found to be significantly
different ($p \leq .001$). The formula used and calculations
can be found in Appendix A4. These results substantiate

the presence of significant recurrent inhibition during
both cyclic passive limb movement and controlled
locomotion and establish that the amount of recurrent
inhibition produced (Fi - Fs) was not a constant as had
been reported on motoneurons that responded to static muscle
stretch with a tonic discharge (Granit and Renkin, 1961).

While the relative change in motoneuron discharge
produced by antidromic ventral root stimulation was not
related to Fi, Δ F was linearly related to Fi with
correlation coefficients of .707 (p $\leq$ .001) for cyclic
passive limb movement and .844 (p $\leq$ .001) for locomotion.
The regression lines are presented in Figure 9B, and it
can be seen from a comparison of the regression coefficients
that there is a trend for the gain in recurrent inhibition
as Fi increases to be greater during locomotion than
during cyclic passive limb movement. The difference
between the two regression lines was not significant,
however.

Although the mean discharge values for Fi were not
greatly different during cyclic passive limb movement
(10.8 spikes/sec) and locomotion (12.4 spikes/sec)
correlation analysis confirmed the lack of relationship

$(r = -.09)$ between the activity rates before stimulation in the two modes of activation for a given motoneuron. Indeed, the data show that while in many cases motoneuron discharge during locomotion was greater than during cyclic passive limb movement, there were also instances where a motoneuron fired at a lower rate during locomotion. The explanation for this most likely resides in the fact that motoneuron activity in response to passive manipulation of the limb does not always mirror the muscle's pattern of involvement in normal locomotion.

In contrast to initial firing frequency, the discharge frequency during antidromic stimulation (Fs) between the two test conditions was correlated $(r = .692)$ and linear $(p \leq .001)$. Thus, while Fi during locomotion could not be predicted from cyclic passive limb movement Fi, recurrent inhibition tended to restrict motoneuron discharge to levels that were similar during cyclic passive limb movement locomotion.

The absolute decrements (ΔF) of motoneuron discharge in response to antidromic stimulation during the two conditions were not correlated. This is consistent with the observation that the ΔF values correlated with Fi

and the Fi values between cyclic passive limb movement
and locomotion were not correlated. In contrast to absolute
decrease in discharge, the relative reductions due to
recurrent inhibition were correlated, r = .622, indicating
that the efficacy of recurrent inhibition was comparable
during cyclic passive limb movement and locomotion.

To further examine the relationship between initial
firing frequency and recurrent inhibition, analysis of
covariance was performed where Fi was the covariate and
Fs the dependent variable. Removal of the regression
effect of Fi essentially allows a comparison of the
relationship between Fi and Fs during cyclic passive limb
movement and locomotion at equal values of Fi. A test of
significant difference between groups (cyclic passive limb
movement and locomotion) revealed that the sum of
deviations around a single population regression line was
not significantly greater than about individual regression
lines for each group; the effect of recurrent inhibition
was the same during cyclic passive limb movement and
locomotion. The analysis of covariance table and the
calculations performed to test for significance of difference
are presented in Appendix A1, A2 and A3. Differences

observed in recurrent inhibition during locomotion as compared to cyclic passive limb movement must be attributed to Fi, therefore, and according to the positive linear relationship between Fi and ΔF, inhibition increases as Fi increases.

(a) Effect of antidromic stimulation parameters on recurrent inhibition

A factorial analysis of variance with two factors, mode of motoneuron activation (cyclic passive limb movement or locomotion) and stimulation frequency, was used to determine the effect of antidromic stimulation frequency on the amount of recurrent inhibition expressed as a percent reduction in motoneuron discharge. The analysis of variance (c.f. Appendix B) revealed that there was a significant difference ($p \leq .001$) in the relative reduction in motoneuron activity produced by the various ventral root stimulation frequencies employed, but that the difference was independent of the mode of motoneuron activity. The means and standard deviations of the percent reductions in motoneuron discharge produced with 10, 20, 30 and 70 Hz stimulation frequencies during cyclic passive limb movement and locomotion are listed in Table 5. Dunn's multiple

comparison test was used to isolate the significant

comparisons. During both cyclic passive limb movement

and locomotion, 10 Hz stimulation of the ventral root

produced significantly lower amounts of recurrent inhibition

than any of the other stimulation frequencies used. There

were no significant differences among the effects of 20,

30 and 70 Hz stimulation on motoneuron discharge.

The effect of antidromic stimulation strength on the

amount of recurrent inhibition was analyzed by comparing

the percent reduction in motoneuron activity produced with

30uA and 300uA stimulation intensities. The means, standard

deviations and analysis of variance tables are presented

in Appendix C. 30uA stimulation produced a mean reduction

of 84% during both cyclic passive limb movement and locomotion,

whereas mean reductions of 71% and 56% were observed during

the two test conditions respectively when a stimulation

strength of 300uA was used. The differences in recurrent

inhibition produced by the two stimulation strengths were

significant during both cyclic passive limb movement

$(p \leq .05)$ and locomotion $(p \leq .001)$.

2. Recurrent facilitation of motoneurons

Two motoneurons displayed higher discharge frequencies

while being antidromically stimulated during cyclic passive limb movement, but neither of these increments was statistically significant. Recurrent facilitation was also seen during 3 individual trials of locomotion in 2 motoneurons, but the mean effect of antidromic stimulation in each case was inhibition. During cyclic stretch there were 6 trials where there was recurrent facilitation; the increases in motoneuron firing ranged from 31.7 to 657%. Recurrent facilitation did not appear to be related to Fi since it occurred at high (19.0 spikes/sec) and low (.3 spikes/sec) initial firing frequencies. In no case was recurrent facilitation seen on every trial within a series even when there was a mean increase of discharge during stimulation. An analysis of the records revealed that in 7 of the 9 trials where there was evidence of recurrent facilitation, the antidromic stimulus train was delivered within the second half of the interburst interval measured from the last burst before stimulation. It is possible, therefore, that the appearance of recurrent facilitation is dependent upon the excitatory state of Ia inhibitory interneurons, which oscillates in relation to phases of activation of antagonistic and agonist muscle groups

(Benecke, et al. 1975; Forrsberg, et al. 1977).

3.   Doublet firing during ventral root stimulation

It was a consistent observation that bursts of
motoneuron activity, whether activated by passive static
and cyclic stretch or locomotion, were usually initiated by
doublet firing. Typically the motoneuron would then
discharge with a fairly constant interspike interval for
the remainder of the burst train. This pattern of motoneuron
firing is in agreement with other reports of motoneuron
firing during MLR-evoked locomotion (Zajac and Young, 1976).
A finding of the present experiments, however, was that
the incidence of doublet firing could be increased during
antidromic stimulation.

Records of the response of motoneurons to ventral root
stimulation during passive cyclic limb movement and
locomotion are presented in Figure 10. During static
stretch (10A, B) it was common to see motoneuron spiking
either inhibited or converted to predominantly doublet
firing while being antidromically stimulated. The doublet
firing did not occur at the same interspike intervals
as the single spiking prior to stimulation, but its
sporadic occurrence prohibits the conclusion that it was

driven by the antidromic stimulation pulses.

Doublet firing was also seen during cyclic passive limb movement (10C) and locomotion (10D), although the consistency of the doublet firing was less during these conditions than during static stretch. Most frequently it appeared that single spike discharge during static stretch was converted to doublet firing during antidromic stimulation, while during cyclic passive limb movement and locomotion, the early initial doublet often remained while the single spikes in the remainder of the train were depressed.

4.   Effect of ventral root stimulation on the locomotor rhythm

In order to test the possibility that ventral root stimulation can perturb the locomotor rhythm, as would be expected if the postulate that Renshaw cells form a part of the locomotion generator is correct (Miller and Scott, 1977), mean normal (prior to antidromic stimulation) burst durations (BD_n) and interburst intervals (IBI_n) were used to predict the timing of burst occurrence in relation to previous burst activity. With this procedure (for details see Methods) it was possible to determine whether the BD or IBI was altered by ventral root stimulation, and if so,

whether the alteration was transient or represented a persistent resetting of the locomotor rhythm.

Only trials which showed highly consistent burst durations and interburst intervals were selected for analysis. In 9 of 39 trials in 7 motoneurons the difference between the actual and predicted occurrence of the first burst of motoneuron activity following cessation of the stimulus exceeded the associated standard error. The alteration consisted of occurrence of the burst either later than predicted (4 trials) or earlier than predicted (5 trials). In only 2 of the trials (both in the same motoneuron) did the actual interval preceding the first burst after cessation of stimulation fall outside the limits of a 95% confidence interval, and these are the only trials for which the difference can be considered significant. Whereas recurrent inhibition observed in any given motoneuron occurred consistently across trials, resetting of the locomotor rhythm as judged by the above criterion was repeatable in only 1 of the 7 motoneurons examined. In 2 additional instances (out of the total 81 trials) antidromic stimulation resulted in cessation of co-ordinated locomotion.

These results suggest that L7 ventral root stimulation does not consistently alter locomotion, in spite of the fact that it produces significant inhibition of individual motoneurons. While not directly related to the capability of ventral root stimulation to reset locomotor rhythm, it should be noted that occassionally antidromic stimulation caused a complete disruption of locomotion rhythm to the extent that co-ordinated locomotion ceased. In one instance, a motoneuron with an Fi of 39 spikes/sec was inhibited by 96.7% during ventral root stimulation. When the antidromic stimulation ceased, the motoneuron began to fire tonically but did not resume its normal locomotor rhythm until 1.8 sec after the removal of the stimulus train.

B.   Comparison of motoneuron firing characteristics during
      controlled treadmill locomotion and fictive locomotion
         The data that will be presented in the remaining sections of the Results were obtained in fictively walking cats. Since a substantial part of this thesis involves attempts to understand the function of various spinal neurons in the generation of the locomotion rhythm by observing their

behavior during fictive locomotion, it was deemed

worthwhile to determine whether the locomotor activity

observed in paralyzed cats was essentially the same as

that seen in cats walking on a treadmill with normal

segmental afferentation. To determine the similarity

in the MLR-evoked locomotion between the two preparations,

the interspike intervals (ISIs) separating the motoneuron

action potentials occurring within a burst were measured

over successive step cycles and averaged. The mean ISIs

for 5 motoneurons recorded from a ventral root filament during

controlled treadmill locomotion and 11 motoneurons recorded

intracellularly or from ventral root filaments during

fictive locomotion are presented in Table 6. Only

motoneurons which produced at least 7 action potentials

per burst were included in this analysis since the intent

was also to examine the pattern of motoneuron firing within

the burst; although many of the 16 motoneurons produced

more than 7 action potentials per burst, only the first 6

ISIs were included in the analysis. A 2-way factorial

analysis was performed on the first 6 ISIs during

controlled treadmill locomotion and fictive locomotion

(c.f. Appendix E), and a Dunn's test of multiple

comparisons was used to isolate the significantly different means.

The mean values for the first 6 ISIs observed during both types of locomotion are plotted in Figure 11. During both controlled treadmill locomotion and fictive locomotion, the first ISI was significantly ($p \leq .01$ and $p \leq .05$, respectively) shorter than the subsequent ISIs, but there was no significant difference in the duration of ISIs 2 - 6. Although the first ISI observed during controlled treadmill locomotion (7.1 msec) was significantly shorter than the 29.2 msec ISI_1 seen during fictive locomotion, there was no overall difference between motoneuron discharge evoked in the two locomotion preparations.

Thus, the data indicates that a similar pattern of motoneuron firing was produced by MLR stimulation in cats with and without phasic segmental afferent information; in general, the motoneuron burst was characterized by an initial short ISI (doublet) with the remaining action potentials separated by a fairly constant ISI averaging 44 msec. There was a tendency for the shorter initial ISIs to be associated with bursts containing a larger number of spikes. A simple linear regression and

correlation analysis confirmed that there was an inverse relationship between ISI_1 and the number of action potentials in the burst, but neither the correlation coefficient ($r = -.414$; $t(15) = 1.71$) or the regression coefficient ($b = -1.84$; $F(1.14) = 2.91$) were statistically significant.

C.   Effect of antidromic ventral root stimulation on

IaIN activity during fictive locomotion

IaINs were recorded in the L5 and L6 segments of the spinal cord with glass micropipettes filled with 3M NaCl and were identified by the criteria set forth by Hultborn, et al. (1971b) (c.f. Methods, 6a). Figure 12 illustrates the standard identification procedures used in these experiments. The interneuron was monosynaptically excited by stimulation of the Q nerve at a strength that was 1.3 times threshold which is in the range for exclusive stimulation of Ia afferents (Eccles, et al. 1957a, b). The shape of the cord dorsum potential, shown in the bottom trace in A-D, also indicates that the stimulus was confined to Ia afferents (Eccles, et al. 1956). Figure 12B shows that the interneuron was able to follow

stimulation of the Q nerve at a rate of 250 Hz which differentiates the cell from motoneurones. A third prerequisite for identification as an IaIN is that the interneuron can be inhibited by antidromic ventral root stimulation. Two methods were used to determine whether the interneuron being tested received recurrent inhibition, and these are shown in 12C, D and E. A condition - test paradigm was used with those interneurons that were not spontaneously active. Figure 12C shows the control response of the interneuron to repeated stimulation of the Q nerve (superimposed traces), and 12D shows that a stimulus pulse delivered to the L6 ventral root 10 msec prior to the stimulation of the Q nerve effectively blocked the activation of the interneuron. Interneurons that were spontaneously active at rest, as this interneuron was, were also tested by trains of ventral root stimulation as shown in 12E (and again in Figure 13A).

The effects of antidromic ventral root stimulation on the spontaneous activity (not evoked) and discharge during fictive locomotion of 6 Q-coupled IaINs are summarized in Table 7. In each cell, the recurrent inhibition of spontaneous activity was tested in 5 trials. In accordance

with accepted criteria for identification of IaINs

(Hultborn, et al. 1971b), ventral root stimulation was

quite effective in inhibiting the spontaneous activity of

IaINs. In the 6 IaINs listed in Table 7, the amount of

recurrent inhibition ranged from 66.6% to 100% and had a

mean of 92.5%. IaINs tended to discharge at higher

frequencies during fictive locomotion as there was a mean

Fi of 60.3 spikes/sec during fictive locomotion as compared

to a mean of 30.2 spikes/sec for spontaneous activity. It

was not uncommon, however, to find some IaINs, such as

IaINs 2 and 4, which had lower frequencies during fictive

locomotion than at rest. This was due to the fact that

some IaINs discharge during locomotion in discrete bursts

with no activity occurring during the portion of the step

cycle in which antagonist motoneurons are active. The

instantaneous frequencies were always higher during fictive

locomotion. The recurrent inhibition of IaINs during fictive

locomotion ranged from 26.7% to 60.0% and had a mean of

34.5%. The difference between Fi and Fs was significant

at an alpha level of at least .01 in each cell as tested

by paired one tailed t-tests.

The recurrent inhibition of spontaneous and MLR-

evoked activity of two Q-IaINs recorded in different experiments is illustrated in Figure 13. The upper trace in A1, A2, B1 and B2 is the extracellular recording from the microelectrode and the lower trace is the recording from an L6 ventral root filament. The IaIN shown in A1 and A2 is cell #1 from Table 7 and the one illustrated in B1 and B2 is IaIN #5. A 200 msec train of .1 msec pulses applied to the L6 ventral root at a frequency of 40 Hz completely inhibited the spontaneous activity of IaIN #1 (A1). The period of ventral root stimulation is indicated in A1 by the stimulus artifact and in A2 by the horizontal bars above the microelectrode trace. The effectiveness of ventral root stimulation in halting the spontaneous activity of IaIN #1 is further demonstrated by the fact that a spurrious ventral root stimulus pulse that was emitted 200 msec after the end of each train of pulses produced about a 60 msec pause in the cell's spontaneous discharge. During fictive locomotion (A2) the mean frequency of discharge for IaIN #1 was 125.1 Hz which was reduced to 87.9 Hz during the 200 msec train of ventral root stimulation. Over the 31 recurrent inhibition trials conducted during fictive locomotion, there was a mean reduction in IaIN #1 activity

of 29.7%. As shown in Figure 13, B1 and B2, IaIN #5 discharged spontaneously at a rate of 5.1 Hz and during fictive locomotion at a rate of 12.7 Hz. Only two action potentials in IaIN #5 appeared during a 10 sec train of pulses delivered to the L6 ventral root at a rate of 30 Hz before the MLR was stimulated (B1). Spontaneous discharge in IaIN #5 was inhibited by a mean of 96.1% by ventral root stimulation. During fictive locomotion, (B2), recurrent inhibition was less effective but still resulted in a mean reduction in discharge of 55.1% which was significant at the .001 level. It can also be seen in B2 that there was a substantial inhibition of the motoneuron activity in the L6 ventral root filament during the 3 sec period of antidromic stimulation of the rest of the L6 ventral root. In this example, the IaIN exhibited much more consistent rhythmic activity than the motoneurons in the ventral root filament.

Multiple correlation and regression analysis revealed that a significant linear relationship existed between F_i and F_s ($r = .993$) and F_i and $\triangle F$ ($r = .967$) but not between F_i and percent reduction in IaIN discharge during fictive locomotion, as shown in Table 8. These results are similar

to the relationships found to exist between motoneuron discharge frequency and recurrent inhibition (Table 4). The regression line describing the relationship between F_i and F_s is shown as the solid line in Figure 14 and the relationship between F_i and $\triangle F$ as a dashed line. The correlation and regression coefficients for each line are also presented in Figure 14, and the standard error of the estimate for each line is drawn in at the means. The regression line of IaIN F_i vs F_s had a steeper slope ($b = .705$) than the line of motoneuron F_i vs F_s ($b = .251$, Figure 9A) indicating that there was a smaller reduction in IaIN firing during ventral root stimulation than was observed in motoneurons. That this was true can also be seen by comparing the regression lines describing the relationship between F_i and $\triangle F$ for IaINs (dashed line, Figure 14) and motoneurons (Figure 9B); the slope of the regression line for IaINs ($b = .293$) was substantially smaller than that for motoneurons ($b = .749$). Although there was a fairly high correlation between F_i and relative amount of recurrent inhibition of IaINs, ($r = .767$), it was not significant at the .05 level or higher.

D.   Modulation of Ia EPSPs during fictive locomotion

Schomburg and Behrends (1978b) reported that in high spinal cats which had been given i.v. injections of Nialamide and L-DOPA Ia EPSPs evoked in flexor and extensor motoneurons by low threshold stimulation of homonymous muscle nerves were found to be largest during each motoneuron's "active phase" of the step cycle. The EPSPs that were evoked during the depolarized or active phase of the step cycle were averaged and compared with the average obtained during the reciprocal or hyperpolarized phase of the step cycle.

These results were confirmed in the present experiments in which Ia EPSPs were evoked in Q motoneurons during fictive locomotion by low threshold stimulation of a branch of the femoral nerve which innervates the vastus medialis. Due to the unavailability of averaging programs, the modulation of the Ia EPSPs during fictive locomotion was analyzed by examining the relationship between the amplitude of the evoked EPSP and the motoneuron membrane potential measured from the DC record at the onset of the EPSP. The results of the linear regression and correlation analysis performed on 4 Q motoneurons which displayed

rhythmic membrane potential oscillations during MLR stimulation are presented in Table 9. In 3 of the 4 motoneurons, there was a significant ($p \leq .01$ or $p \leq .001$) positive correlation between EPSP amplitude and membrane potential. In all 3 of these motoneurons, the F test of the significance of the regression coefficient revealed that the relationship between these two variables was linear ($p \leq .01$). It can be seen, however, that there was a substantial amount of variability in the values of the correlation and regression coefficients among the 3 motoneurons.

Examples of the modulation of the Ia EPSPs in 2 motoneurons, Ia-26 #5 and Ia-8 #3, are illustrated in Figure 15A and B, respectively. Figure 16 shows the regression lines describing the relationship between EPSP amplitude and motoneuron membrane potential for motoneurons Ia-26 #5 (broken line) and Ia-8 #3 (solid line). Again, the disparity in the slopes of the 2 lines is evident; the change in the amplitude of the EPSP was much greater for a given change in membrane potential of motoneuron Ia-8 #3 than the other motoneuron despite the fact that they both had similar mean membrane potentials (Table 9).

Motoneuron Ia-8 #3 showed the greatest modulation in amplitude as indicated by its larger standard deviation ($\pm$ 2.1 mv). Likewise, it should be noted that the 3 motoneurons in which there was a significant linear relationship between EPSP amplitude and membrane potential also showed greater shifts in their DC potentials, as indicated by their standard deviations, than did motoneuron I1-27-6 #3.

It was also of interest to compare the range of EPSP amplitudes observed during fictive locomotion to the control values, i.e., the amplitude of the EPSP obtained during resting conditions. If amplitudes smaller than the control value were obtained during locomotion, this would suggest the presence of some inhibitory processes directed at either the pre- or postsynaptic level.

Table 10 presents the control EPSP amplitude and the largest and smallest values obtained during fictive locomotion for the 4 motoneurons analyzed. In all cases, except for motoneuron Ia-8 #3, the amplitude of the control EPSP was of a value that was intermediate between the largest and smallest values seen during locomotion. The amplitude of the EPSPs varied during locomotion within a range of

2.5 to 3.0 mv. In motoneuron Ia-8 #3, the amplitude of
the EPSP was consistently larger than the control value
during locomotion, and it displayed the largest range
of variation (6.7 mv) of the 4 motoneurons. It should
be noted that this motoneuron was also found to have the
tightest coupling between EPSP amplitude and membrane
potential variation (r = .884) and, interestingly, the
smallest mean membrane potential (E_M = 63.7 mv) during
locomotion.

E.   Modulation of Ia IPSPs during fictive locomotion

Ia IPSPs were evoked at various points throughout the
step cycle to determine whether there was any evidence of
phasic recurrent inhibition of IaINs during locomotion.
These tests were conducted on just 2 Q motoneurons both
of which received Ia IPSPs from the nerve to TA. The 2
motoneurons were recorded in different experiments. The
experimental procedure used is illustrated in Figure 17.
The upper trace in panels A, B and C is the AC coupled
record from the microelectrode, and the middle trace is
the cord dorsum potential recorded at the L5 dorsal root
entry zone. The cell was identified as being a motoneuron

(Ia-2 #6) by the single action potential evoked in response to stimulation of the proximal portion of the cut L5 ventral root (panel B). Stimulation of the nerve to Q via the nerve cuff evoked an EPSP in this motoneuron (panel A) which, according to the cord dorsum potential, was evoked at group I stimulation strength and at a monosynaptic latency (0.5 msec); the motoneuron was thus identified as a Q motoneuron. Panel C shows the 5 mv IPSP that was evoked in the motoneuron by group I strength stimulation of the nerve to TA. The IPSP can be classified as a Ia IPSP since it was evoked at group I strength and at a latency indicating a disynaptic pathway (2 msec).

Panel D shows the DC coupled recording of the motoneuron's activity during fictive locomotion. The A gates shown in the trace just above the DC record were generated from the oscilloscope simultaneously with the stimulus pulse to the TA nerve and provide a marker of the evoked IPSP. The IPSPs numbered 1 - 4 are shown at higher amplification and an expanded time base in the bottom trace of the figure. It is obvious that despite the somewhat irregular firing of the motoneuron during fictive locomotion, the IPSPs that were evoked when the motoneuron membrane

potential was more depolarized (IPSPs 1 and 2) were

substantially larger than those evoked during the

hyperpolarized phase of the step cycle. In both motoneurons

tested, linear regression analysis revealed that there was a

significant inverse relationship between TA Ia IPSP

amplitude and Q motoneuron membrane potential. The

correlation and regression coefficients and regression

lines for motoneurons Ia-2 #6 (solid line) and I1-27-6-78

#10 (broken line) are presented in Figure 18. The lower

X axis is associated with the solid regression line and

the upper X axis with the broken regression line. All

regression and correlation coefficients were significant

at the .001 level. The slopes of the two lines are quite

different (b = -.392 and -.152) indicating that the change

in membrane potential had a smaller effect on the amplitude

of the TA IPSP in motoneuron I1-27-6-78 #10 than in the

other motoneuron. There was also a substantial difference

in the resting membrane potential (E_R) of the two cells,

I1-27-6-78 #10 being the more depolarized of the two

(E_R = -50 mv). This may indicate a poorer impalement or

that the cell was damaged by the penetration of the

microelectrode and may explain the smaller modulation in the

size of the IPSP. This motoneuron exhibited a greater amplitude in its membrane potential oscillation during fictive locomotion, however, than did motoneuron Ia-2 #6, indicating that it had a healthy response to MLR stimulation.

In the present study, the amplitude of TA Ia IPSPs was greatest during Q motoneuron depolarization which occurs out of phase with TA motoneuron activity and thus at a time when the recurrent inhibition of TA IaINs projecting to Q motoneurons by TA Renshaw cells should be minimal or nonexistent. The variations in TA Ia IPSP amplitude that occur during fictive locomotion could be due to changes in motoneuron membrane potential and to the phasic recurrent inhibition of IaINs. The data presented here do not allow decisions to be made regarding the relative contributions of each of these influences.

As was seen to be true in most cases with monosynaptic EPSP amplitude during fictive locomotion, the control Ia IPSP amplitudes were intermediate between the largest and smallest IPSPs observed during locomotion. Motoneuron Ia-2 #6 had a mean control TA IPSP of 4.3 mv but the IPSP

values ranged from 2.8 to 11.3 mv during locomotion. The
mean TA IPSP evoked in motoneuron Il-27-6-78 #10 at rest
was 5.4 mv but ranged from 2.4 to 6.8 mv during locomotion.
The smallest IPSPs were evoked during the hyperpolarized
phase of the Q motoneuron step cycle at a time when the
TA IaIN should be maximally active. The fact that some
of these IPSPs were smaller than those evoked at rest may
be partly explained by the greater membrane potential during
this phase, but, as is shown in Figure 18, only motoneuron
Il-27-6-78 #10 hyperpolarized below resting membrane
potential levels during the hyperpolarized phase of the step
cycle. It is also possible that the inhibitory effect of
the IaIN on the motoneuron was less prominent during the
hyperpolarized phase of the step cycle than at rest because
of shunting due to direct inhibition of the motoneuron
during this phase.

F. Membrane potential oscillations observed in flexor and
    extensor motoneurons during fictive locomotion
    When plotting the membrane potential oscillations
relative to the resting membrane potential (E_R) that
occurred during fictive locomotion in flexor and extensor

motoneurons, it appeared that there were basic differences in the behavior of the 2 populations of motoneurons that might be related to differences in their synaptic input from the spinal locomotion generator. Both flexor and extensor motoneurons displayed membrane potential oscillations during fictive locomotion such that they were depolarized during their active phase and hyperpolarized during their inactive phase of the step cycle. The main difference was that the membrane potential of the extensor motoneurons was often tonically shifted in a depolarized direction during fictive locomotion; the motoneuron membrane potential during the hyperpolarized phase of the step cycle was still more depolarized than the E_R. Conversely, flexor motoneurons consistently exhibited membrane potential oscillations above and below the E_R. The contrast between flexor and extensor motoneurons is evident when comparing the membrane potential oscillations that occurred during fictive locomotion, plotted in a normalized step cycle, in 2 Q motoneurons shown in the top 2 panels of Figure 19 with those of the 3 flexor motoneurons illustrated in Figure 20.

Systematic analysis of all motoneurons which could be identified as being a flexor or an extensor, which had an E_R of -50 mv or greater and which were rhythmically active during fictive locomotion confirmed these observations but failed to reveal causative variables. Table 11 contains information on the membrane potential changes that were observed during fictive locomotion in 12 extensor and 3 flexor motoneurons. All of the flexor motoneurons but only 3 of the 12 extensor motoneurons hyperpolarized below the E_R during locomotion. Figure 19 shows the membrane potential plots in normalized step cycles of 4 Q motoneurons. The plots in the top 2 panels (motoneurons 9 and 6, respectively, from Table 11) are characteristic of the membrane potential oscillations observed in the majority of the extensor motoneurons. The third and fourth panels illustrate the DC shifts seen in 2 motoneurons (motoneurons 2 and 3, respectively) which did hyperpolarize below the resting level.

It was noted that the extensor motoneurons which did hyperpolarize below E_R tended to have a higher E_R than the other extensor motoneurons as well as the flexor motoneurons. Motoneurons 2 and 3 had an E_R of -130 and -80 mv,

respectively, and it can be seen from Table 11 that 2
of the 3 extensor motoneurons that behaved in this manner
had an E_R greater than the motoneurons which were tonically
depolarized during locomotion. To determine if the
difference in membrane potential behavior during locomotion
was primarily related to the general health of the cell
or the quality of the impalement, a multiple correlation
analysis was performed on the following variables which
are listed in Table 11: motoneuron E_R; action potential
height; amplitude of the membrane potential oscillations
during locomotion; whether the motoneuron hyperpolarized
below E_R (coded as either a 0 (no) or a 1 (yes)) and the
difference between the level of peak hyperpolarization
(PHP) and the E_R (E_R-PHP). The analysis was performed
on the 9 motoneurons for which all of the data was
available. Whether or not the motoneuron hyperpolarized
below E_R was inversely related to E_R ($r = -.49$, $p \leq .05$)
and to E_R - PHP ($r = -.67$, $p \leq .01$). There was also a
significant inverse relationship between E_R - PHP and the
amplitude of the E_M oscillation ($r = -.83$, $p \leq .05$). Thus,
motoneurons which did hyperpolarize below E_R tended to
have a larger E_R and a greater fluctuation in E_M during

fictive locomotion.

It is still difficult to determine whether the differences in the membrane potential oscillations observed are a function of the different populations of motoneurons and represent different modes of synaptic activation during locomotion or simply reflect the quality of the recording conditions. All of the extensor motoneurons which hyperpolarized below E_R were recorded in different experiments; motoneurons 4 and 16 were recorded in the same preparation. Only one motoneuron with an E_R above -60 mv remained depolarized above E_R throughout the step cycle. However, motoneurons 5 and 7, which displayed membrane potential oscillation amplitudes of 10.3 and 15.0 mv respectively, which indicates a healthy response to MLR stimulation, were tonically depolarized during locomotion.

G. Phase relationships among Renshaw cell, IaIN and
    motoneuron activity during fictive locomotion

The phase relationships among Renshaw cell, IaIN and motoneuron activity were determined by comparing the frequency (frequency histograms) and the onset and offset

times of neuron activity that occurred during individual step cycles which had been normalized to allow comparisons among cells recorded at different times throughout an experiment as well as in different experiments.

Figures 21 - 26 illustrate the normalized data from 6 experiments in which successful recordings were made from more than one cell type and in which the fictive locomotion activity in the ventral root filament was of sufficient quality to permit analysis. The bursts of motoneuron activity had to be discrete enough to allow finite measurements of the beginning and end of a step cycle for the phasing analysis to be performed. Figure 27 shows the raw data that is presented in Figure 21 to exemplify the transformation of the data that occurs with normalization. Figures 21 - 26 will be referred to repeatedly in the following sections in which the details of the phase relationships between activity in Renshaw cells and motoneurons, IaINs and motoneurons and Renshaw cells and IaINs will be presented.

1. Phase relationships between periods of Renshaw cell and motoneuron activity

Table 12 compares the burst durations and the onset
and offset times of E-RC and F-RC activity with extensor
and flexor motoneuron activity respectively for 10 Renshaw
cells in which it was possible to identify the motoneuron
activity in the ventral root filament as being either
flexor or extensor so that the phasing of Renshaw cell
activity during fictive locomotion could be determined.
Six of the Renshaw cells could be identified in terms of
their orthodromic input as they responded to stimulation
of various peripheral nerves. The other 4 cells either
could not be excited by stimulation of the peripheral
nerves via the nerve cuffs or were recorded in experiments
in which the dorsal roots were cut. In these 4 cells,
it was still possible to identify them as F-RCs or
E-RCs by relating their periods of activity to that in
the identified filament. Identification in this manner
was based on data that Renhsaw cells are active in phase
with the motoneurons from which they receive their axon
collateral input (McCrea and Jordan, 1976a). The number
of the figure in which the cell is illustrated, if
it is, is in parenthesis next to the cell number. The
identification of the cells included in Table 12 can be

found in Appendix H.

Inspection of the data presented in Table 12 enables one to determine for each Renshaw cell and each group of Renshaw cells the temporal relation between Renshaw cell activity and the activity of motoneurons with which the Renshaw cells are coupled. The values presented in columns 4 - 6 (cycle duration; duration of the extension or flexion phase of the step cycle; and the onset (X) and offset (Y) times of the extension and flexion phases of the step cycle) are the mean values obtained from the n trials (step cycles) in which the Renshaw cell data was obtained. The population values for the ventral root filament that are drawn in normalized form in the respective figures represent the mean from all the step cycles measured for all of the cells presented in each figure. For example, RC #1 in Table 12 was measured over 8 step cycles, but the values listed in columns 4 - 6 for this cell are the means of the 60 total step cycles measured for the 6 cells analyzed in that experiment. Thus, there will be some discrepancy between the values listed in column 6 and the X and Y values for the extensor and flexor filament records, respectively, that are shown in the normalized diagrams.

2. E-RCs and extensor motoneurons

RC #1 became active almost coincidentally with the extensor filament; the Q-RC started its activity at 0.62 and the filament commenced activity at 0.63 of the step cycle. The Renshaw cell was active longer than both the extensor filament and the intracellularly recorded Q motoneuron that was also recorded in this experiment (Figure 21). The 1.03 y value for the Renshaw cell shows that its activity extended through the extension phase of the step cycle into the subsequent flexor phase. RC #4 (y = 1.07) also was active into the next flexor phase although, unlike RC #1, its burst of activity was shorter than that of the extensor motoneurons in the filament; while the extension phase started at 0.60, the Q-RC did not become active until 0.93 of the step cycle. RC #3 was active for a much longer period than the extension phase of the step cycle as it became active midway through the flexion phase (0.33) but continued to discharge almost to the end of the extension phase (y = 0.99). Thus, as can be seen in Figure 23, the offset of RC #3's activity almost coincided with the termination of activity in the extensor filament and occurred after the cessation of activity in

2 Q motoneurons. RC #2 had the earliest offset time
(y = 0.70) of the extensor coupled E-RCs analyzed.
This cell became active before the Q motoneuron that was
also recorded in that experiment and stopped firing just
after the middle of the Q motoneuron burst. LG-RC #7
shown in Figure 25 was active for 264 msec of the 472
msec long extension phase of the step cycle being active
for about the first two thirds of the extension phase. This
Renshaw cell stopped discharging fairly early in the step
cycle (y = 0.83) in relation to other E-RCs. The other 2
LG-RCs included in Table 12, RCs #5 and #6, both became
active shortly after the onset of the extension phase and
stopped discharging near the end of the extension phase
(y = 0.93).

The means of the data presented in Table 12 are
presented in schematic form in Figure 28 which illustrates
the relationship between the onset and offset of E-RC and
F-RC activity and the activity periods of extensor and
flexor motoneurons, respectively. In summary, the extension
phase of the step cycle occupied a mean 368 msec of the
717 msec step cycle and had a mean onset time of 0.50.
On the average, the duration of the E-RC burst of activity

(249 msec) was shorter than the extension phase. The mean
onset of E-RC activity (0.55) occurred just slightly after
the onset of the extension phase of the step cycle. The
offset time of E-RC activity is of particular interest
insofar as it relates the cessation of extensor motoneuron
activity and E-RC activity. If RCs contribute to the
turning off of motoneurons, as has been postulated, then
it would be necessary for them to be active at the time
that motoneuron activity ceases. Three of the 5 E-RCs
(RCs #1, 3 and 4) which were recorded in experiments in
which extensor motoneuron activity was also recorded, had
activity periods that extended just beyond the offset of
activity in extensor motoneurons. The mean offset time
for E-RC activity was 0.93. There was more variability
associated with the onset time of E-RC activity than with
the offset time as shown by the respective standard
deviations in Figure 28.

(b) F-RCs and flexor motoneurons

The relationship between F-RC and flexor motoneuron
activity was similar to that observed between E-RCs and
extensor motoneurons. RC #8 became active during the end
of the previous extension phase (x = -0.12) and continued

to discharge well into the subsequent extension phase
($y = 0.61$), overlapping the onset of activity in the Q
motoneuron and the extensor filament. The onset and
offset of activity in RC #9 practically coincided with the
beginning and end of activity in the flexor filament; it
started to fire at 0.04 of the step cycle and ceased firing
just after cessation of activity in the filament. RC #10
commenced activity ($x = 0.12$) about 75 msec after the
initiation of activity in the flexor filament and in an
St motoneuron (Figure 25). It stopped firing just after
the flexor motoneuron activity in the filament ceased (see
Table 12) but long before the St motoneuron stopped
discharging.

As can be seen in Figure 28, the F-RCs were, on the
average, active for a longer period than flexor
motoneurons (the exception being the St motoneuron in
Figure 24). The mean duration of the flexion phase of
the step cycle was 290 msec and the mean duration of F-RC
activity was 306 msec. F-RCs tended to become active very
shortly after the onset of activity in flexor motoneurons
($x = 0.04$) which is similar to the behavior seen in E-RCs
in relation to extensor motoneurons. Again, the important

parameter in terms of attempting to interpret the function of Renshaw cells during locomotion, is the relationship between the cessation of activity in flexor motoneurons and F-RCs.   All 3 F-RCs stopped firing just after the cessation of flexor motoneuron activity in the ventral root filament.   The offset times for the flexor filament and the F-RC activity were 0.44 and 0.47, respectively.

2.   Relationship between Renshaw cell discharge pattern and motoneuron activity

(a)   Comparison of frequency histograms

In 3 experiments it was possible to compare frequency of Renshaw cell activity that occurred throughout the fictive step cycle with that observed in single motoneurons that were recorded intracellularly.   In most cases, the ventral root filament records contained more than one motor axon which made it difficult to construct a frequency histogram describing its activity during locomotion.   Figure 25 shows the frequency histograms of a Pb-RC (RC #10) an St motoneuron displayed their peak discharge frequencies from 0.1 to 0.2 of the step cycle.   The frequency of firing in the Pb-RC remained elevated during the subsequent bin while the frequency of firing in the St motoneuron fell

to 50% of the rate it had displayed in the previous bin.
The Pb-RC burst ended abruptly as the St motoneuron
continued to fire for some time further at progressively
declining rates. The important comparison between these
2 cells is, however, the fact that maximal activity in the
Pb-RC coincided with the reduction of activity in the St
motoneuron. One would expect to see a close correlation
between the activity of Pb-RCs and St motoneurons since
it has been shown that RIPSPs are produced in St motoneurons
primarily by stimulation of the nerves to St and Pb muscles
(Eccles, et al. 1961a) and that Renshaw cells which receive
input from St motoneurons are also excited by axon
collaterals form Pb motoneurons (Eccles, et al. 1961b).
RC #9 was, in fact, excited by stimulation of the St nerve,
although it responded maximally to stimulation of the nerve
to Pb.

The frequency histograms of an E-RC (RC #2) and a Q
motoneuron are presented in Figure 22. The Renshaw cell
became active after the onset of activity in the Q
motoneuron and displayed its maximal firing rate at the
beginning of its burst. Again the maximal firing in the
Renshaw cell coincided with the peak discharge rate seen

in the motoneuron (period of 0.6 to 0.7). The pattern

of discharge in the 2 cells then paralleled one another

as there was a steady decline in discharge frequency

throughout the remainder of their activity periods.

Figure 23 presents another comparison between the

activity in an E-RC (RC #3) and a Q motoneuron. The

motoneuron starts to fire early in the step cycle and

continues to discharge at a fairly steady rate for the

remainder of the step cycle. The E-RC became active almost

simultaneously with the Q motoneuron and discharged at a

moderate rate until the end of the extension phase at

which time there was a dramatic increase in the rate of

RC firing; the period of maximal activity in the Renshaw

cell just preceded the cessation of activity in the Q

motoneuron. The frequency histograms shown in Figure 28

represent the mean discharge frequencies of E-RCs and

F-RCs during fictive locomotion. E-RCs displayed elevated

discharge rates from 0.5 to 1.1 of the step cycle but were

maximally active at the end of the extension phase (0.9 -

1.0). F-RC activity was maximal during middle to late

flexion, i.e., from 0.1 - 0.4 while moderate activity

occurred from 0 - 0.5 of the step cycle. The mean offset

time of flexor motoneuron activity was 0.44.

(b) Relationship between Renshaw cell discharge and

membrane potential in associated motoneurons

Some information about the effects of Renshaw cells

on motoneurons during fictive locomotion can also be derived

from comparisons of the pattern of Renshaw cell discharge

and the relative depolarization and hyperpolarization of

the membrane potential in associated motoneurons. The

frequency histograms of activity in E-RC #3 can be compared

with the membrane potential oscillations observed in 2 Q

motoneurons during locomotion in Figure 23. It can be seen

that the maximal rate of discharge in the Renshaw cell

coincides with the onset of hyperpolarization in both Q

motoneurons. The same is true with E-RC #2 and the Q

motoneuron shown in Figure 22.

(c) Relationship between the period of maximal Renshaw

cell activity and motoneuron activity

E-RC #3, Q-RC #1 and the Pb-RC #10 all exhibited their

highest frequency of firing at the end of their burst of

activity which coincided with the reduction of activity in

motoneurons to which the Renshaw cells were functionally

related. The discharge frequencies and patterns of Q-RC #1

and E-RC #3 are particularly similar to one another. The
flexor F-RCs #8 and #9 were maximally active during the
middle of their burst and the 2 E-RCs, RC #2 and Q-RC
#4, discharged at maximal rates at the beginning of their
active periods. The consistent observation was that the
periods of most vigorous Renshaw cell firing were temporally
related to the onset of the reduced activity in motoneurons
to which they were functionally related. The Q-RC #1
(Figure 21) is maximally active at the very end of the
extension phase of the step cycle. E-RC #2 (Figure 22)
is maximally active during the period in which the Q
motoneuron is becoming hyperpolarized. F-RC #9 displayed
its highest frequency of discharge from 0.1 - 0.3 of the
step cycle and the flexor motoneuron activity in the ventral
root filament ceased at 0.31. The onset of hyperpolarization
in 2 Q motoneurons occurred at the very end of the extension
phase of the step cycle coincidental with the termination
of extensor motoneuron activity in the filament and the
period of maximal activity in E-RC #3 (Figure 23). Q-RC
#4 (Figure 24) was active for only a brief portion of the
step cycle; it fired most vigorously at the onset of its
active period and this period of maximal activity

coincided with the end of the extension phase when activity in extensor motoneurons would be declining. Maximal activity in Pb-RC #10 (Figure 25) coincided with the onset of reduced rates of discharge in an St motoneuron and just preceded cessation of activity of flexor motoneurons in the ventral root filament.

3.   Phasing of rhythmic activity in Renshaw cells associated with antagonistic motoneuron pools

In 3 experiments, the activity of Renshaw cells that were coupled to antagonistic motoneuron pools was recorded during fictive locomotion. Figure 21 shows the frequency histograms of Q-RC #1 and F-RC #8 activity during fictive locomotion. It can be seen that their discharge profiles are almost exact mirror images of one another. The bar graph at the bottom of the figure confirms the fact that these 2 Renshaw cells were active completely out of phase with one another. The same was found to be true with F-RC #9 and E-RC #2 as can be seen in Figure 22. The F-RC activity had dropped to negligible levels when the E-RC became active. Again the bar graph shows that there was no overlap in the activity of the F-RC and the E-RC. The same pattern was observed with Pb-RC #10 and the LG-RC #7 in Figure 25.

4.   Phase relationship between IaIN and motoneuron activity

Phasing analysis was performed on a total of 6 IaINs all of which were Q coupled. Despite the fact that all of these cells met the established criteria for identification as IaINs (see Methods) and were coupled to the same motoneuron pool, no consistent pattern of Q-IaIN discharge during fictive locomotion was evident.

Figure 26 presents the frequency histograms describing the activity of 3 Q-IaINs along with the active period of Q motoneurons in the ventral root filament and the membrane potential oscillation in a Sart motoneuron (an antagonist of Q motoneurons). All 3 Q-IaINs were maximally active during the extension phase of the step cycle as was reported by Feldman and Orlovsky (1975). Q-IaINs C and D discharged in discrete bursts; their firing rate fell to zero at some point during the flexion phase of the step cycle. The bar graph at the bottom of the figure illustrates the onset and offset points for the activity periods of Q-IaINs C and D. The activity of Q-IaIN C almost perfectly matched that of the Q motoneurons in the ventral root filament. Q-IaIN D, however, was active for a much shorter time, becoming active after and quiescent before the Q motoneurons in the filament. All 3 Q-IaINs exhibited

their most vigorous firing in either the early or middle portion of the extension phase. It would seem to be reasonable to expect that maximal Q-IaIN activity would occur during these periods of the step cycle, since it would not be beneficial for the flexor motoneurons to be maximally inhibited at the end of the extension phase when the transition from extension to flexion would be taking place. It can be seen in Figure 26 that the elevation in Q-IaIN B's firing rate coincided with the onset of hyperpolarization in a Sart motoneuron, and all 3 Q-IaINs discharged maximally throughout the Sart motoneuron's hyperpolarized phase. The Sart motoneuron's membrane potential began to shift in a depolarized direction at 0.95 of the step cycle; this depolarization occurs simultaneously with a reduction in the discharge of Q-IaINs C and D.

The frequency histograms describing the activity of 2 Q-IaINs during fictive locomotion are presented in Figure 21. Q-IaIN A was active throughout the step cycle; it was not possible, therefore, to determine the x and y values for this cell. Q-IaIN A displayed elevated discharge frequencies from late flexion (0.3) to the middle of the

extension phase (0.7). Q-IaIN B became active in the latter part of the flexion phase (x = 0.35) and continued to discharge until the end of the extension phase (y = 1.0). Unlike the other Q-IaINs thus far mentioned, Q-IaIN B was maximally active at the end of the extension phase. As discussed previously, this would seem to be inefficient since at this part of the step cycle the flexor motoneurons would be getting readied, i.e., becoming depolarized and approaching threshold, for the initiation of activity. It must be remembered, however, that the extension phase of the step cycle is being defined in this case by the motoneuron activity in the ventral root filament. Since extensor motoneurons are likely to be recruited before and after those contained in the filament, the duration of the extension phase of the step cycle, as shown here, must be considered a "best approximation" rather than an absolute value. So, the period of maximal activity in Q-IaIN B could fall in the middle of other Q motoneuron's burst of activity.

The last Q-IaIN analyzed, which is illustrated in Figure 23, displayed an atypical profile of activity during locomotion. As can be seen in the bar diagram, Q-IaIN D

became active at the very end of the extension phase and continued to discharge throughout the flexion phase and into the next extension phase. Its period of maximal activity occurred during the flexion phase of the step cycle, but it should be noted that this Q-IaIN discharged at fairly low rates even during its period of most vigorous firing.

In summary, 4 of the 6 analyzed Q-IaINs were maximally active during the early and middle portions of the extension phase of the step cycle. One Q-IaIN discharged primarily at the end of extension, and one cell discharged at low rates throughout the step cycle with its most vigorous firing occurring during the flexion phase. The 2 Q-IaINs that discharged at fairly high rates throughout the step cycle (Q-IaIN A, Figure 21 and Q-IaIN B, Figure 26) tended to be most active at the transition of flexion to extension. Those cells that discharged in a more burst-like fashion were maximally active in the middle or late portions of the extension phase. The phase relationships between the period of Q-IaIN activity and extensor motoneuron activity for the 4 Q-IaINs that displayed discrete onsets of activity during fictive locomotion are presented in Table 13. The

mean X and Y values for these 4 Q-IaINs are diagramatically presented in Figure 29 along with the mean discharge frequencies of all 6 Q-IaINs studied. The mean duration of Q-IaIN activity (40.13 msec) exceeded that of extensor motoneuron activity (334.5 msec). The mean onset of extensor motoneuron activity was 0.55 while Q-IaIN activity started at 0.32. The dashed lines, which represent one tail of the standard deviation, shows that the onset of Q-IaIN was quite variable. The mean offset of Q-IaIN activity (0.86) occurred well before the end of extension, but as shown in Table 13, 2 Q-IaINs were active until the end of extension. The mean X and Y values for those Q-IaINs that were maximally active during the extension phase were 0.43 and 0.92, respectively. The frequency histogram shown in Figure 29 shows that the discharge rate of Q-IaINs was elevated from late flexion through extension into early flexion (0.4 - 0.1) with the periods of peak activity occurring during early extension (0.5 - 0.7).

5. Discharge frequency of IaINs during spontaneous activity and fictive locomotion

Four of the 6 Q-IaINs analyzed in the present experiments discharged tonically at rest at frequencies

ranging from 15 - 65 Hz. In these IaINs, it was of interest to determine whether the discharge frequency that was observed during the period of the step cycle in which the cell was minimally active was lower than that observed at rest (before the MLR stimulation was started). Discharge frequencies during locomotion that were lower than those seen at rest could be evidence that direct inhibition of IaINs contributes to the modulation of their discharge during fictive locomotion.

Table 14 summarizes the findings obtained in the 4 Q-IaINs that were spontaneously active at rest. The frequencies listed in the table were determined by dividing the interspike interval measured at rest and during the periods of maximum and minimum firing during fictive locomotion into 1000 msec. All 4 of the Q-IaINs displayed discharge frequencies during the period of the step cycle in which they were minimally active that were lower than those exhibited at rest. The mean frequency of spontaneous discharge was 33.8 Hz, while the mean minimum frequency was 7.3 Hz. Maximal discharge frequencies seen during locomotion ranged from 50 - 250 Hz and had a mean of 156.3 Hz.

The evidence thus suggests that rhythmic excitation and inhibition of IaINs is involved in the modulation of their activity during fictive locomotion.

6.   Relationship between IaIN and Renshaw cell discharge frequencies

In 2 experiments, it was possible to record the activity of Q-IaINs and E-RCs during fictive locomotion. Figure 21 illustrates the activity in 2 Q-IaINs and a Q-RC. Since it has been shown that transmission in the Ia inhibitory pathway to Q motoneurons is diminished by antidromic stimulation of the Q nerve (Hultborn, et al. 1971c), one would expect to find that the discharge frequency of Q-IaINs and Q-RCs are reciprocally related, that is, when Q-RCs are most active, Q-IaINs would display a reduction of activity. This relationship was found to exist between the Q-RC and Q-IaIN A as revealed by comparing their respective frequency histograms. Q-IaIN A is tonically active throughout the step cycle, shows an acceleration in firing at the onset and a reduction at the end of the extension phase. The decline in Q-IaIN A discharge parallels the increase in activity in the Q-RC. The same interrelationship was not evident between the Q-RC

and Q-IaIN B, however, as the discharge patterns of these 2 cells were almost identical to one another. The period of most vigorous Renshaw cell firing (0.9 - 1.0) did just precede a reduction in Q-IaIN B's activity, however. There was an inverse relationship between the discharge pattern of the F-RC and the 2 Q-IaINs which might reflect a significant functional relationship.

There was also a reciprocal relationship between the activity in an E-RC and Q-IaIN D in Figure 23. This Q-IaIN was surprisingly maximally active during the flexion phase of the step cycle. It is possible that the cell was only weakly excited by the spinal locomotion generator, as indicated by its fairly low discharge rates, and that the inhibitory effects of E-RCs outweighed the excitatory drive from the generator during the extension phase.

A comparison of the discharge patterns of the Q-RC in Figure 21, the Q-RC in Figure 24 and the E-RC in Figure 23 with those of the 3 Q-IaINs illustrated in Figure 26 further suggests that Renshaw cells may function to diminish the activity in IaINs projecting to antagonistic motoneurons. Again, the accelerated activity in the Renshaw cells at the end of extension coincides with a reduction in Q-IaIN

discharge. If these 2 phenomena are causally related, this would result in a disinhibition of flexor motoneurons in late extension that would facilitate the onset of the flexion phase. The E-RC in Figure 22 was maximally active at the beginning of extension, perhaps indicating that it was coupled to a small motoneuron that would be recruited earlier in the extension phase than other larger motoneurons (Henneman, et al. 1965a, b). Maximal Renshaw cell firing during this period of the step cycle still can be related to decreased activity in IaINs projecting to antagonistic motoneurons as can be seen by comparing the frequency histogram of E-RC #2 in Figure 22 with those of Q-IaINs A and B in Figure 21 and Q-IaIN D in Figure 23.

7. Activity of unidentified interneurons

Since this study mainly dealt with the function of spinal interneurons that might function as part of a spinal locomotion generator, although the identified interneurons, Renshaw cells and IaINs have been focused upon, the activity of other unidentified interneurons that exhibited rhythmic behavior during fictive locomotion are also of interest. The behavior of 5 unidentified interneurons will be discussed briefly.

The frequency histogram of an interneuron that received a monosynaptic Ia EPSP from the nerve to LG and followed high frequency stimulation (250 Hz) but was not inhibited by antidromic stimulation of the L5 - S1 ventral roots is presented in Figure 7. The rhythmic membrane potential changes that occurred in a TA motoneuron are also shown in Figure 7. As shown in the bar diagram, the LG-IN and the TA motoneuron were active out of phase with one another, the TA motoneuron being active during flexion and the LG-IN being active from late flexion through extension. A comparison of the frequency histogram of the LG-IN and the membrane potential oscillation of the TA motoneuron shows a close correspondence between the period of maximal activity in the LG-IN and the hyperpolarization of the TA motoneuron. It is not possible from the data available to tell whether there is a causal relationship between the 2 cells, since the LG-IN could be an inhibitory or an excitatory interneuron. Interneurons in the intermediate zone of the lumbar spinal cord have been found that are monosynaptically excited by Ia afferents but do not receive recurrent inhibition (Hultborn, et al. 1971b). However, this cell was located 3.6 mm along a dorso-ventral

plane in the L6 segment of the spinal cord, which is an area in which the IaINs that were recurrently inhibited were found. Another possibility is that it is an excitatory interneuron that provides drive to LG motoneurons during locomotion. This is an interesting possibility in light of recent evidence (Pratt, et al. 1979) that the spinal locomotion generator must contain some source of excitation to motoneurons during their active period, since disinhibition alone did not appear to be an adequate explanation of motoneuron depolarization.

A Q-IN that was active during the flexion phase of the step cycle is illustrated in Figure 21. Q-IN E was excited at a short latency (1 msec) by low threshold stimulation of the Q nerve. Ventral root stimulation had no effect on this interneuron. This cell became active coincidental with the start of the flexion phase of the step cycle. A possible explanation for this pattern of discharge may be that these INs were primarily excited by group II afferents, which are active during muscle stretch rather than during the period of muscle contraction during locomotion (Loeb and Duysens, 1979). Perhaps this excitatory drive to Q motoneurons during the flexion phase

serves to offset the effects of reciprocal inhibition which should be focused on Q motoneurons during flexion.

A recording of another interesting interneuron that was recorded in the same experiment as the cells shown in Figure 23 is presented in Figure 30 along with the normalized data. This data was not included in Figure 23 because it was an unidentified cell. The behavior of this cell during fictive locomotion was recorded before the identification procedures were conducted and the cell was unfortunately lost during the locomotion trials. It began to discharge just after the onset of extensor motoneuron activity in the ventral root filament and fires for about 260 msec at a frequency of about 50 Hz. This interneuron appears to be inhibitory to the extensor motoneurons in the filament, which discharged directly in phase with intracellularly recorded Q motoneurons, since there is an obvious reduction in motoneuron activity in the filament during the burst of interneuron activity. It is interesting that the extensor motoneuron discharge that did persist in the filament during the period of interneuron firing was characterized by an unusual preponderance of doublet firing; this phenomenon was also observed when motoneurons that

were activated by muscle stretch or MLR stimulation were subjected to antidromic ventral root stimulation.

Inhibition of extensor motoneurons at the beginning of the stance phase would seem to be counterproductive to the smooth transition between the phases of the step cycle. During an earlier locomotion trial, the discharge of this interneuron seemed to be more coupled to the flexor phase of the step cycle and out of phase with extensor motoneurons. In later trials, as shown in Figure 30, extensor motoneuron activity commenced earlier in the step cycle and overlapped that of the interneuron. The activity period of the interneuron (0.2 to 0.42) occurs primarily during what is normally the flexion phase of the step cycle. It might be, therefore, that this interneuron is a flexor coupled inhibitory interneuron and that the extensor motoneurons were recruited unusually early in the step cycle. Thus, the extensor motoneurons were inhibited during the period of the interneuron firing. This interneuron may be a flexor coupled IaIN or Renshaw cell since its rhythmic activity during fictive locomotion was completely abolished by antidromic ventral root stimulation.

H.   Effect of antidromic ventral root stimulation on the

     rhythmic activity of Renshaw cell during fictive

     locomotion

     In a separate series of experiments conducted in our

laboratory, the possibility that Renshaw cells were

inhibited during fictive locomotion was tested by comparing

the number of action potentials produced within a Renshaw

cell burst evoked in response to a single stimulus pulse

delivered to a cut ventral root during various phases of

the step cycle and at rest (McCrea and Jordan, 1976a).

It was noticed that, in some cases, Renshaw cells which

had discharged rhythmically during locomotion before ventral

root stimulation commenced, discharged only in response

to the ventral root stimulation and no longer displayed

any rhythmic activity coupled to the activity of motoneurons

in a ventral root filament. An example of this phenomenon,

observed in an intracellularly recorded E-RC (RC #3 in

Table 12 and in Figure 23), is presented in Figure 31.

The Renshaw cell responses that were evoked by antidromic

stimulation of a cut L6 ventral root is shown in A (middle

trace), and the extensor motoneuron activity in the ventral

root filament is shown in the bottom trace. The upper

trace shows the A gate pulses that mark the time of ventral

root stimulation. The cycle interval of ventral root

stimulation was 360 msec. The Renshaw cell responded to

each ventral root stimulus with a burst of activity

typical of that produced by antidromic stimulation (Renshaw,

1946; Eccles, et al. 1954), and there is a reduction in

extensor motoneuron activity in the filament coincidental

with the Renshaw cell discharge. The spontaneously occurring

(not evoked by ventral root stimulation) activity of the

same Renshaw cell during fictive locomotion is illustrated

in B, and it is apparent that the Renshaw cell discharge

is rhythmic and occurs throughout the extension phase of

the step cycle (see Figure 23 for the normalized data).

As shown in A, however, when the ventral root is

stimulated during fictive locomotion, the Renshaw cell

discharge is almost entirely entrained to the stimulus

rate except for a few single action potentials which occur

near Renshaw cell bursts 3, 4 and 6.

A possible explanation for the inhibition of spontaneous

Renshaw cell activity during fictive locomotion by ventral

root stimulation is Renshaw cell - Renshaw cell inhibition,

which was described by Ryall (1970) and reported to have a

duration of less than 500 msec. Thus, the relationship between the rate of ventral root stimulation and the inhibition of spontaneous Renshaw cell discharge during fictive locomotion was determined to see whether there was some cycle interval above which rhythmic Renshaw cell activity persisted and below which it was abolished. In other words, was there evidence that there was Renshaw cell - Renshaw cell inhibition which had a finite duration.

Table 15 summarizes the results of this analysis. When the ventral root stimuli were delivered at intervals of 220 msec or greater, Renshaw cells continued to display rhythmic behavior during locomotion. When 200 msec or less separated the stimulus pulses, rhythmic Renshaw cell activity was abolished in 5 of 6 cells. The evidence does suggest, therefore, that rhythmic Renshaw cell activity during fictive locomotion can be inhibited by ventral root stimulation, and suggests that Renshaw cell inhibition of other Renshaw cells usually lasts about 200 msec. RC #6 from Table 15 was tested at ventral root stimulation rates with cycle intervals of 1400 msec and 200 msec; it displayed rhythmic activity amidst the evoked responses when there were 1400 msec separating the stimulus pulses but did not at the

faster stimulation rate when there were only 200 msec between stimulus pulses. Another cell, RC #5, was also tested at high and low stimulation rates, but this cell continued to exhibit rhythmic activity even when there was only 80 msec between stimulus pulses. Since there is some organization to the distribution of the mutual inhibition among Renshaw cells (Ryall, 1970, Ryall, et al. 1971) with the most powerful inhibition being obtained by stimulation of adjacent ventral roots (Ryall, et al. 1971), the failure of RC #5 to show evidence of inhibition may be related to the anatomical or functional characteristics of Renshaw cell - Renshaw cell inhibition.

DISCUSSION

A. Efficiency of transmission in the recurrent inhibitory
    pathway

1. Recurrent inhibition of motoneurons during controlled
treadmill locomotion

Before the recurrent inhibition of motoneurons produced
by antidromic ventral root stimulation during controlled
treadmill locomotion is discussed in detail, it is
important that it be established that the changes in
motoneuron discharge that were observed during ventral
root stimulation were mediated by Renshaw cells. It is well
established that stimulation of a cut ventral root
monosynaptically excites Renshaw cells (Renshaw, 1946;
Eccles, et al. 1954; Jankowska and Lindstrom, 1971; Ross,
et al. 1976) and results in a disynaptic inhibition of
certain motoneurons (Eccles, et al. 1954, 1961a; Willis
and Willis, 1966; Bergmans, et al. 1969; McCrea and Jordan,
1976b). Antidromic ventral root stimulation has been used
to study the recurrent inhibition of tonically active
motoneurons (Granit, et al. 1957, 1960; Granit and Rutledge,
1960; Granit and Renkin, 1961; Fromm, et al. 1977) and has

been shown to be effective in inhibiting motoneuron
activity. There has been no direct evidence heretofore,
however, that the reduction in motoneuron activity that
occurs with antidromic ventral root stimulation is due
to the activation of Renshaw cells. Figure 31A, which
shows the effect of periodic antidromic ventral root
stimulation on an intracellularly recorded Renshaw cell
(middle trace) and motoneuron activity recorded
simultaneously in an L6 ventral root filament, establishes
the cause and effect relationship between ventral root
stimulation and the inhibition of motoneurons. It can be
seen that coincidental with the stimulus pulse delivered
to the L6 ventral root (indicated by the onset of the A
gate pulse in the upper trace) there is a characteristic
high frequency Renshaw cell response and an interruption
in the motoneuron activity in the filament that is of the
same approximate duration as the Renshaw cell burst. On
the basis of this illustration and the aforementioned
literature, it is deemed justifiable to attribute reductions
in motoneuron activity seen during antidromic ventral root
stimulation to the recurrent inhibitory effects of Renshaw
cells on motoneurons.

The present data demonstrate conclusively that antidromic stimulation of the L7 ventral root can significantly reduce motoneuron discharge during MLR-evoked treadmill locomotion, as well as when motoneurons are activated by cyclic passive limb movement. In 19 motoneurons which were tested during both test conditions, there was a mean reduction in motoneuron activity of 72.3% during cyclic passive limb movement and 68.5% during controlled treadmill locomotion during the period of antidromic ventral root stimulation. An analysis of covariance showed that the relationship between Fi and Fs, which is an expression of recurrent inhibition, was the same during cyclic passive limb movement and locomotion after removal of the effects of initial discharge frequency. Thus, the recurrent inhibitory pathway is equally effective on motoneuron activity produced during either of the test circumstances.

These results do not confirm those of Severin, et al. (1968) obtained on 7 motoneurons using a similar experimental paradigm. There are several possible explanations for the difference between these findings and those reported by Severin, et al. (1968). Foremost

on the list is the possibility that cells which are less
likely to be significantly inhibited by ventral root
stimulation may have predominated their small sample.
Recurrent inhibition is maximal when the tested motoneuron
is a member of the muscle nerve that is antidromically
stimulated (Renshaw, 1941; Eccles, et al. 1961). In
contrast to the recurrent inhibition of agonists, antidromic
stimulation results in the facilitation, or disinhibition,
of antagonists due to Renshaw cell inhibition of IaINs
(Wilson, 1959; Wilson, et al. 1960, 1962). Antidromic
stimulation of an entire ventral root will activate
agonist and antagonist motoneurons and produce a mixture
of recurrent inhibition and facilitation. The response
of an individual motoneuron to ventral root stimulation
will be determined by its functional relationship to the
population of motor nerves arising from that root and the
net facilitation or inhibition that results from the
anatomical pattern of convergence. The data of Severin,
et al. (1968) did, in fact, show one motoneuron (motoneuron
#6 in their Table 1) which was recurrently facilitated
during controlled treadmill locomotion. Given the
variability that can be expected when a mixed population

of motor axons in a ventral root is stimulated, the negative findings of the Russian authors may be explained as a sampling phenomena related to the limited number of motoneurons investigated.

Another difference between the two studies was the range of ventral root stimulation frequencies employed; Severin, et al. (1968) utilized frequencies of 70 - 100 Hz while in the present experiments, stimulation frequencies of 20 - 30 Hz were used most often (in 73% of the 75 trials during cyclic passive limb movement and in 63% of the 80 controlled treadmill locomotion trials). One motoneuron was also tested using 70 Hz stimulation. The frequency of antidromic ventral root stimulation has been shown in the present study and in another (Granit and Renkin, 1961) to affect the efficacy of recurrent inhibition. Granit and Renkin (1961) showed that there was a positive linear relationship between ΔF and the frequency of antidromic ventral root stimulation. Maximal recurrent inhibition of tonically active motoneurons was obtained with stimulation frequencies of 30 - 40 Hz; at higher frequencies there was no further increase in ΔF. In the present experiments on rhythmically active motoneurons,

the efficacy of recurrent inhibition steadily increased

from 10 Hz to 30 Hz stimulation of the ventral root.

In the one motoneuron tested at 70 Hz, the recurrent

inhibition was less than that produced at 30 Hz during

cyclic passive limb movement but greater than that

produced at 30 Hz during locomotion. The efficacy of

recurrent inhibition was significantly greater when rates

of 20 - 70 Hz were used as opposed to 10 Hz, but there were

no significant differences among the results obtained with

20, 30 and 70 Hz. Thus, due to a lack of observations,

it is not possible to confirm with the present data, the

plateau in recurrent inhibition that occurred beyond

stimulation frequencies of 30 - 40 Hz as described by

Granit and Renkin (1961).

There is additional evidence available that suggests

that the effects of antidromic ventral root stimulation may

be less powerful when higher rates of ventral root

stimulation are employed. Eccles, et al. (1954) reported

that submaximal Renshaw cell responses were evoked by

ventral root stimuli when stimulation rates in excess of

10 Hz were used, and in a later study (Eccles, et al.

1961b), it was reported that there was a 60% decrease in

the amplitude of the EPSP in Renshaw cells when there was

less than 150 msec between antidromic ventral root stimulus pulses. The probable explanation for these findings surfaced in 1971 when Ryall provided evidence to suggest that there was mutual inhibition among Renshaw cells. The duration of this Renshaw cell - Renshaw cell inhibition was reported to be less than 500 msec (Ryall, 1970). In the present experiments, it was found that the rhythmic activity of Renshaw cells during fictive locomotion was abolished in 5 of 6 cells by antidromic ventral root stimulation when there was less than 200 msec between stimulus pulses. It is somewhat surprising, on the basis of this data, that 10 Hz ventral root stimulation was less effective in inhibiting motoneurons than 20 - 30 Hz, but it does seem likely that the 70 - 100 Hz stimulation frequencies used by Severin, et al. (1968) were not optimal for producing recurrent inhibition.

A significant linear relationship was found to exist between initial firing frequency (Fi) and ΔF which represents the absolute amount of recurrent inhibition in spikes/sec. Thus, the greater the frequency of motoneuron discharge, the greater the reduction during antidromic stimulation. The positive linear relationship between Fi

and $\triangle F$, which is illustrated in Figure 9B, conflicts
with results obtained during tonic motoneuron activity
(Granit and Renkin, 1961). Granit, et al. (1960a)
tetanized (114 pulses/second) the proximal ends of cut
nerves to the medial and lateral gastrocnemius muscles
of a denervated hindlimb and triggered the antidromic
stimulus pulses delivered to the ventral root by the
motoneuron action potential recorded in the ventral root
filament. Their data showed that recurrent inhibition
was cumulative, reaching complete effectiveness after 50
seconds. In addition, when the antidromic stimulus train
was presented 9.5 seconds after stretch, the rate of
inhibition was greater than if the stimulus was presented
1 - 3 seconds after the commencement of tonic stretch.
Based on this evidence, the authors concluded that at the
initial stage of motoneuron activation there was a surplus
of synaptic excitation exerted upon the motoneuron in
excess of that needed to sustain a tonic discharge. The
"surplus excitation" which opposed the hyperpolarizing
effects of Renshaw cells, diminished over the course of
activity, thus leading to a subsecuent increase in
recurrent inhibition.

During tonic muscle stretch, Granit, et al. (1960) reported different slopes defining the relationship between ΔF (fn - fi in their text) and Fi. In some cases, the slopes were positive, indicating that recurrent inhibition increased as Fi increased, while in other instances negative slopes were found. Their explanation for this variability was based on the effect of surplus excitation such that when Fi approached F max., "a surplus excitation" was minimal and recurrent inhibition was proportional to Fi. Conversely, when Fi was less than F max., the presence of surplus excitation antagonized the effects of antidromic stimulation and the greatest recurrent inhibition occurred at the lowest values of Fi. In accordance with this hypothesis, Granit and Rutledge (1960) stated that because of greater surplus excitation associated with low-threshold tonically active motoneurons, these motoneurons often showed greater resistance to recurrent inhibition than rapidly-firing, high-threshold motoneurons. This observation would explain the discrepancy between the earlier study and the present one regarding the relationship of ΔF to Fi. In the present experiments, recurrent inhibition was tested only on

rhythmically active motoneurons as opposed to the tonic motoneuron activity studied by Granit, et al. (1957, 1960a, b, 1961). It was shown by Hellweg, et al. (1974) that 90% of the Renshaw cells studied discharged in a burst-like manner during the dynamic phase of ramp stretch of the GS muscle and were usually active in parallel with phasically active motoneurons rather than the tonically active motoneurons recorded simultaneously in a ventral root filament. The authors concluded that Renshaw cells are primarily driven by the larger, phasically active motoneurons. The correlation observed during fictive locomotion between Renshaw cell activity and the larger amplitude action potentials in the ventral root filament shown in Figure 30B is consistent with this postulate. The linear relationship between ΔF and F_i may be specific to populations of rhythmically active motoneurons and might not be an accurate description of events occurring during conditions of tonic motoneuron activity evoked by static muscle stretch. It also appears that motoneurons discharging during locomotion may operate at levels approaching F max. with less surplus excitation and would thus demonstrate greater recurrent inhibition as the frequency of discharge increases.

Another major difference between the studies of Granit and coworkers and the present study was that antidromic ventral root stimulation was synchronized with motoneuron discharge in the former studies while a stimulus train of constant rate was employed in the present experiments. Since the severed peripheral nerve was stimulated at a rate of 114 Hz, it is probable that the efficacy of recurrent inhibition produced by ventral root stimulation coupled to the ventral root activity was submaximal.

Motoneuron discharge during antidromic stimulation was linearly related to Fi, a finding which is in agreement with the relationship between Fi and Fs observed by Granit, et al. (1960) and Granit and Renkin (1961) during tonic motoneuron activity. The regression lines reported by the latter authors are substantially different from those found in the current study during cyclic passive limb movement and locomotion, however, (Figure 9A). Granit and Renkin (1961) showed that the slopes of the regression lines for Fi vs. Fs were parallel to a theoretical regression line of slope b=1 (the case when Fi = Fs). They concluded that the amount of recurrent inhibition was a constant

and independent of Fi.

It is obvious from inspection of the regression lines for cyclic passive limb movement and locomotion in Figure 9A that the slopes are significantly different from a theoretical slope of b=1. This fact was confirmed by statistical analysis. During rhythmic motoneuron activity, Fs definitely appears to be dependent upon Fi, which was, in fact shown to be the most effective predictor of Fs. The available evidence suggests that Renshw cell activity is closely correlated with motoneuron activity.

There was no correlation between initial motoneuron discharge frequency during cyclic stretch and locomotion, but there was a linear relationship between motoneuron discharge in the 2 test conditions during antidromic stimulation, i.e., between C-FS and LFs. The correlation analysis may indicate that recurrent inhibition operates to reduce motoneuron firing rates below a critical level specific to each motoneuron that would result in a motor unit or muscle's elimination from the expression of a motor act.

Severin, et al. (1968) reported average motoneuron discharge frequencies of 32.5 spikes/sec for static muscle

stretch, 33.9 spikes/sec for cyclic muscle stretch and
47.7 spikes/sec during controlled treadmill locomotion.
These discharge rates are in agreement with ranges of
motoneuron discharge observed by Shik, et al. (1966)
during MLR-evoked treadmill locomotion in decerebrate cats,
but they are substantially higher than these reported in
the present study. The explanation resides in different
methods employed to measure motoneuron activity. Severin,
et al. (1968) as well as Shik, et al. (1966) determined
motoneuron discharge frequency by dividing the duration of
the motoneuron burst of activity by the number of action
potentials that occurred in that burst and then expressed
this figure in frequencies per second. In the present
study, the number of action potentials occurring in 1
sec intervals before (Fi) and during (Fs) periods of
antidromic ventral root stimulation were counted. Since
the duration of a walking step cycle is usually less than
1 sec, each 1 sec interval contained a period of
motoneuron activity as well as a period of inactivity.
It is understandable, therefore, that the frequencies of
motoneuron discharge obtained by the latter method will
be consistently lower than those based upon frequency/burst

measurements. Analysis of the present data using the counting method employed by the other investigators indicates that the motoneuron discharge rates are comparable with those reported elsewhere (Shik, et al. 1966; Severin, et al. 1968; Zajac and Young, 1976). Furthermore, according to the highly significant ($p \leq .01$) linear relationship between Fi and ΔF observed in the present study, greater amounts of recurrent inhibition would be predicted with higher initial motoneuron discharge rates. Multiple linear regression and correlation analysis revealed that Fi was the most weighted predictor of ΔF, and analysis of covariance indicated that any difference in treatment effects (recurrent inhibition) between cyclic passive limb movement and locomotion were attributable to initial firing frequency. The reported lack of recurrent inhibition by Severin, et al. (1968) cannot be explained, therefore, on the basis of more vigorous motoneuron activity.

Severin, et al. (1968) postulated that the diminished efficiency of recurrent inhibition during MLR-evoked locomotion could be attributed to either direct inhibition of Renshaw cells or to the presence of surplus excitation

that would render motoneurons more resistant to inhibition. Neither of these rationales is supported, however, by recently acquired data. Evidence from the present study suggests that surplus excitation is not a critical factor affecting recurrent inhibition in phasically active motoneurons. This issue has already been discussed in some detail. It was first shown by McCrea and Jordan (1976a) and confirmed in the present studies that Renshaw cells are rhythmically active during fictive locomotion. This does not support the notion that Renshaw cells are inhibited during locomotion. Furthermore, the recurrent inhibitory pathway to motoneurons was found to be functional during all phases of the fictive step cycle (McCrea and Jordan, 1976b). Since significant recurrent inhibition of motoneuron activity was observed during controlled treadmill locomotion in the present study, it has now also been established that the discrepancy between the absence of inhibition of Renshaw cells during fictive locomotion (McCrea and Jordan, 1976a) and the failure of the Russian workers to observe recurrent inhibition during treadmill locomotion cannot be due to Renshaw cell inhibition from afferent activity originating

in the moving limb or from supraspinal centers.

The reason for the increased occurrence of doublet firing during antidromic stimulation is not clear. During static stretch, single motoneuron spikes seem to be converted to doublet spikes if they occur at all. As can be seen in Figure 10B, the doublets occur at longer interburst intervals than the interspike intervals present before stimulation. The doublets seem neither phase locked to the antidromic stimulus nor do they maintain a constant interburst interval with other doublets. Some insight into the phenomenon of doublet firing produced by antidromic stimulation was provided by Granit, et al. (1957). After interruption of tonic motoneuron activity by antidromic stimulation of a cut ventral root, resumption of motoneuron activity was usually initiated by doublet firing. The authors also found that the occurrence of doublet firing was dependent upon recurrent inhibition of the motoneuron, and it was postulated that the rebound doublet reflected a faster rate of depolarization arising from a hyperpolarization than when the cell is maintained near threshold.

Motoneuron discharge can be converted from single

action potentials to doublets by normally occurring inhibition that is orthodromically rather than antidromically evoked. Figure 29 shows an apparent, but unidentified, flexor coupled inhibitory interneuron that was rhythmically active during fictive locomotion. The extensor motoneurons in the ventral root filament began to discharge with a pair of doublets before the interneuron became active. During the period of interneuron discharge, the extensor motoneuron activity was diminished and was restricted to doublet firing.

Motoneurons that were recorded intracellularly during fictive locomotion or extracellularly in a ventral root filament during controlled treadmill locomotion were found to commence rhythmic activity with an initial doublet while the action potentials in the remainder of the burst were separated by a longer and fairly constant ISI. Zajac and Young (1976) also reported observing initial doublet firing in rhythmically active motoneurons during controlled treadmill locomotion. Intracellular stimulation of motoneurons with constant currents evokes a train of motoneuron discharge that begins with a doublet (Baldissera and Gustafsson, 1974; Calvin, 1974).

Intracellular stimulation of motoneurons resulted in greater amounts of muscle tension when stimuli were 3 - 10 msec apart than if single stimuli or trains of longer spaced stimuli were used (Burke, et al. 1970). It has been suggested that motoneuron discharge that commences with an initial doublet represents a mechanism for optimizing tension production that would facilitate the initiation of muscle activity (Zajac and Young, 1976; R.B. Stein, personal communication).

There is some evidence that the initial doublet is a function of the amount of excitatory drive to the motoneuron, as was suggested by Granit, et al. (1957), since there was an obvious, but statistically insignificant, inverse relationship between the duration of the initial ISI and the number of action potentials in the burst. Also, the initial ISI was substantially shorter in motoneurons that were active during controlled treadmill locomotion than during fictive locomotion.

The doublet firing produced by antidromic stimulation during cyclic passive limb movement and locomotion in the present study could be a rebound phenomenon, but it is also possible that antidromic stimulation was less

effective in inhibiting the initial doublet or triplet than
in depressing the remainder of the burst. It is
noteworthy that Renshaw (1946) reported observing a small
number of motoneurons that responded to a single antidromic
ventral root stimulus with two successive action potentials.
These antidromically evoked doublets may be related to the
effects of recurrent inhibition or possibly to motor axon
collaterals which have been found to terminate on
motoneuron somas and dendrites (Cullheim, et al. 1977).

Aside from inhibition during the stimulus train and
the appearance of doublets, L7 ventral root stimulation
produced only minimal effects on motoneuron discharge during
walking on the treadmill. It is possible that this is
due to tight coupling between the motoneurons affected
by the stimulus and parts of the locomotion system which
are unaffected by such a restricted stimulus. Nevertheless,
these findings do not support the assertion by Miller and
Scott (1977) that the spinal locomotion generator consists
of antagonistic systems of motoneurons, Ia inhibitory
interneurons and Renshaw cells. Significant resetting of
the locomotor rhythm by the stimulus was observed in only
1 motoneuron, in spite of the presence of marked recurrent

inhibition in 68% of the motoneurons during locomotion. Even in cases where inhibition was present, but rhythmic bursting of the cell could still be observed (Figure 8A, D), resetting of the locomotor rhythm during the stimulus period was not produced. Large doses of mecamylamine and atropine, which block the nicotinic and muscarinic ACh receptors, respectively, did not produce any noticeable impairment of MLR-evoked treadmill locomotion (McCrea and Jordan, 1976b), and in the present study, ventral root stimulation eliminated rhythmic RC activity during fictive locomotion without affecting the rhythmic activity of motoneurons (Figure 31). These findings render the model proposed by Miller and Scott unlikely.

2.   Recurrent inhibition of IaINs during fictive locomotion

In 1975, Feldman and Orlovsky reported that the recurrent inhibition of IaINs was diminished during fictive locomotion which they suggested was consistent with previous work (Severin, et al. 1968) indicating that Renshaw cells were depressed during locomotion. Only 4 IaINs were tested in the 1975 study, however; the efficacy of recurrent inhibition was reduced in 2 Q-IaINs and was not changed in another Q-IaIN and a PB-ST-IaIN.

In the present study, the efficacy of antidromically-evoked recurrent inhibition was tested in 6 Q-IaINs during

fictive locomotion. In all 6 IaINs, the difference between the discharge frequency of IaINs during the period of antidromic ventral root stimulation (Fs) was significantly lower than that observed during locomotion without ventral root stimulation (Fi). The mean total reduction in IaIN discharge by ventral root stimulation was only 34.5% as compared to the powerful recurrent inhibition of IaIN spontaneous activity (92.5%). However, the mean ΔF obtained during fictive locomotion (20.8 spikes/sec) was fairly similar to the mean amount of recurrent inhibition of spontaneous activity (27.6 spikes/ sec) despite the fact that IaINs discharged at twice the rate during fictive locomotion (60.3 Hz) than at rest (30.2 Hz).

As in motoneurons, the amount of recurrent inhibition of IaINs was linearly related to Fi, but the increase in recurrent inhibition per increase in IaIN discharge was much smaller than was observed in motoneurons. The recurrent inhibition of IaINs during fictive locomotion ($\Delta F = 20.8$ spikes/sec) was actually greater than the observed recurrent inhibition of motoneurons during controlled treadmill locomotion ($\Delta F = 8.4$ spikes/sec).

The less effective recurrent inhibition of IaINs (34.5%) as compared to motoneurons (64.8%) primarily reflects the greater discharge rate of IaINs (60.3 Hz) during fictive locomotion than that of motoneurons during treadmill locomotion and the fact that Renshaw cell activity is not coupled to that of IaINs (vis a vis the difference in the slopes of the regression lines showing the relationship between Fi and ΔF for motoneurons and IaINs in Figures 9B and 14B, respectively). This really is not a surprising finding, since one would expect to see a closer correlation between Renshaw cell and motoneuron activity than between Renshaw cell and IaIN activity.

Hellweg, et al. (1974) demonstrated that Renshaw cell activity during ramp stretch of the tendon to the G-S muscle was more closely correlated with that of phasically active rather than tonically active motoneurons. Benecke, et al. (1975) then speculated and confirmed that the recurrent inhibition of IaINs was more potent during the dynamic phase of ramp and hold G-S muscle stretch than during the static phase. Renshaw cells were primarily, if not exclusively active during the dynamic phase of muscle stretch, and their burst of activity was temporally

related to a reduction in or cessation of IaIN activity. IaINs were spontaneously active at rates of 25 - 35 Hz; these rates are in agreement with those observed in the present experiments and others (Hultborn, et al. 1971b; Feldman and Orlovsky, 1975). During ramp stretch in a decerebrate cat which had received an i.v. injection of DHβE, which blocks the activation of Renshaw cells via nicotinic synapses, a IaIN discharged at a frequency of 88 Hz. Before the administration of DHβE, the same IaIN discharged at a frequency of only 20 Hz during the same period of ramp stretch. These data indicate that during movements involving dynamic muscle stretch, orthodromically activated Renshaw cells can effectively inhibit IaINs discharging at moderate frequencies.

The data suggests that a critical factor affecting the efficacy of recurrent inhibition is the rate at which the IaINs are discharging. Table 14 shows that the mean frequency of IaIN activity was 33.8 Hz during spontaneous activity and 156.3 Hz during the periods of maximal activity during fictive locomotion. (These frequencies were determined on the basis of interspike intervals while those in Table 7 were based on the number of action potentials

that actually occurred within 1 sec). The frequency of

IaIN discharge during ramp stretch was intermediate between

these two rates. Ventral root stimulation was most

effective in inhibiting IaINs when they were spontaneously

active. During ramp stretch, Benecke and coworkers (1975)

reported almost a 100% decrease in IaIN activity

coincidental with an 80% increase in Renshaw cell activity

(c.f. their Figure 8). IaIN activity was reduced to well

below spontaneous levels during the period of augmented

Renshaw cell activity. During fictive locomotion,

antidromic ventral root stimulation produced a smaller

mean reduction in IaIN activity, but all 6 Q-IaINs tested

were significantly inhibited by ventral root stimulation

during fictive locomotion. The frequency of discharge

of 2 Q-IaINs (#4 and #6 in Table 7) was lower during

antidromic ventral root stimulation during fictive

locomotion (Fs) than at rest, although the mean Fs

during fictive locomotion (38.5 Hz) was greater than the

mean rate of spontaneous activity (30.2 Hz). It is

apparent that the spinal locomotion generator provides a

very powerful phasic drive to IaINs during fictive

locomotion, as increases in instantaneous frequency of

235 Hz (IaIN Ia-33 #15 in Table 14) above the spontaneous
discharge frequency have been observed.   The fact that
antidromic ventral root stimulation is capable of blocking
about 66% of the augmentation of IaINs by the locomotion
generator, as evidenced by the fact that the Fi during
fictive locomotion was 30 Hz greater than at rest and the
Fs during fictive locomotion showed a reduction of 20 Hz
(Table 7), is evidence that the recurrent inhibitory
pathway is functional during fictive locomotion.

Although evidence of recurrent facilitation of
motoneuron activity in ventral root filaments was observed
in individual trials of antidromic ventral root stimulation
during controlled treadmill locomotion, none of the 19
motoneurons tested in that series of experiments exhibited
a mean Fs that was larger than Fi.   The reason for this
probably relates to the fact that ventral root stimulation
resulted in a mean reduction in IaIN discharge of just
34.5% during fictive locomotion.   A 20% increase in the
monosynaptic peroneal reflex was observed by Benecke,
et al. (1975) when there was almost a 100% reduction in
IaIN discharge during ramp stretch.   Thus, it would appear
that the amount of recurrent facilitation of motoneurons

resulting from the recurrent inhibition of IaINs during fictive locomotion is probably minimal.

The results from the present study have established that IaINs are consistently and significantly inhibited by antidromic ventral root stimulation during fictive locomotion. These data further support the findings of McCrea and Jordan (1976a) that Renshaw cells are not depressed during locomotion and that the recurrent inhibitory pathway is functional during fictive locomotion (1976b). In fact there is evidence that indicates that Renshaw cells probably function optimally during motor tasks like locomotion since they appear to be most effectively recruited by large, phasically active motoneurons, as shown in Figure 31B and as discussed by Hellweg, et al. (1974).

The decrease in the efficacy of recurrent inhibition of IaINs produced by ventral root stimulation during fictive locomotion appears to be primarily related to the very high frequency of IaIN discharge that occurs during locomotion. There was a positive linear relationship between the amount of recurrent inhibition (ΔF) and the activity of motoneurons (r = .844) and IaINs (r = .967),

but the "gain" of the recurrent inhibitory effects on
motoneurons was greater (b = .749) than on IaINs
(b = .293). The explanation for the more effective
recurrent inhibition of motoneurons than of IaINs may
reside in the fact that the rate of antidromic ventral
root stimulation used (30 Hz) more closely matched the
firing frequency of motoneurons than of IaINs.

Of course, it must be remembered that antidromic
activation of Renshaw cells is highly unphysiological and
really only provides information regarding the degree to
which the pathway is functional during locomotion and the
relative susceptibility of the target cells to the effects
of recurrent inhibition. However, there is indirect
evidence available that suggests that the recurrent
inhibition of IaINs that results from the orthodromic
activation of Renshaw cells is more related to the activity
of motoneurons than that of IaINs. Hultborn and Lundberg
(1972) showed that when a monosynaptic PbSt reflex was
conditioned by stretch of the tendon to the Q muscle,
there was a positive linear relationship between stretch
of the muscle and the discharge of a Q Ia afferent (which
provides input to the Q-IaINs that reciprocally inhibit
PbSt motoneurons). Within a certain range of stretch,

there was also a linear increase in the percent reciprocal
inhibition of the PbSt reflex, but after the tension in
the Q muscle increased, the amount of reciprocal
inhibition of the PbSt reflex plateaued. This result
was interpreted to indicate that at a certain point of
muscle stretch, the Ia drive to the Q-IaINs was matched
by recurrent inhibition of the Q-IaINs, thus preventing
the depth of reciprocal inhibition from increasing with
increased Ia afferent activity. Hultborn (1976) often
saw that the plateau was temporary, and that there was a
resumption in the increase in reciprocal inhibition with
continued muscle stretch. Thus, although there was a
maintained acceleration in Ia drive to the Q-IaINs, the
amount of reciprocal inhibition varied due to some nonlinear
recruitment of motoneurons (Grillner and Udo, 1971) and
subsequent Renshaw cell activity levels. At increasing
levels of stretch, larger motoneurons are recruited
(Henneman, et al. 1965b). Thus, within the range of
muscle stretch in which there was a balance between Ia
drive and recurrent inhibition of Q-IaINs, the largest
motoneurons might have been recruited, providing the
maximum drive to Renshaw cells and resulting in a period

of most efficacious recurrent inhibition. As stretch continued, the drive to Renshaw cells could not keep pace with the continued increase in Ia afferent activity, and the amount of reciprocal inhibition once again became defined by the amount of Ia afferent activity.

The issue of the efficacy of recurrent inhibition of IaINs and motoneurons and its relationship to the input to Renshaw cells, IaINs and motoneurons is an important one in view of the demonstration that a number of descending and segmental pathways have parallel effects on motoneurons and IaINs (Hultborn, 1972; Hultborn and Udo, 1972; Hultborn, et al. 1976b, c). It follows that the answers to questions regarding the impact and the role of recurrent inhibition during locomotion are dependent upon information about the input to all three cell types during locomotion. It is obvious from a comparison of the discharge rates of IaINs and motoneurons that their input is not identical during fictive locomotion. Supraspinal and segmental input to Renshaw cells will also likely affect the "gain" of the system. In a later section, the activity patterns of motoneurons, Renshaw cells and IaINs, which reflect their input during locomotion, will be

discussed in an attempt to decipher the contributions
of IaINs and Renshaw cells to the control of locomotion.

3.   Recurrent inhibition of Renshaw cells during fictive
locomotion

The finding in the present experiments that the
rhythmic activity of Renshaw cells could be abolished by
antidromic ventral root stimulation confirms observations
made in chloralose, anesthetized spinal cats that Renshaw
cells inhibit other Renshaw cells and further supports
the conclusion that Renshaw cells are not inhibited
during fictive locomotion.

In 5 of 6 Renshaw cells tested, rhythmic activity
was abolished when there was 200 msec or less separating
the ventral root stimulus pulses, which suggests that this
is the upper range for the duration of the mutual inhibition
among Renshaw cells. This finding is consistent with
reports (Eccles, et al. 1954, 1961b) that in barbituate,
spinal cats, Renshaw cells responses were submaximal when
there was less than 100 - 150 msec between stimulus pulses.

B.   Membrane potential oscillations in flexor and extensor
     motoneurons during fictive locomotion

Essential to the understanding of the spinal mechanisms involved in the generation of locomotion is the determination of the synaptology impinging on alpha motoneurons that induces rhythmic activity. Are the rhythmic depolarizations and hyperpolarizations caused by postsynaptic excitation and inhibition or due to presynaptic disinhibition and disfacilitation, respectively? Evidence based upon chloride injections and hyperpolarizing currents in some motoneurons suggests that the interburst hyperpolarization is due to postsynaptic inhibition (Edgerton, et al. 1976; Menzies, et al. 1978). Strychnine, which blocks the postsynaptic inhibitory effects of Ia inhibitory interneurons and Renshaw cells (Lodge, et al. 1977), promotes walking when given in sub-convulsive doses (Hart, 1971). Pratt, et al. (1979) found that i.v. strychnine removed the interburst hyperpolarization in motoneurons that remained phasically active during fictive locomotion. The evidence thus suggests that the interburst hyperpolarization is due to the postsynaptic action of some inhibitory interneuron(s) on motoneurons, but the fact that removal of this inhibition does not disrupt the rhythmicity of alpha motoneuron firing raises questions regarding the function of this inhibition in controlling

the pattern of motoneuron firing during locomotion.

Although one model of the spinal locomotion generator, has been proposed which assumes that motoneuron depolarization results from disinhibition alone (Miller and Scott, 1977), recent evidence suggests that the spinal rhythm generator must contain some source of phasic excitatory input to motoneurons as well. Menzies, et al. (1978) injected hyperpolarizing current pulses through the recording microelectrode while intracellularly recording the activity of alpha motoneurons during fictive locomotion in mesencephalic cats. By comparing the amplitude of the current pulses produced throughout the step cycle it was possible to determine whether the conductance change associated with depolarization was less than that seen during the hyperpolarized phase; this would be expected if the depolarized phase were due to disinhibition. There was no significant differencebetween the conductance measured during hyperpolarization and depolarization,which would indicate the presence of similar amounts of synaptic activity. Pratt, et al. (1979) found that numerous EPSPs, that were modulated in amplitude, were unveiled during fictive locomotion after i.v. injection of strychnine,

indicating the presence of phasic excitatory input to motoneurons. Although motoneurons are rhythmically active without an interburst hyperpolarization during fictive locomotion in strychnine treated cats, when 4 sec depolarizing currents were injected through the microelectrode, the motoneurons fired tonically and no longer displayed any rhythmic activity during fictive locomotion (Pratt, et al. 1979). The fact that depolarizing current abolished motoneuron rhythmicity is further evidence of phasic excitatory input to motoneurons during fictive locomotion.

The available data thus suggests that motoneuron rhythmicity during fictive locomotion is due to periodic excitation and inhibition of motoneurons. Most of the data discussed was obtained in extensor motoneurons, however, and it is not known whether the same spinal mechanisms are responsible for the generation of rhythmicity in both flexor and extensor motoneurons. There are some reasons to suspect that there might be essential differences in the spinal locomotion circuitry for extensor and flexor motoneurons. The dendritic branching of flexor motoneurons appears to be different than that of extensors since the

dendrites of medial motoneuron pools (mostly flexor motoneurons) tend to have more contralateral projections than do those of more lateral motoneuron pools (mostly extensor motoneurons). The relative contribution from various species of afferent input to flexor and extensor motoneurons might differ since it was observed that flexor motoneurons were less stable than extensor motoneurons after deafferentation (Zangger, 1978). Andersson, et al. (1978) reported that the membrane potential oscillations of flexor motoneurons were greater than those in extensor motoneurons in acute spinal cats treated with DOPA.

An analysis of the membrane potential oscillations that occur in flexor and extensor motoneurons during fictive scratching (Berkenblit, et al. 1978b) revealed that the spinal circuitry for flexor and extensor motoneurons can indeed be asymmetrical. During fictive scratching in decerebrate cats flexor motoneurons were tonically depolarized; as they became rhythmically active, the membrane potential phasically hyperpolarized to about their resting level during the extension phase of the scratch cycle. Extensor motoneurons, on the other hand, were not tonically depolarized but rather displayed a brief

depolarization followed by a hyperpolarization that was equal to or greater than the resting membrane potential. The membrane potential fluctuations in extensor motoneurons and flexor motoneurons and the relative durations of the flexor and extensor phases of the scratch cycle are just the opposite of that seen in flexor and extensor motoneurons during a fictive walking step cycle. In the latter case, the extension phase of the step cycle is longer than the flexion phase (Engberg and Lundberg, 1969; Goslow, et al. 1973; Tokuriki, 1973) and thus extensor motoneurons tend to be depolarized for a longer period than are flexor motoneurons. It is apparent, therefore, that the activity of flexor and extensor motoneurons can be programmed with a high degree of specificity by different spinal rhythm generators that reflect the demands of particular motor tasks.

The results obtained in the present study do not provide conclusive evidence of the nature of the flexor and extensor motoneuron spinal locomotion generators and whether they are similar or dissimilar. The majority of extensor motoneurons (9 of 12), all of which were Q motoneurons, were tonically depolarized during fictive

locomotion, while all 3 of the flexor motoneurons hyperpolarized below resting membrane potential levels. Based on percentages (100% of the flexors and 75% of the extensors), one might venture to speculate that, as in the rhythmic act of scratching, flexor and extensor motoneurons receive different types of synaptic input from their respective rhythm generators that reflects their specialized roles during locomotion; extensor motoneurons are involved in the maintenance of posture and maintained support during the stance phase of the step cycle while flexor motoneurons are phasically involved in the transfer of the limb. Thus, it might be that there is a tonic excitation or disinhibition of extensor motoneurons during locomotion that serves to keep their membrane potential closer to threshold so they are kept ready for activation. The rhythmic activity of flexor and extensor motoneurons seems to be due to periodic excitation and inhibition (Menzies, et al. 1978; Pratt, et al. 1979), but it might be that these cyclic processes are superimposed on a tonically depolarized membrane potential in extensor motoneurons and on the resting membrane potential in flexor motoneurons.

Since whether a motoneuron hyperpolarized below the resting level was correlated to variables that could be indicators of the general health of the cell and the quality of the impalement or could reflect fundamental differences in synaptic input from flexor and extensor rhythm generators, it is difficult to know whether the difference between the flexor and extensor motoneurons analyzed was due to the recording conditions or to the actions of specific interneuronal circuits. Further study of a broader range of flexor and extensor motoneurons associated with the different hindlimb joints is necessary to resolve this issue. This study, which represents the initial efforts to conduct a systematic quantitative analysis of motoneuron membrane potential oscillations during MLR-evoked fictive locomotion, does indicate that further efforts in this direction are warranted.

C. Activity and function of IaINs and Renshaw cells

The discussion of the possible functions of Renshaw cells and IaINs, as indicated by an analysis of their activity patterns and phase relationships during fictive locomotion, that follows is based on an acknowledged limited sample of data. A thorough analysis would necessitate

having data on the activity of Renshaw cells, IaINs

and motoneurons associated with flexor and extensor

muscles at all hindlimb joints. The moderate yield from

these experiments is understandable considering the

difficulties associated with obtaining recordings of

several identified cell types in a fictively walking

cat in which the quality of locomotion was adequate

to permit normalization of the data. Thus, while the data

presented here may be considered "anecdotal" examples of

the pattern and interrelationships of activity in Renshaw

cells, IaINs and motoneurons, they are considered to be

valuable well-documented descriptions that are significant

contributions to the existing rather scanty data base on

the activity of identified spinal neurons during locomotion.

1.   IaINs

All of the 6 Q-IaINs that were analyzed in the present

study displayed rhythmic activity during MLR-evoked

fictive locomotion. These results confirm reports by

Feldman and Orlovsky (1975) and Edgerton, et al. (1976)

that IaINs are phasically active during locomotion in the

absence of cyclic afferent input. Since IaINs have been

shown in other studies to be rhythmically active in spinal,

paralyzed cats (Edgerton, et al. 1976) and in decerebellate,

de-efferented cats (Feldman and Orlovsky, 1975), it is now well established that the modulated activity of IaINs during locomotion is at least partly centrally programmed. The axonal projections of IaINs to motoneuron nuclei were found to be organized with a high degree of specificity (Jankowska and Roberts, 1972b) which supports available electrophysiological evidence that IaIN activity is coupled with that of particular motoneuron pools.

Feldman and Orlovsky (1975) reported that 22 of 23 Q-IaINs studied were rhythmically active with maximal activity occurring in the stance phase of MLR-evoked locomotion in cats which had been bilaterally de-efferented from L3 on caudally. Most of these Q-IaINs (18 of 22) were silent during the flexion phase of the step cycle. In the present study, 5 of 6 Q-IaINs were found to be maximally active during the stance phase and 4 of the 6 Q-IaINs were completely inactive during the reciprocal phase of the step cycle. Those Q-IaINs that discharged in discrete bursts during fictive locomotion and were maximally active during extension became active at the end of flexion ($x = 0.43$) and discharged until late extension ($y = 0.92$).

One Q-IaIN discharged at low rates throughout the step cycle but was maximally active during flexion. One possible explanation for this unexpected firing pattern seen in Q-IaIN D in Figure 23 is that this Q interneuron was primarily excited by group II afferents. As has been pointed out by others (Stauffer, et al. 1976; Watt, et al. 1976), there is some unspecificity when graded electrical stimulation is used to selectively activate various populations of afferents contained in peripheral nerves. There is some chance, therefore, that the stimulation of the Q nerve was of sufficient intensity to include group II afferents. The group II afferents arise from slowly adapting stretch receptors in the muscle spindles, and it has been shown by Loeb and Duysens (1979) that group II afferents are active when the muscle containing the parent muscle spindle is stretched during normal walking in intact, unrestrained cats. Thus, one would expect to find that interneurons receiving input from Q group II afferents would be active during flexion when the Q muscles would be stretched. Another possibility is that this Q-IaIN was inhibited by Q-RCs which are active during the stance phase. It is also possible that this cell is a VSCT cell

since some of these cells are monosynaptically excited by

Ia afferents (Lundberg and Weight, 1971; Gustafsson and

Lindstrom, 1973; Lindstrom and Schomburg, 1973), and

about 3% of these are also inhibited by Renshaw cells

(Lindstrom and Schomburg, 1973). VSCT cells have been

shown to be phasically active during locomotion in

deafferented cats (Arshavsky, et al. 1972b). VSCT cells

are thought to transmit information regarding the

activity of inhibitory and excitatory interneurons in

spinal reflex pathways (Lundberg, 1971) functioning as

an integrator of several modes of information rather than

transmitting discrete sensory signals like the DSCT.

Thus, it is difficult to speculate about the phasing and

firing patterns that would be expected in VSCT cells

during fictive locomotion.

Frequency histograms describing Q-IaIN activity

during fictive locomotion were analyzed to allow maximal

IaIN activity to be correlated with that of associated

motoneurons, in an attempt to discern causal relationships.

Four Q-IaINs displayed their periods of maximal activity

during the early and middle parts of the extension phase

while one Q-IaIN was maximally active at the end of the

stance. Assuming that the frequency of Q-IaIN firing is directly related to the amount of reciprocal inhibition focused on antagonistic motoneurons, then it would appear that maximal inhibition of knee flexors by Q-IaINs would occur most often during the first half of the extension phase and occasionally at the end of extension.

IaINs have been shown to be active in phase with motoneurons receiving similar Ia input and simultaneously with the inactive period of antagonistic motoneurons indicating that IaIN-mediated reciprocal inhibition (Hongo, et al. 1971b) is a feature of fictive locomotion. As can be seen in Figure 26, Q-IaINs are active in phase with Q motoneurons in a ventral root filament, and the onset of their activity coincides with the onset of hyperpolarization in a Sart motoneuron which is an antagonist of Q motoneurons. These results are consistent with the findings of Pratt, et al. (1979) that strychnine, which blocks the inhibitory effects of IaINs (Lodge, et al. 1977), removes the interburst hyperpolarization of phasically active motoneurons during fictive locomotion.

The activity of IaINs during normal locomotion in

intact cats would be determined by central programming, descending information (c.f. Intro. 3a) and input from Ia afferents. Of course, phasic contributions from the latter source were absent in the present study, but under normal conditions the Ia afferents would probably provide tonic and phasic excitatory input to IaINs. Feldman and Orlovsky (1975) found that Q-IaINs had a lower mean frequency of discharge during their active periods of locomotion when the ventral roots had been cut $(\overline{X} = 120$ Hz) as opposed to when they were intact and the cat's hindlimbs were free to move $(\overline{X} = 150$ Hz). Static stretch of the Q muscle in the former preparation resulted in a substantial enhancement of Q-IaIN activity during locomotion, but the pattern of rhythmicity was not altered.

The first recordings of the activity of muscle primaries during locomotion were obtained in decerebrate cats by Severin and coworkers (1967a). Ia afferents were phasically active with their burst of activity coinciding with ankle extensor EMG activity. Primary endings from ankle flexors were also active during homonymous muscle contraction but displayed peak frequency of discharge when their parent muscle was passively stretched. The

demonstration that Ia activity continued without pause during extrafusal muscle fiber contraction, particularly in the ankle extensors, was interpreted to indicate the presence of α-γ coactivation. Such pronounced and consistent coactivation of α and γ motoneurons has not been observed, however, during locomotion in intact, awake animals (Prochazka, et al. 1976, 1977, 1979; Loeb and Duysens, 1979). These data were obtained by chronic recordings of Ia afferents during normal, unrestrained locomotion in healthy, awake cats. While there was evidence of moderate fusimotor activity during periods of active muscle shortening during locomotion, much less pronounced α-γ coactivation was observed than that seen in ankle extensor Ia afferents in decerebrate cats (Severin, et al. 1967a). The concept of invariable α and γ motoneuron coupling does not appear feasible on the basis of reported examples of obvious independent α and γ motoneuron control (Prochazka, et al. 1977; Loeb and Duysens, 1979). Loeb, et al. (1979) observed examples of Ia afferent activity which occurred in the absence of or inversely with EMG activity in the receptor bearing muscles during normal locomotion. These observations of fusimotor activity

which occurred out of phase with α motoneuron firing were restricted to muscles which were not operating as prime movers at the time.

The available evidence supports the notion that the nervous system is capable of employing various strategies of α and γ motoneuron control depending upon the muscle being observed and the task the muscle is engaged in performing (Loeb and Duysens, 1979; Prochazka, et al. 1979). Loeb, et al. (1979) suggested that prime movers engaged in "stereotyped, cyclical, nonballistic movements" are typically coactivated with fusimotor motoneurons causing modest firing levels of primary afferents to persist throughout muscle shortening. The velocity of muscle shortening has also been proposed as the primary factor determing the amount of coactivation of α and γ motoneurons (Prochazka, et al. 1979). Movements associated with muscle velocities less than .2 resting lengths per second may receive more powerful fusimotor support than those incorporating more vigorous movements, such as walking (Prochazka, et al. 1979).

Since Ia afferent activity is less tightly coupled to a particular phase of locomotion in intact cats than in

decerebrate cats walking on a treadmill, it might be that the primary responsibility for the regulation of IaIN activity during normal locomotion resides with the spinal locomotion generator. The evidence suggests that the modulation of IaIN activity by the spinal locomotion generator involves periodic excitation and inhibition of IaINs. The frequency of IaIN discharge at rest was found to be intermediate between the rates seen during the periods of maximum and minimum activity during fictive locomotion. Also, the amplitude of Ia IPSPs evoked in Q motoneurons by stimulation of the TA nerve during fictive locomotion were greater than control Ia IPSPs when Q motoneurons were maximally depolarized and were smaller than controls when Q motoneurons were hyperpolarized.

There is also evidence indicating that Ia input is modulated during fictive locomotion which also probably contributes to the regulation of IaIN activity.

Feldman and Orlovsky (1975) found that stimulation of the Q nerve in de-efferented cats evoked monosynaptic reflexes in Q-IaINs only during the phase of the step cycle in which the Q-IaINs were active. Ia EPSPs were shown by Schomburg and Behrends (1978b) and in the present

study to be maximal when motoneurons were most depolarized, i.e., during their active phase of the step cycle. Polysynaptic Ia EPSPs have been reported (Watt, et al. 1976; Schomburg and Behrends, 1978b), but they occur only during the phase of motoneuron depolarization in paralyzed, spinal DOPA treated cats (Schomburg and Behrends, 1978b). Henneman (1977, 1979) has proposed that transmission in large Ia afferents with profuse terminal branching is normally less efficacious than in smaller Ia afferents because of the slowing of conduction at a greater number of branch points. Post-tetanic potentiation was reported by Henneman (1979) to have a greater effect on larger Ia afferents. Thus, it might be that during the active phase of the step cycle temporal summation increases the number of active Ia afferent terminals and transmission to IaINs and motoneurons is facilitated.

During normal locomotion, IaIN activity is probably regulated by Ia afferent activity, the presynaptic inhibition of Ia afferent terminals and/or changes in the efficacy of transmission in the terminals, supraspinal centers and the spinal locmotion generator. The present data, in accord with previous reports, (Feldman and

Orlovsky, 1975; Edgerton, et al. 1976) supports the
concept of $\alpha - \gamma$ - linked reciprocal inhibition that was
forwarded by Hongo, et al. (1969) and indicates that the
programming for this basic feature of locomotion is
contained within spinal interneuronal circuits.

2.   Renshaw cells

(a)   Coupling with motoneurons

The activity of 7 E-RCs and 3 F-RCs during fictive
locomotion was analyzed in detail in the present study.
In addition to confirming a previous report (McCrea and
Jordan, 1976a) that Renshaw cells are rhythmically active
during fictive locomotion in phase with the motoneurons
that excite them, the present study provides the first
quantitative assessment of the phasing of Renshaw cell
activity during locomotion. This analysis was performed
in hopes of learning more about the input to Renshaw cells
as well as the function of recurrent inhibition in the
control of locomotion.

Both F-RCs and E-RCs became active just after the
onset of the flexion and extension phases of the step cycle
(Figure 28), indicating that they are activated by and
secondarily to flexor and extensor motoneurons,

respectively. Of particular interest was the relationship between the offset of Renshaw cell activity and the cessation of motoneuron activity. All 3 F-RCs stopped discharging just after the end of the flexion phase of the step cycle. The mean offset for the flexion phase was 0.44 and for F-RCs it was 0.47. The mean offset point for E-RCs (y = 0.93) just preceded the end of the extension phase, but 3 of 5 E-RCs that were recorded in experiments in which an extensor motoneuron was also recorded, were active beyond the offset of extensor motoneuron activity.

F-RCs were active throughout virtually all of the flexion phase while E-RCs discharged throughout about 75% of the extension phase. The fact that Renshaw cell activity was usually spread across either phase of the step cycle suggests that there is a convergence onto Renshaw cells from small and large motoneurons that are recruited sequentially (Henneman, et al. 1965b) during their active period. A substantial convergence onto Renshaw cells was reported by Eccles, et al. (1954) since as many as 10 axons from 5 motor nerves were seen to converge onto a single Renshaw cell. There is evidence in the present

data that, as suggested by Hellweg, et al. (1974),
Renshaw cells are most effectively driven by large
phasically active motoneurons that are the last to be
recruited. Eight of the 10 Renshaw cells studied were
either recruited or were maximally active at the end of
their particular active phase of the step cycle.

The fact that a majority of flexor and extensor
coupled Renshaw cells were maximally active at the end of
the flexion or extension phase of the step cycle and had
activity periods that extended beyond the cessation of
motoneuron activity suggests that Renshaw cells may
function to help terminate motoneuron activity at the
transition points in the step cycle. As can be seen in
Figures 22 and 23, there was a correspondence between
maximal activity in E-RCs and the onset of hyperpolarization
in Q motoneurons. F-RCs and E-RCs were found by Delyagina
and Feldman (1978) to be active at the end of the flexion
and extension phases, respectively, of the fictive scratch
cycle.

Since Renshaw cell activity largely coincided with
that in coupled motoneurons, it is likely that, as suggested
by Eccles, et al. (1954), Renshaw cells serve to curtail

motoneuron discharge during their active period to promote

fluid movement and to prevent highly intense, spastic

movement during locomotion. Strychnine, which reportedly

blocks the postsynaptic inhibition mediated by Renshaw

cells and IaINs (Lodge, et al. 1977) is a convulsant which

can facilitate walking in spinal dogs (Hart, 1971).

Strychnine also abolishes the interburst hyperpolarization

in motoneurons that continue to display rhythmic activity

during fictive locomotion (Pratt, et al. 1979). The

activity patterns of Renshaw cells indicate that the

interburst hyperpolarization is not the result of the

recurrent inhibition of motoneurons. Thus, the convulsive

effect of strychnine might result from the blockage of the

IaIN-mediated interburst hyperpolarization and the removal

of Renshaw cell regulation of motoneuron discharge. There

is a substantial amount of evidence now available that

suggests that the regulation of motoneuron activity during

fictive locomotion must involve both postsynaptic

excitation and inhibition (Menzies, et al. 1978; Pratt,

et al. 1979). The removal of the excitatory drive to

motoneurons plus the increased activity of Renshaw cells

at the end of the active phase may combine to shut off

motoneuron activity prior to the onset of the subsequent

phase of the step cycle. It does not appear, however,

that Renshaw cells are essential to the proper phasing

of motoneuron activity since they are active concurrently

with their associated motoneurons and since motoneuron

phasing is not disrupted by strychnine. McCrea and

Jordan (1976b) also reported that large doses of

mecamylamine and atropine did not noticeably impair

controlled treadmill locomotion. Furthermore, as can be

seen in Figure 31, when antidromic ventral root stimulation

results in an inhibition of the rhythmic activity of

Renshaw cells during fictive locomotion, the rhythmicity

of the motoneurons in the filament is not perturbed.

Since Renshaw cells are active in parallel with the

motoneurons which excite them and which they recurrently

inhibit, it would seem that there are 3 possible ways that

Renshaw cells contribute to the control of locomotion.

Firstly, Renshaw cells probably act as a "quality control

system" acting to keep motoneuron discharge within moderate

rates. The recurrent inhibition of motoneurons may help

ensure that motoneuron activation is normally asynchronous

so as to prevent spastic movements. Secondly, since

Renshaw cell activity is apparently related to the discharge frequency of motoneurons as indicated by the linear relationship between Fi and Δ F, Renshaw cells may function as an efferent monitor of motoneuron activity. It would seem that supraspinal centers, in particular the cerebellum, would require information of the activity in various motoneuron pools that was not coded in proprioceptive signals such as are carried by the DSCT. Renshaw cells have been shown to affect transmission in the VSCT (Gustafsson and Lindstrom, 1973; Lindstrom and Schomburg, 1973), and Lundberg (1971) has proposed that this ascending pathway may provide a means for supraspinal monitoring of the activity of spinal neurons. The third function of Renshaw cells might be to contribute to the phasing of other interneurons, such as IaINs, that might be associated with the spinal rhythm generator.

(b) Coupling with IaINs

Benecke, et al. (1975) suggested that the recurrent inhibition of IaINs projecting to antagonist motoneurons would disinhibit those motoneurons and facilitate the switching between antagonist motoneuron pools during fast alternating movements. Both IaINs and Renshaw cells have

been found to be rhythmically active during fictive

locomotion in phase with motoneurons to which they are

coupled, i.e., Q-IaINs and Q-RCs are active in phase with

Q motoneurons. This phase relationship would not be

consistent with the idea that the recurrent inhibition

of IaINs contributes to the control of alternating

movements such as walking since, for instance, Q-IaINs

projecting to knee flexor motoneurons would be recurrently

inhibited by Q-RCs during Q motoneuron activity; thus,

knee flexors would be disinhibited during the stance

phase of the step cycle.

In order to determine the interrelationship between

IaIN and Renshaw cell activity, it is important to consider

the relative discharge frequencies of these interneurons

that are observed at various points of the step cycle.

IaINs are most effectively recurrently inhibited by

antidromic stimulation of the peripheral nerve containing

the Ia afferent that excites the IaIN (Hultborn, et al.

1971c). Thus, Q-IaINs receive the most powerful

recurrent inhibition from Q-RCs. One would therefore

expect to find a reciprocal relationship between the

activity of Q-RCs and Q-IaINs. A comparison of the

frequency histograms of Q-IaIN and E-RC activity during
fictive locomotion shows that 5 of the 6 Q-IaINs
analyzed exhibited a decrease in discharge frequency
at the end of the extension phase which was coincidental with
increased rates of firing in 5 of the 7 E-RCs analyzed
(2 Q-RCs, 2 E-RCs and 1 LG-RC). This finding would
suggest that the most powerful recurrent inhibition of
IaINs occurs during late extension. Q-IaINs which
discharged in discrete bursts during fictive locomotion
were active from 0.43 to 0.92 of the normalized step cycle.
E-RCs were active from 0.55 to 0.93. These data are
consistent with the possibility that E-RCs function to turn
off the activity in Q-IaINs during late extension.
Disinhibition of knee flexor motoneurons at this point
in the step cycle would facilitate the smooth transition
between the extension and flexion phases.

During fictive locomotion the amplitude of Ia IPSPs
evoked in Q motoneurons by stimulation of the nerve to
TA were largest when the Q motoneuron was depolarized
during the stance phase of the step cycle. During flexion,
when the Q motoneuron is hyperpolarized, TA-IaINs should
be recurrently inhibited by TA-RCs. Thus, the larger TA

IPSPs seen in Q motoneurons during extension could be due to the depolarization of the membrane potential and to the fact that the TA-IaINs are not inhibited by Renshaw cells at that time. The fact that the amplitude of TA IPSPs was smaller during flexion than control values obtained at rest, is further evidence for direct inhibition of TA-IaINs.

The recurrent inhibition of IaINs during fictive locomotion does not appear to be very potent. The recurrent facilitation of motoneurons was only occasionally and inconsistently observed when an entire ventral root was stimulated during controlled treadmill locomotion. A similar procedure did result in the recurrent facilitation of one motoneuron in the work of Severin, et al. (1968), however. Also, activation of a massive population of Renshaw cells by stimulation of a ventral root could reduce IaIN discharge by only 34.5% during fictive locomotion. Thus, it does not seem likely that the recurrent inhibition of IaINs during locomotion results in a significant facilitation of antagonist motoneurons. It is more probable that Renshaw cells limit the activity of IaINs projecting to antagonist motoneurons in a fashion

similar to their control of motoneuron discharge. The

suggestion that the recurrent inhibition of IaINs

functions to keep the level of reciprocal inhibition of

antagonist motoneurons fairly constant despite increases

in Ia afferent activity during static stretch (Hultborn

and Lundberg, 1972; Hultborn, 1976) might pertain to

locomotion as well. It is obvious that the recurrent

inhibition of IaINs is not powerful enough to block the

reciprocal inhibition of antagonist motoneurons since 5

of 6 Q-IaINs were maximally active during the stance phase,

the exception being the Q-IaIN in Figure 23. It was

reasoned by Hultborn (1972, 1976) that the depression

of Renshaw cells during locomotion that had been reported

(Severin, et al. 1968; Bergmans, et al. 1969; Feldman

and Orlovsky, 1975) was necessary for α - γ - linked

reciprocal inhibition to operate during locomotion. It

is now well established that Renshaw cells are not

inhibited during locomotion, however. Instead the

coincidence of E-RC and Q-IaIN activity can be interpreted

to indicate that the recurrent inhibition of IaINs does

not interfere with the timing of α - γ - linked reciprocal

inhibition, just as the recurrent inhibition of motoneurons

does not appear to affect their rhythmicity, but rather merely serves as a brake that prevents the reciprocal inhibition from becoming too intense. The data also suggests that the increase in E-RC activity at the end of extension may contribute to the termination of activity in Q-IaINs.

3. Summary

Motoneurons, Renshaw cells and IaINs have all been shown to be rhythmically active during fictive locomotion in the absence of cyclic afferent input. Motoneurons (Edgerton, et al. 1976; Andersson, et al. 1978, Schomburg and Behrends, 1978a, b) and IaINs (Edgerton, et al. 1976) have also been found to be rhythmically active in spinal, paralyzed cats treated with DOPA. Thus, the modulation of motoneuron and IaIN activity during locomotion appears to be centrally programmed by the spinal locomotion generator, and the evidence suggests that this modulation involves direct periodic excitation and inhibition. The activity patterns of Q-IaINs during fictive locomotion suggests that $\alpha - \gamma$ - linked reciprocal inhibition of antagonist motoneurons is also a centrally programmed feature of locomotion. Q-RCs and other E-RCs were active

throughout the extension phase simultaneously with Q motoneurons and Q-IaINs and tended to exhibit an acceleration of activity at the end of their active period. Recurrent inhibition of Q motoneurons and Q-IaINs by Q or E-RCs most likely serves to keep the discharge rates of these neurons within moderate ranges so as to prevent spastic, synchronous activity of knee extensors and extreme amounts of reciprocal inhibition of knee flexors, respectively. The active periods of E-RCs suggest that they might also function to help terminate activity in Q motoneurons and Q-IaINs at the end of extension, most probably in conjunction with the removal of excitation by the spinal generator. Since rhythmic motoneuron activity persists after strychnine administration but is abolished by depolarizing current injection after strychnine (Pratt, et al. 1979), it would appear that the most significant element in the regulation of rhythmic activity in motoneurons and perhaps IaINs is the phasic excitatory drive from the spinal locomotion generator. IaINs may contribute to the appropriate phasing of motoneuron activity during alternating movement, but Renshaw cell activity seems to be more involved in "quality control" rather than phase control. Renshaw

cells may contribute to phase control, however, by providing ascending pathways with immediate, reliable information of the activity in various motoneuron pools.

D.   Summary and Conclusions

1.   The recurrent inhibitory pathway to motoneurons was found to be functional during controlled treadmill locomotion in postmammillary cats. 19 motoneurons isolated in left L7 ventral root filaments were activated by both cyclic passive movement of the left hindlimb and MLR stimulation. Antidromic stimulation of a cut L7 ventral root produced a mean reduction in motoneuron activity during cyclic passive limb movement of 72.3% and of 68.5% during controlled treadmill locomotion in these 19 motoneurons. There was no significant difference between the amount of recurrent inhibition produced during these two test conditions. The rate of firing was significantly reduced by ventral root stimulation during cyclic passive limb movement in 15 of the 19 motoneurons and in 13 of the 19 motoneurons during controlled treadmill locomotion.

2.   There was a significant positive linear relationship between the initial firing rate of phasically active

motoneurons (Fi) and the amount by which their discharge was reduced by antidromically-evoked recurrent inhibition (ΔF). These results contrast with previously reported data on the recurrent inhibition of tonically active motoneurons (Granit, et al. 1960; Granit and Renkin, 1961).

3. The locomotor rhythm displayed by hindlimb motoneurons in the L7 ventral root filament was not affected by antidromic stimulation of the ipsilateral L7 ventral root. The initiation of motoneuron activity occurred within predicted latencies following cessation of the stimulus train; and the ensuing pattern of motoneuron activity was not significantly different from that observed in the filament prior to antidromic stimulation.

4. The firing pattern of motoneurons that responded tonically to static muscle stretch was altered by antidromic stimulation of the ipsilateral L7 ventral root. In addition to significant reductions in motoneuron firing frequency, motoneuron activity was often seen to switch from single spikes appearing at a constant interspike interval to doublets with a variable interburst interval. Cyclic movement of the passive hindlimb and stimulation

of the MLR evoked bursts of motoneuron activity in the L7

ventral root filament characterized by an initial doublet

which was less effectively inhibited than the remainder

of the burst during antidromic stimulation of the

ipsilateral L7 ventral root.

5. It is concluded that Renshaw cells respond to

antidromic stimulation with equal effectiveness during

cyclic passive limb movement and controlled treadmill

locomotion. None of the evidence indicates that Renshaw

cells are depressed during controlled treadmill locomotion,

as had been previously reported (Severin, et al. 1968).

These results further illustrate that the discrepancy

between the absence of inhibition of Renshaw cells during

fictive locomotion observed by McCrea and Jordan (1976a)

and the failure to observe recurrent inhibition during

controlled treadmill locomotion (Severin, et al. 1968)

cannot be due to Renshaw cell inhibition by rhythmically

active segmental afferents not present in the paralyzed

preparation.

6. Antidromic ventral root stimulation produced a

significant reduction in Q-IaIN activity during fictive

locomotion. The amount of recurrent inhibition ranged

from 26.7% to 60.0% and had a mean of 34.7%. All 6 Q-IaINs tested were significantly recurrently inhibited during fictive locomotion. As was found to be true in motoneurons, a positive linear relationship existed between $\triangle F$ and Q-IaIN Fi.

7. Antidromic ventral root stimulation during fictive locomotion evoked typical high frequency Renshaw cell discharges throughout the step cycle which could be shown to occur concurrently with depressions of motoneuron activity in ventral root filaments.

8. Antidromic ventral root stimulation could abolish the rhythmic activity of Renshaw cells when rates of ventral root stimulation in excess of 5 Hz were used. The duration of Renshaw cell - Renshaw cell inhibition appeared to be about 200 msec.

9. The results of this study confirm the findings of McCrea and Jordan (1976a, b) that Renshaw cells are not depressed during fictive locomotion. The recurrent inhibitory pathways from Renshaw cells to motoneurons, IaINs and Renshaw cells were found to be functional during fictive locomotion. In agreement with McCrea and Jordan (1976a), Renshaw cells were found to be rhythmically

active during MLR-evoked fictive locomotion in
postmammillary cats.   Rhythmic Renshaw cell activity
has also been observed during spontaneous fictive
locomotion in thalamic cats (McCrea and Jordan, 1976a).
Recurrent inhibitory effects have been observed in these
preparations and in non-paralyzed cats during controlled
treadmill locomotion.   Thus, it is concluded that Renshaw
cells are not inhibited by supraspinal or segmental
mechanisms during locomotion in cats that have been
decerebrated at the precollicular-postmammillary or more
rostral levels.

10.   The amplitude of Ia EPSPs evoked by stimulation of
the femoral nerve was found to be greatest when quadriceps
motoneurons were most depolarized during fictive locomotion.
This finding is in agreement with data reported by Schomburg
and Behrends (1978b) obtained in high spinal, DOPA-treated
cats.   There was a positive linear relationship between
Ia EPSP amplitude and motoneuron membrane potential in
3 of the 4 quadriceps motoneurons studied.   In 3 of 4
motoneurons, the amplitude of the control EPSP was of a
value that was intermediate between the largest and
smallest EPSPs seen during fictive locomotion.   The

modulation of Ia EPSPs during fictive locomotion may be
due to phasic presynaptic inhibition of Ia terminals
by either the spinal locomotion generator or descending
pathways.

11. A significant inverse linear relationship was found
to exist between the amplitude of Ia IPSPs evoked in 2
quadriceps motoneurons by stimulation of the nerve to
tibialis anterior during fictive locomotion and motoneuron
membrane potential. TA Ia IPSPs were greatest when
quadriceps motoneurons were active and the recurrent
inhibition of TA-IaINs by quadriceps Renshaw cells should
be maximal. Motoneuron membrane potential oscillations
appears to affect the amplitude of Ia IPSPs to a greater
extent than does the recurrent inhibition of IaINs.

12. Analysis of the membrane potential oscillations
that occurred relative to the resting membrane potential
(E_R) in flexor and extensor motoneurons during fictive
locomotion revealed that in all 3 flexor motoneurons and
in 3 of 12 extensor motoneurons the membrane potential
depolarized above E_R during the motoneuron's active
phase and hyperpolarized below E_R during the inactive
phase; in 9 of 12 extensor motoneurons the membrane

potential was tonically shifted above E_R throughout the entire step cycle such that the depolarized and hyperpolarized periods all occurred above E_R. Whether or not motoneurons hyperpolarized below E_R during fictive locomotion was inversely related to E_R and to the difference between E_R and peak hyperpolarization during fictive locomotion. Since these variables might also be a function of the health of the cell and/or the quality of the impalement, these results cannot be interpreted to conclusively show that there may be basic differences in the synaptic input to flexor and extensor motoneurons during fictive locomotion.

13. In addition to confirming the findings of McCrea and Jordan (1976a) that Renshaw cells are rhythmically active during fictive locomotion and discharge in phase with motoneurons from which they are excited, the present study has provided the first detailed quantitative assessment of the phase relationships between Renshaw cell and homonymous motoneuron activity during fictive locomotion. Both flexor and extensor coupled Renshaw cells became active after the onset of flexor and extensor motoneuron activity, respectively, indicating that Renshaw

cells are activated primarily by motoneurons during fictive locomotion. On the average, the extension phase extended from 0.5 to 1.0 while the 7 extensor Renshaw cells analyzed were active from 0.55 to 0.93 of the step cycle. Maximal activity in extensor Renshaw cells occurred at the end of the extension phase (0.8 - 1.0) and could be seen to correspond to the onset of hyperpolarization in extensor motoneurons and decreases in extensor motoneuron discharge.

The flexion phase extended from 0 - 0.44 while the mean onset and offset points of flexor Renshaw cell activity were 0.04 and 0.47, respectively. All 3 flexor Renshaw cells studied stopped firing just after the end of the flexion phase. Maximal flexor Renshaw cell activity occurred during middle to late flexion (0.1 - 0.4) and could be seen to correspond to the onset of reduced flexor motoneuron activity.

14. All 6 Q-IaINs studied in the present experiments were rhythmically active during fictive locomotion which confirms the findings of Feldman and Orlovsky (1975) and Edgerton, et al. (1976). The mean period of Q-IaIN activity exceeded that of the extension phase of the step cycle;

the former extended from 0.55 to 1.0 and the latter from 0.32 to 0.86 of the step cycle. Two Q-IaINs displayed elevated but modulated discharge rates throughout the step cycle while the other 4 Q-IaINs fired in discrete bursts. On the average, Q-IaINs displayed elevated discharge rates from 0.4 to 1.1 of the step cycle with maximal activity occurring during early extension (0.5 - 0.7). Maximal activity in Q-IaINs could be shown to be coincidental with the onset of hyperpolarization in sartorious motoneurons. Thus, in general, the activity pattern of Q-IaIN activity indicated that α - γ linked reciprocal inhibition is a centrally programmed feature of fictive locomotion. The variability seen in Q-IaIN activity patterns during fictive locomotion may have been related to functional subdivisions of Q-IaINs, i.e., coupling to one of the 4 component quadriceps muscles.

15. All 4 Q-IaINs that were tonically active at rest displayed discharge frequencies during their inactive phase which were lower than their spontaneous discharge frequencies. These 4 Q-IaINs had a mean frequency of spontaneous discharge of 33.8 Hz while the mean minimum and maximum frequencies seen during fictive locomotion

were 7.3 and 156.3 Hz, respectively. Further evidence
of periodic inhibition of IaINs during fictive locomotion
was provided by the fact that the amplitude of control
Ia IPSPs were larger than the smallest IPSPs evoked
during fictive locomotion. These data suggest that the
modulation of IaINs during fictive locomotion is due to
periodic inhibition and excitation directed at either the
IaIN and/or at excitatory interneurons that impinge upon
them. The modulation of Ia EPSPs during fictive locomotion
indicates that there is a phasic presynaptic inhibition of
Ia afferent terminals which could also contribute to the
modulation of IaINs during fictive locomotion.

16. 5 of the 6 Q-IaINs analyzed exhibited a decrease in
discharge frequency at the end of the extension phase which
was coincidental with increased rates of firing in 5 of
the 7 extensor Renshaw cells analyzed. Q-IaINs which
discharged in discrete bursts during the extension phase
were active from 0.43 - 0.92 of the step cycle. Extensor
Renshaw cells were active from 0.55 to 0.93 and maximally
active from 0.9 - 1.0. These data are consistent with the
possibility that extensor Renshaw cells contribute to the
reduction in Q-IaIN discharge with the most powerful

recurrent inhibition occurring during late extension.

Since Q-IaINs were mainly active during extension, the

recurrent inhibition of Q-IaINs by Renshaw cells obviously

is not powerful enough to interfere with the reciprocal

inhibition of antagonist motoneurons, but it might be

instrumental in preventing the amount of reciprocal

inhibition from becoming too intense.

17. The analysis of the patterns and phase relationships

of motoneuron, Renshaw cell and IaIN activity has provided

some insight into the possible functions of the inhibitory

interneurons in the control of locomotion at the spinal

level. IaINs appear to mediate $\alpha - \gamma$ - linked reciprocal

inhibition during fictive locomotion thereby contributing

to the hyperpolarization of antagonist motoneurons during

their inactive phase. The data suggest that Renshaw cells

may function to limit motoneuron and IaIN activity during

their active phase, and, in conjunction with the removal

of phasic excitatory input, help to terminate activity

in these neurons prior to the onset of the subsequent

phase of the step cycle. This role of Renshaw cells could

facilitate the transition of activity betwen pools of

antagonist motoneurons as was suggested by Benecke, _et al_.

(1975). The recurrent inhibition of IaINs would limit but not interfere with the appropriate phasing of $\alpha - \gamma$ - linked reciprocal inhibition. Phasic excitatory input to motoneurons during fictive locomotion appears to be primarily responsible for controlling the rhythmic onset of activity in appropriate motoneuron pools and the removal of this excitation along with the activity of Renshaw cells and IaINs together function to suppress motoneuron discharge during the reciprocal phase of the step cycle. These interpretations are consistent with the finding that strychnine removes the interburst hyperpolarization but does not abolish motoneuron rhythmicity during fictive locomotion (Pratt, et al. 1979). In addition, Renshaw cells may function as an efferent monitor of alpha motoneuron activity and could relay this information to the cerebellum via the ventral spinocerebellar tract. This information could be essential in co-ordinating fine movements and/or setting the level of gamma bias.

18. No significant difference was found in the basic pattern of motoneuron discharge observed during controlled treadmill locomotion and fictive locomotion. In both

cases, motoneuron discharge was characterized by a short

initial interspike interval (ISI) while the action

potentials in the remainder of the burst were separated

by a longer and fairly constant ISI ($\overline{X}$ = 44.0 msec).

The mean initial ISI was shorter during controlled

treadmill locomotion (7.1 msec) than during fictive

locomotion (29.2 msec) and may be related to the magnitude

of the excitatory drive to the motoneuron. The similarity

in the pattern of motoneuron locomotor activity in

paralyzed and non-paralyzed cats suggests that the phasing

data obtained during fictive locomotion may also pertain

to conditions in which locomotion occurs in the presence

of rhythmic afferent input.

Table 1

Comparison Between Mean Motoneuron Discharge Frequencies
(spikes/sec) Before (Fi) and During (Fs) Antidromic
Stimulation of the L7 Ventral Root in Motoneurons
Tested During Cyclic Passive Limb Movement and
Controlled Treadmill Locomotion

Motoneurons	$^+$n	Cyclic			n	Locomotion		
		Fi	Fs			Fi	Fs	
1	5	9.0	4.9		7	7.4	4.9	*
2	5	16.8	7.6	**	7	12.3	9.1	**
3	5	12.3	4.4		5	3.7	2.0	
4	3	9.8	0.5	**	4	4.6	1.0	**
5	5	0.9	0.8		5	7.9	4.6	*
6	3	1.1	0.1	***	4	12.4	1.5	***
7	4	7.2	1.4	**	6	14.3	4.6	**
8	3	4.0	0.6		7	13.1	2.0	***
9	5	10.7	1.8	***	6	14.0	10.2	*
10	5	8.2	1.0	**	6	11.6	0.6	***
11	1	19.2	19.3		1	26	16	
12	5	14.5	7.1	**	4	30.2	0.4	**
13	5	16.7	1.9	***	4	1.9	0.1	*
14	4	12.9	0.3	**	1	18.3	0.6	
15	4	3.9	0	**	1	1.0	0	
16	4	25.5	3.1	***	1	2	0.3	
17	7	11.7	0.07	***	5	15.7	3.1	*
18	3	8.8	0.17	**	5	21.2	6.8	**
19	3	11.2	5.6	*	2	18.3	9.0	
$\overline{X}$		10.8	3.2			12.4	4.0	

$^+$n = Number of trials. Each trial consisted of 1 recurrent
inhibition test, i.e., the motoneuron activity during
the 3 second train of ventral root stimulation (Fs)
and the normal motoneuron activity in the 3 second
period preceding (Fi) and following (Fp) the ventral
root stimulation. Each trial normally had about 3
cycles of motoneuron activity during Fi and Fs and 1
during Fp.

* Indicates level of significance between Fi and Fs tested
with a paired t test (* = $p \leq .05$; ** = $p \leq .01$;
*** = $p \leq .001$).

Table 2

Effects of Antidromic Stimulation of the

L7 Ventral Root on Motoneuron Discharge During

Cyclic Passive Limb Movement and Locomotion

	Cyclic	Locomotion
Number of motoneurons	19	19
Number of trials	79	81
[a] F_i	10.8	12.4
[b] F_s	3.2	4.0
[c] ΔF	7.6	8.4
Relative reduction	72.3%	68.5%
Significant t-tests	15	13

[a] Mean initial motoneuron discharge frequency (spikes/second).

[b] Mean motoneuron discharge frequency during L7 ventral root stimulation.

[c] $\Delta F = F_i - F_s$.

Table 3

Means and Standard Deviations of Motoneuron

Discharge Frequencies and Changes in Frequency

During Ventral Root Stimulation

Parameters	Number of Spikes (per sec)
* C - Fi	10.8 ± 6.2
C - Fs	3.2 ± 4.6
C - (Fi - Fs/Fi)	0.72 ± 0.30
C - ΔF	7.6 ± 5.4
+ L - Fi	12.4 ± 8.2
L - Fs	4.0 ± 4.4
L - (Fi - Fs/Fi)	0.69 ± 0.26
L - ΔF	8.4 ± 7.2

* C - Cyclic passive limb movement.

+ L - Controlled treadmill locomotion.

Table 4

Results of Multiple Correlation and Regression Analysis on Eight

Test Variables Listed in Table 3

Comparisons	r [a]	T test [b]	b [c]	F test [d]
[1] C-Fi vs. C-Fs	.524	.05	.391	.05
C-Fi vs. C-ΔF	.707	.001	.617	.001
C-Fi vs. C-(Fi-Fs/Fi)	.095	N.S. [e]		N.S.
C-Fi vs. [2] L-Fi	-.090	N.S.		N.S.
C-Fs vs. L-Fs	.692	.01	.659	.001
C-ΔF vs. L-ΔF	.299	N.S.		N.S.
C-(Fi-Fs/Fi) vs. L-(Fi-Fs/Fi)	.622	.01	.554	.01
L-Fi vs. L-Fs	.465	.05	.251	.05
L-Fi vs. L-ΔF	.844	.001	.749	.001
L-Fi vs. L-(Fi-Fs/Fi)	.061	N.S.		N.S.

a   r = correlation coefficient
b   Test of significance of r
c   b = regression coefficient
d   Test of significance of linear regression
e   N.S. = not significant
1   C - cyclic passive limb movement
2   L - controlled treadmill locomotion

Table 5

Effect of Antidromic Stimulation Frequency on the

Amount of Recurrent Inhibition Expressed as a

Percent Reduction in Motoneuron Discharge

Stimulation Frequency	Number of Trials	(Fi-Fs/Fi) x 100	Standard Deviation
Cyclic Passive Limb Movement			
10 HZ	17	52.5%	19.2
20 HZ	13	84.7%	22.6
30 HZ	42	85.2%	15.9
70 HZ	3	81.3%	14.8
Controlled Treadmill Locomotion			
10 HZ	24	36.3%	18.9
20 HZ	12	74.8%	13.7
30 HZ	38	77.1%	26.9
70 HZ	7	84.6%	7.4

Table 6

Comparison of Motoneuron Firing Characteristics During

Controlled Treadmill Locomotion and Fictive Locomotion

[a] MN	[b] ISI1	ISI2	ISI3	ISI4	ISI5	ISI6	# of spikes per burst	[c] n
Treadmill								
1	4	4.1	7.9	11.3	14.8	18.6	8.4	7
2	11	69.5	36.7	51.7	52.2	36.2	14.8	6
3	6	37.0	37.0	35.5	45.0	29.3	19.0	4
4	7	107.8	57.7	94.8	61.9	87.3	11.7	9
5	7.5	36.6	38.3	28.6	35.0	38.8	13.6	8
Mean	7.1	51.0	35.5	44.4	41.8	42.0	13.5	
[d] S.D.	2.6	39.3	17.8	31.7	18.0	26.5	3.9	
Fictive								
6	12.9	46.9	41.4	40.0	42.9	44.6	10.3	10
7	9.0	23.6	18.9	24.1	21.9	28.6	16.0	7
8	6.4	12.3	30.9	28.5	42.1	37.1	14.2	15
9	19.4	46.4	45.6	44.4	34.0	43.0	9.2	9
10	38.7	39.1	57.7	60.0	70.2	70.7	6.8	10
11	35.4	65.2	76.1	82.5	85.9	90.0	6.2	10
12	10.7	20.7	30.0	25.0	31.7	31.7	8.7	3
13	21.7	43.8	38.3	30.0	28.8	28.8	13.6	8
14	66.5	64.4	57.3	52.2	45.0	45.0	8.8	10
15	27.9	36.4	39.4	39.2	34.2	34.2	23.4	10
16	72.9	66.9	55.0	58.4	72.5	72.5	6.6	14
Mean	29.2	42.3	44.6	44.0	46.3	47.8	12.4	
S.D.	22.6	18.5	16.0	18.0	20.7	20.6	3.7	

[a] MN = Motoneuron
[b] ISI = Interspike interval
[c] n = Number of trials
[d] S.D. = Standard deviation

Table 7

Effect of Antidromic Ventral Root Stimulation on IaIN Spontaneous Activity

and Discharge During Fictive Locomotion

IaINs	+n	Spontaneous Activity				Fictive Locomotion				
		Fi	Fs	ΔF	% Decrease	+n	Fi	Fs	ΔF	% Decrease
1	5	71.9	0.0	71.9	100.0%	31	125.1	87.9***	37.2	29.7%
2	5	33.5	2.5	31.0	92.5%	24	19.2	12.2**	7.0	36.5%
3	5	16.0	0.0	16.0	100.0%	16	65.5	36.4***	29.1	44.4%
4	5	15.0	0.0	15.0	100.0%	3	8.8	3.7**	5.1	60.0%
5	5	5.1	0.1	4.9	96.1%	19	12.7	5.7***	7.0	55.1%
6	5	40.1	13.4	26.7	66.6%	4	49.0	29.0**	20.0	40.8%
X̄		30.2	2.7	27.6	92.5%		60.3	39.5	20.8	34.5%

+n = Number of trials

* Indicates level of significance between Fi and Fs during fictive locomotion tested with a paired t test (* = p ≤ .05; ** = p ≤ .01; *** = p ≤ .001).

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Table 8

Results of Multiple Correlation and Regression Analysis on Relationships

Among Initial IaIN Firing Rate (Fi), Discharge During

Antidromic Ventral Root Stimulation (Fs), Absolute

(ΔF) and Relative ((Fi - Fs/Fi) 100) Amount of Recurrent

Inhibition During Fictive Locomotion

Comparisons	r [a]	T test [b]	b [c]	F test [d]
Fi - Fs	.993	.001	.705	.01
Fi - ΔF	.967	.01	.293	.05
Fi - (Fi-Fs/Fi) 100	.767	N.S. [e]	-.170	N.S.

a   r = correlation coefficient
b   T test = test of significance of r
c   b = regression coefficient
d   F Test = test of significance of b
e   N.S. = Not significant

Table 9

Relationship Between Monosynaptic EPSP Amplitude and Motoneuron

Membrane Potential (E_M) During Fictive Locomotion

Motoneuron	EPSP (mv)	E_M (mv)	r [a]	T [b]	b [c]	F [d]
Ia-26 #5	2.74 (± 0.7)	69.7 (± 2.7)	.485	.001	.118	.01
Ia-8 #3	4.13 (± 2.1)	63.9 (± 2.3)	.884	.001	.863	.01
I1-27-6 #3	3.12 (± 0.6)	78.9 (± 0.6)	.034	N.S. [e]	.040	N.S.
Ia-24 #9	2.50 (± 1.8)	79.3 (± 3.8)	.444	.01	.215	.01

a   r = correlation coefficient
b   T = test of significance of r
c   b = regression coefficient
d   F = test of significance of b
e   N.S. = Not significant

Table 10

Comparison of Monosynaptic EPSP Amplitudes Obtained

at Rest (Control) with the Largest and Smallest

Values Obtained During Fictive Locomotion

Motoneuron	Control	Locomotion	
		Smallest	Largest
Ia-26 #5	2.0 mv	1.1 mv	3.6 mv
Ia-8 #3	2.0	2.0	8.7
I1-27-6 #3	3.5	1.5	4.5
Ia-24 #9	3.0	1.4	4.1
Mean	2.6 ± 0.8	1.5 ± 0.4	5.2 ± 2.4

Table 11

Comparison of Flexor and Extensor Motoneuron Membrane Potential

Oscillations During Fictive Locomotion

*Motoneuron	Type	1 Hyp. Below E_R	2 E_R-PHP	3 Ampl. of E_M	E_R	Action Potential
1	Q	0	3.8 mv	3.0 mv	- 50 mv	60 mv
2	Q	1	- 2.8	7.2	- 130	90
3	Q	1	- 0.4	5.8	- 80	90
4	Q	0	0.5	3.5	- 55	50
5	Q	0	5.0	10.3	- 55	80
6	Q	1	- 1.5	1.6	- 72	
7	Q	0	9.0	15.0		60
8	Q	0	2.5	5.1		60
9	Q	0	0.2	2.5		50
10	Q	0	0.8	1.0		
11	Q	0	1.5	1.5	- 70	85
12	Q	0	2.4	2.0	- 60	110
Flexor Motoneurons						
13	TA	1	- 15.0	24.7	- 60	50
14	SART	1	- 1.0	11.4	- 50	70
15	SART	1	- 3.2	7.2	- 62	

1   Whether motoneuron hyperpolarized below E_R: 1 = yes,   0 = no.
2   Difference between the peak hyperpolarization and the E_R.
3   Amplitude of the membrane potential oscillation during locomotion.
*   The identification of these motoneurons is contained in Appendix F.

Table 12

Phase Relationships Between Extensor-Coupled Renshaw Cell (E-RC) and Extensor

Motoneuron Activity and Between Flexor-Coupled Renshaw Cell (F-RC) and

Flexor Motoneuron Activity During Fictive Locomotion

RC	RC ID	n[1]	Cycle Duration	Ext. Phase Duration	x (Ext)[2]	RC Burst Duration	x (RC)[2]	y (RC)[3]
1 (21)	Q-RC	8	709 msec	264 msec	0.63	291 msec	0.62	1.03
2 (22)	E-RC	5	532	282	0.47	118	0.48	0.70
3 (23)	E-RC	10	630	265	0.58	404	0.33	0.99
4 (24)	Q-RC	10	881	350	0.60	185	0.93	1.07
5	LG-RC	5	645	340	0.53	119	0.64	0.93
6	LG-RC	8	921	589	0.36	365	0.40	0.93
7 (25)	LG-RC	10	700	472	0.33	264	0.45	0.83
Means			717	368	0.50	249	0.55	0.93

RC	RC ID	n[1]	Cycle Duration	Flex Phase Duration	y (Flex)[2]	RC Burst Duration	x (RC)[2]	y (RC)[3]
8 (21)	F-RC	10	564	334	0.59	344	-0.12	0.61
9 (22)	F-RC	10	748	300	0.40	312	0.04	0.42
10 (25)	Pb-RC	9	707	238	0.34	261	0.12	0.37
Means			673	291	0.44	306	0.04	0.47

1 n = Number of trials (step cycles) in which RC activity was measured.
2 x = Time of onset of activity in normalized step cycle.
3 y = Time of offset of activity in normalized step cycle.

Table 13

Phase Relationship Between Extensor Motoneuron Activity and the Activity of

Q-IaINs that Displayed Bursting Behavior During Fictive Locomotion

Q-IaIN	n [1]	Cycle Duration	Ext. Phase Duration	[2] x (Ext)	Q-IaIN Burst Duration	x (Q-IaIN)	[3] y (Q-IaIN)
Ia-10 #5	10	623 msec	266 msec	0.57	408	0.35	1.006
Ia-3 #2	7	651	195	0.70	467	-0.06	0.66
Ia-33 #11	6	868	370	0.57	552	0.41	1.04
Ia-33 #15	7	845	507	0.40	178	0.54	0.75
Means		746.8 msec	334.5 msec	0.55	401.3	0.32	0.86

1 n = Number of step cycles in which Q-IaIN activity was measured.
2 x = Time of onset of activity in normalized step cycle.
3 y = Time of offset of activity in normalized step cycle.

Table 14

Comparison of [*] Frequency of IaIN Discharge at Rest

(Spontaneous Activity) with the Minimum and

Maximum Frequencies Observed During Fictive Locomotion

IaIN	Spontaneous Activity	Locomotion	
		Minimum	Maximum
Ia-10 #1	65 Hz	5 Hz	200 Hz
Ia-2 #7	15	2	50
Ia-33 #11	40	20	125
Ia-33 #15	15	2	250
Mean	33.8	7.3	156.3

[*] Frequency = 1000 msec/interspike interval

Table 15

Effect of Antidromic Ventral Root Stimulation at

Various Frequencies on the Spontaneous Rhythmic

Behavior of Renshaw Cells During Fictive Locomotion

* Renshaw Cell	Cycle Interval	Rhythmic Activity During Ventral Root Stimulation	
		Yes	No
1	2300 msec	+	
2	2280	+	
3	2200	+	
4	2052	+	
5	1140	+	
6	1200	+	
7	920	+	
8	550	+	
9	360	+	
10	220	+	
4	220	+	
6	200		+
11	200		+
12	136		+
13	100		+
14	80		+
5	80	+	

*   Identification of Renshaw cells listed in this table
    is provided in Appendix I.

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Figure 1: Schematic drawing summarizing the synaptic connections among motoneurons (Mns), Renshaw cells (RCs) and Ia inhibitory interneurons (IaINs) within a given motoneuron pool (i.e., FMns, FRCs and FIaINs) and between pools of antagonist motoneurons. Excitatory synapses are represented by a bar and inhibitory synapses by a filled circle. Flexor coupled neurons are indicated by a hatched soma and extensor coupled neurons by a clear zone.

Illustrated at the bottom of the figure is a hypothetical approximation of the phasing that might be expected to occur during locomotion among the phasic activity periods of the elements shown. The traces read from left to right.
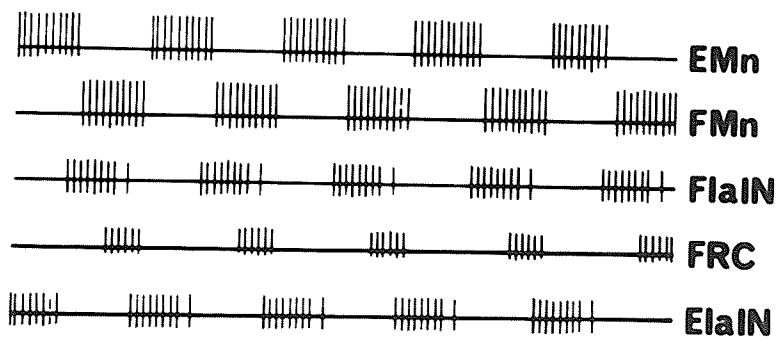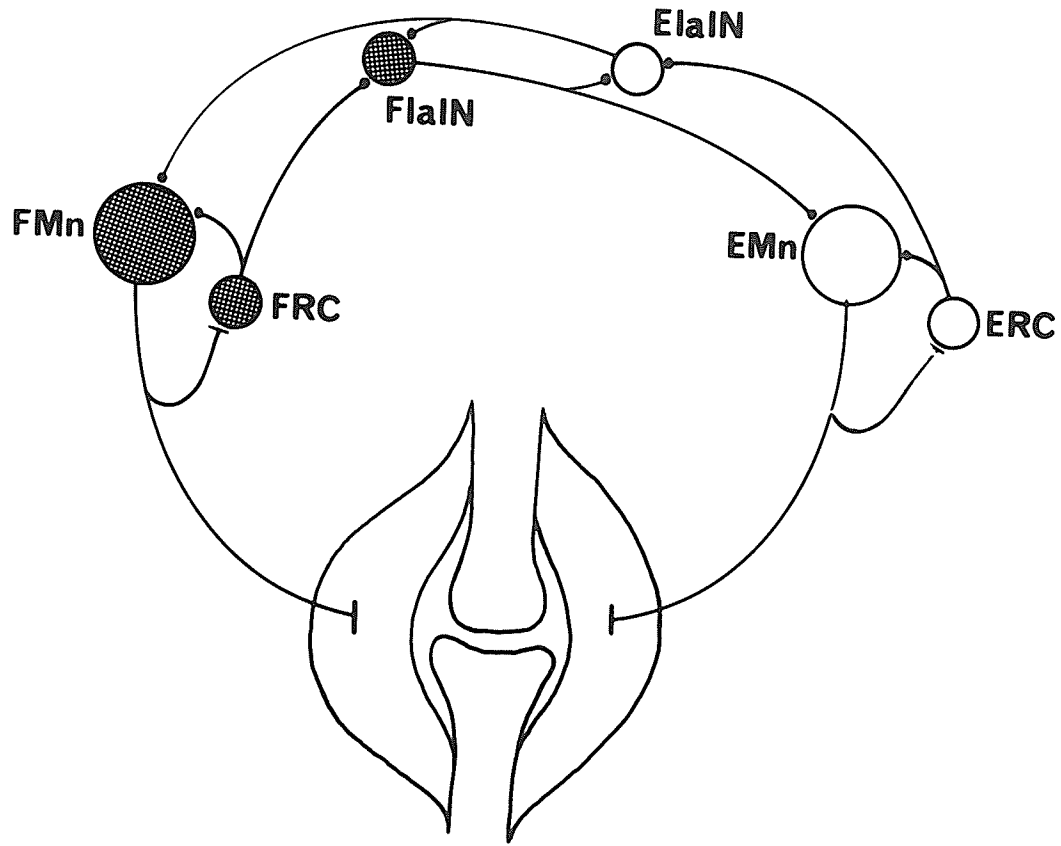
Figure 2: Schematic diagram of a saggital section of the cat brainstem 4.1 mm from the midline. The dorsoventral broken line indicates the plane of the transection used in these experiments. These mesencephalic preparations do not walk spontaneously but will when an area below the inferior colliculus (IC), designated the mesencephalic locomotor region (MLR), is electrically stimulated. The effective site for producing locomotion is indicated in the diagram by a solid black spot between the cuneiform nucleus (CNF) and the brachium conjunctivum (BC). Other abbreviations used in the diagram are:

MB   mammillary bodies

OT   optic tract (chiasm)

PG   pontine gray

R   red nucleus

SN   substantia nigra

SUB   subthalamic nucleus

3n   exit of third cranial nerve

LAT 4.1

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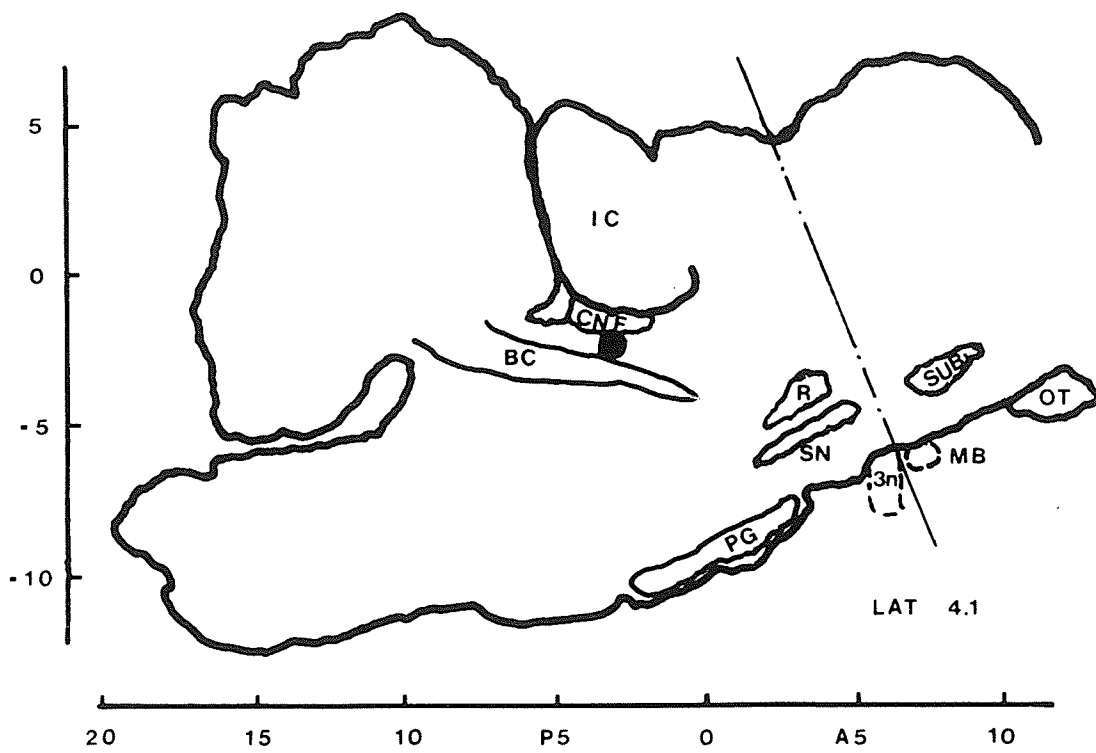
Figure 3: Diagramatic representation of the experimental
arrangement used in the controlled treadmill locomotion
experiments. The cat was supported above the treadmill
belt by the sterotaxic head holder and clamps on the
iliac crests and the L3 spinous process. Constant current
square wave pulses were delivered to a monopolar electrode
in the MLR to induce locomotion. The L7 ventral root (VR)
was mounted on bipolar stimulating electrodes. Signals
from the L7 VR filament were led to a high gain AC
differential preamplifier and then to an oscilloscope and
an audio monitor.

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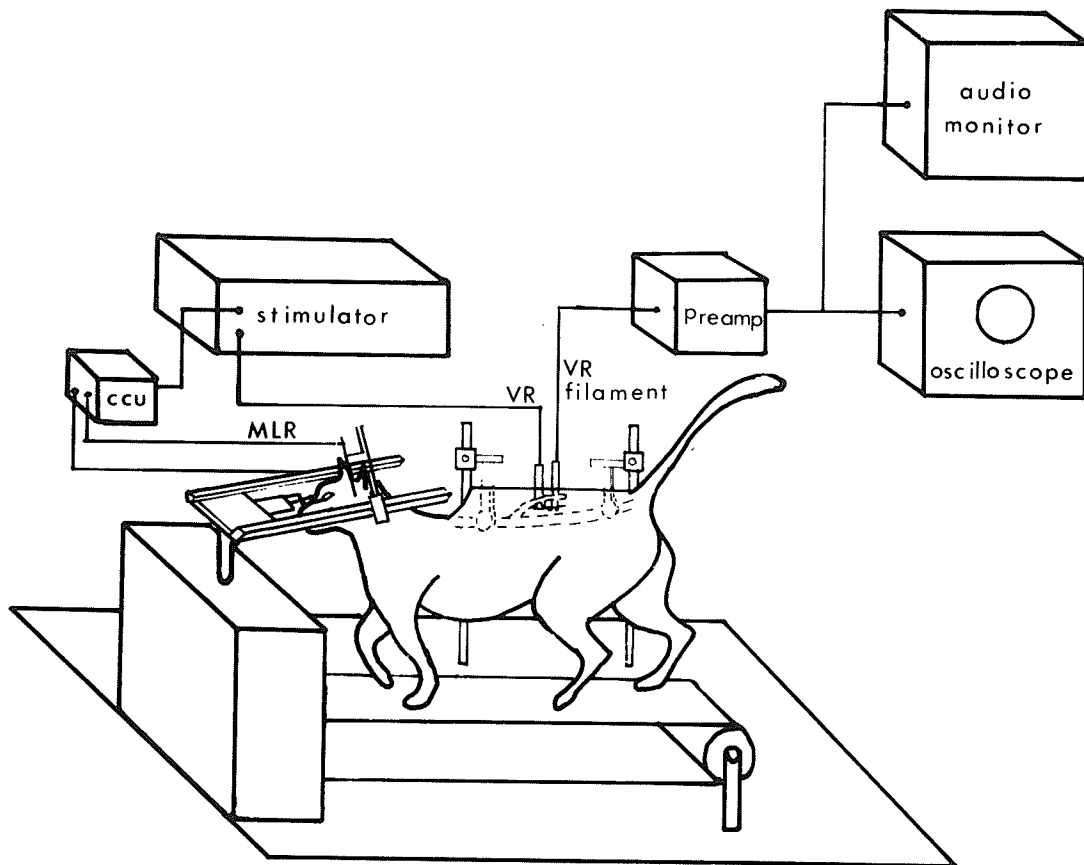
Figure 4: MLR evoked locomotion before and after
paralyzation with gallamine triethiodide. Examples of
motoneuron firing recorded from ventral root filaments
to the left hindlimb in 2 cats (A and B) are shown before
(pre) and after (post) administration of a paralyzing
dose of gallamine triethiodide. The middle trace (R-HL)
in A is a record of right hindlimb movement (flexion
upwards) derived from a potentiometer attached by a lever
to the ankle joint. Flexion of the right hindlimb is
synchronous with activity of quadriceps motoneurons in
the left L7 ventral root filament. The stimulus to the
MLR was applied at the time indicated by the stimulus
artifact in A and at the beginning of the record in B.
The stimulus was maintained throughout the illustrated
periods in both cases.

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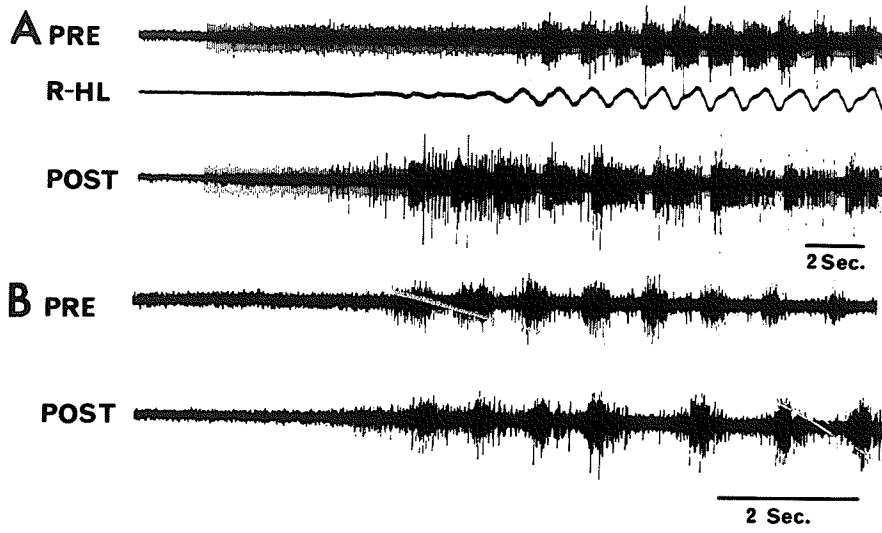
Figure 5: Schematic representation of the experimental arrangement used in the fictive locomotion experiments. Constant current square wave pulses were delivered to a monopolar electrode in the MLR to induce locomotion. The L5-S1 ventral roots (VRs) were cut and mounted on bipolar stimulating electrodes. Nerve cuffs were fastened about the nerves to the muscles listed. A silver tipped ball electrode was placed at the dorsal root entry zone to record cord dorsum potentials. Signals from a VR filament were led to a high gain AC differential preamplifier (W.P.I. DAM 5A) and then to an audio monitor and the oscilloscope. The activity of spinal neurons was recorded by a microelectrode which was connected to an electrometer (W.P.I. Model 4A). The output of the electrometer was led to AC and DC coupled vertical differential amplifiers (Tektronix 5A22N) and to an audio monitor. The circle in the upper right hand corner represents the face of the oscilloscope (Tektronix 565 dual beam) on which the AC and DC coupled records from the microelectrode and the cord dorsum potential were displayed at a fast sweep speed and the VR filament at a slow sweep speed. The data was stored on a 4 channel FM tape recorder (Hewlett Packard 3690) and/or photographed from the oscilloscope with a camera (Grass C4N Kymograph).

FM TAPE
RECORDER

AUDIO
MONITOR

VR FILAMENT

L5-S1
VR5

SURAL
TA
LG
SM
ST
PB
GRAC
SART
QUAD
VENTRAL ROOTS **LEG NERVES**

DR5

CORD
DORSUM

CONST. CURR.
STIMULATOR

CONST. CURR.
STIMULATOR

MLR

SLOW

FAST

FAST

FAST

DC

AC

AUDIO
MONITOR

279
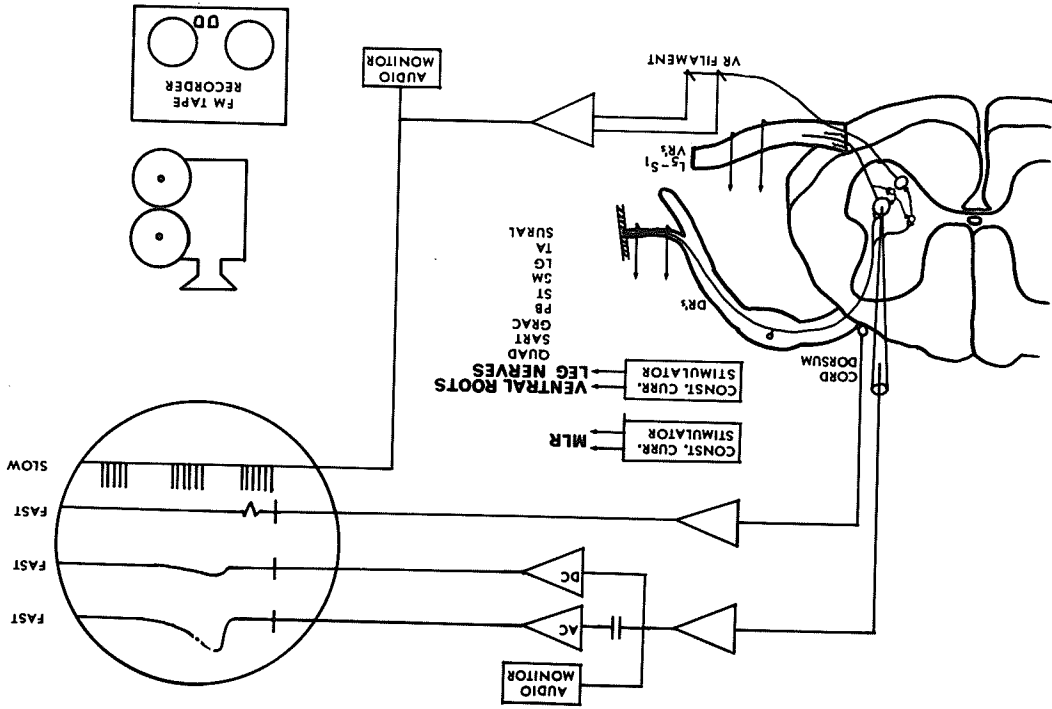
Figure 6: Illustration of procedure used to construct the frequency histograms, activity periods and membrane potential oscillation plots in normalized step cycles. The total duration of the step cycle (T) is measured from the onset of the flexor motoneuron burst in the ventral root filament (upper trace). F refers to the flexion phase and E the extension phase of the step cycle. The bottom trace is the intracellular record of a tibialis anterior motoneuron (TA-MN). Both recordings were obtained simultaneously during fictive locomotion in a mesencephalic cat. The initiation of neuronal activity (X) is measured from the onset of the step cycle to the onset of cell activity. Y represents the point at which the cell stops firing. The step cycle is divided into 10 equal intervals and the frequency of cell activity and the membrane potential is measured in each bin.

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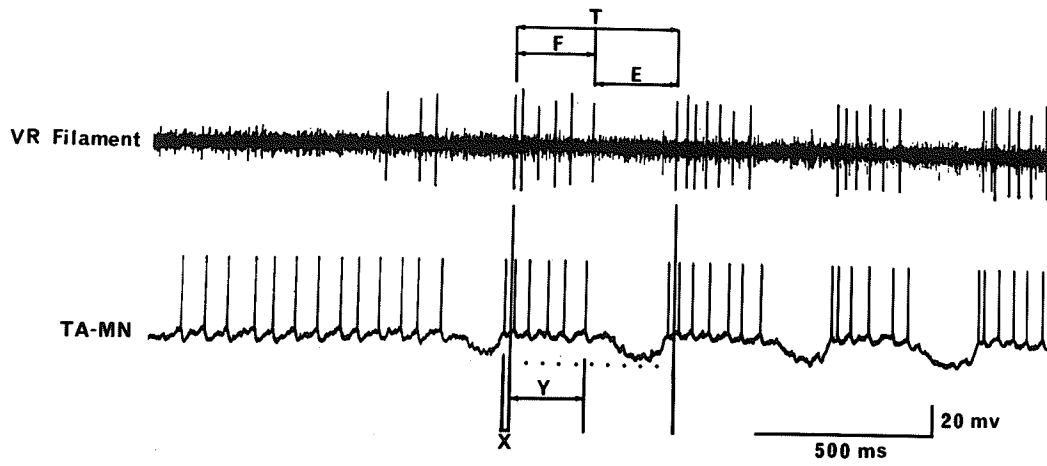
Figure 7: Examples of measurements from a tibialis
anterior (TA) motoneuron and an interneuron monosynaptically
excited by stimulation of the lateral gastrocnemius nerve
(LG-IN) are illustrated. Graphs were constructed from means
of measurements at 10 separate points during 10 consecutive
step cycles for each cell. The upper trace shows the
motoneuron membrane potential depolarization (upward
deflection) or hyperpolarization relative to the membrane
potential prior to the initiation of locomotion (E_R). The
histogram shows the firing frequency (spikes/second) of
the LG-IN during the step cycle. The lower panel shows the
duration of activity in the 2 cells relative to the firing
of a TA MN in the L7 ventral root filament. The non-
normalized activity of the TA MN and the ventral root
filament are illustrated in Figure 6.

TA-MN

DC
4
2
(E_r) 0
-2
-4
-6
-8

TA-MN

Frequency
2
1

LG-IN

Frequency
14
12
10
8
6
4
2
0

TA-MN
LG-IN
Filament

0 .1 .2 .3 .4 .5 .6 .7 .8 .9 1.0

STEP CYCLE

283
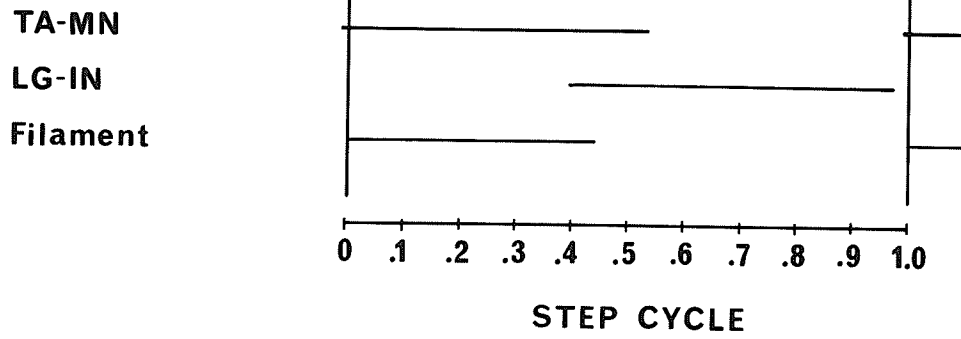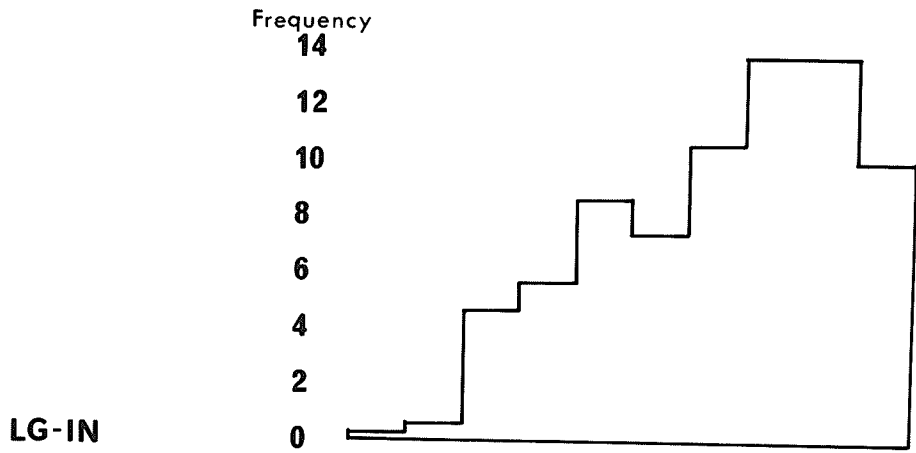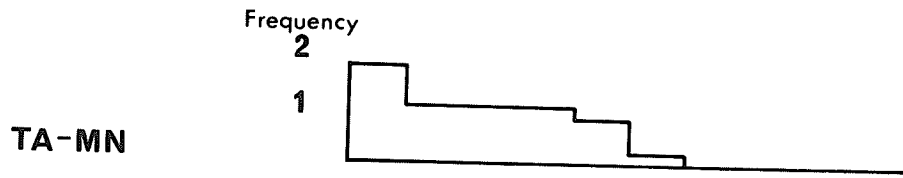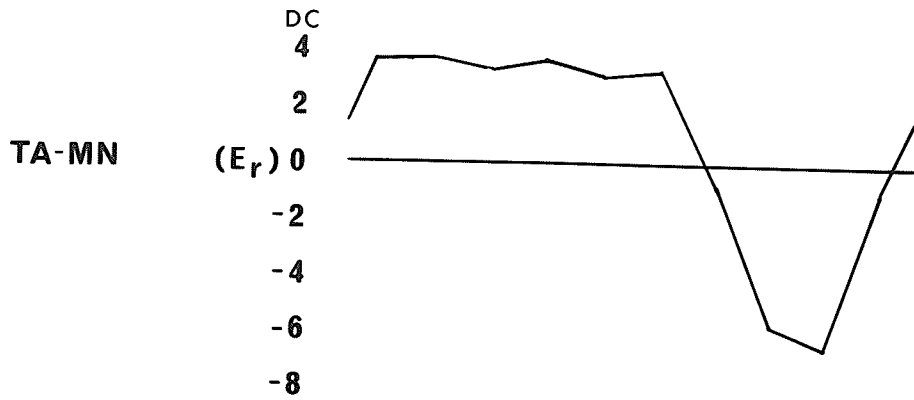
Figure 8: Effects of antidromic stimulation of the left L7 ventral root on three motoneurons of the left hindlimb recorded in an L7 ventral root filament during cyclic passive limb movement (1A, 1C) and during locomotion (1B, 1D). Records A and B are from a different filament than C and D. The larger amplitude spike in 1A and 1B (motoneuron #14 in Table 1) is a knee extensor. An ankle extensor (smaller amplitude spike) is also activated during cyclic passive limb movement but not during locomotion. Two motoneurons are identifiable in 1C and 1D, both ankle extensors. The larger amplitude spike (motoneuron #9) shows greater reduction during cyclic passive limb movement than during locomotion while motoneuron #10 was almost completely inhibited during both conditions. The stimulus artifact above each record marks the period of antidromic stimulation.

1 sec.

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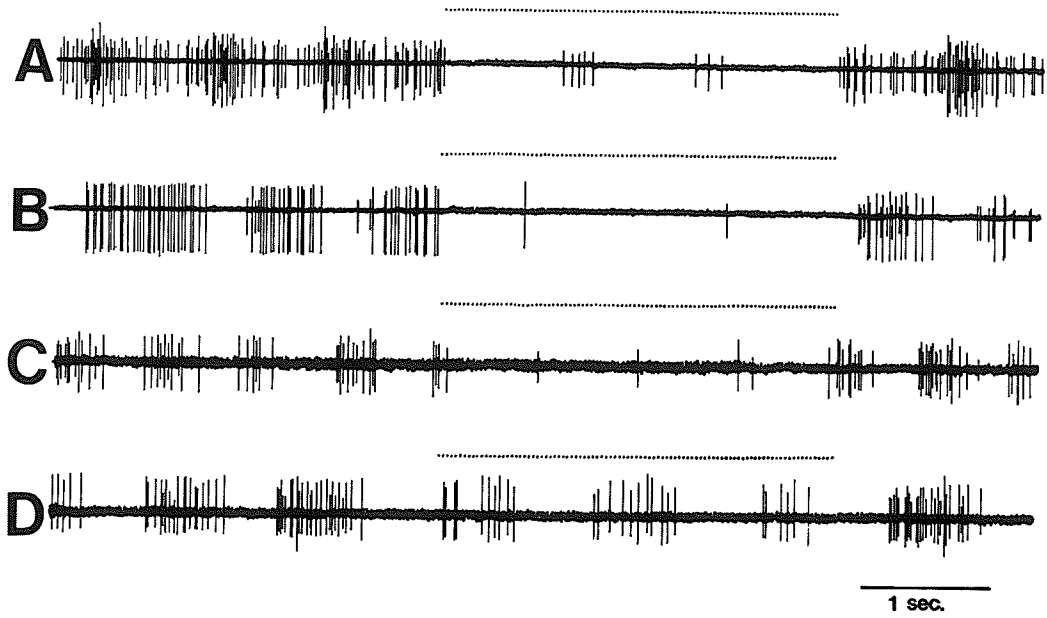
Figure 9: Relationship between motoneuron discharge

frequency (spikes/second) before (Fi) and during (Fs)

antidromic stimulation of an L7 ventral root (9A);

9B shows the relationship between Fi and the amount of

recurrent inhibition ($\Delta F = Fi - Fs$). Data obtained

during cyclic passive limb movement and controlled

treadmill locomotion are illustrated. The correlation

coefficients (r) and regression coefficients (b) are

indicated in each graph. The standard error of the

estimate is drawn at the means for each regression line.
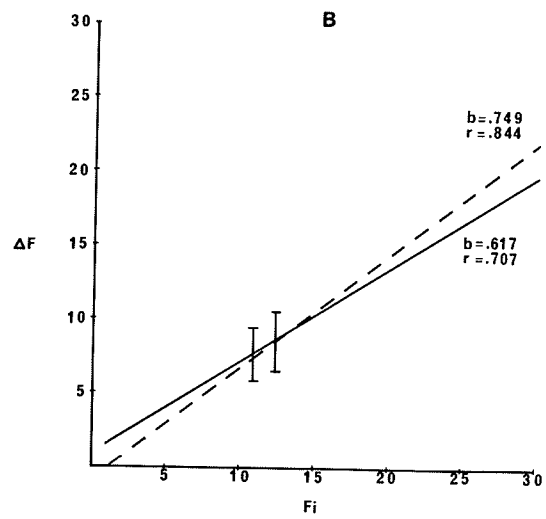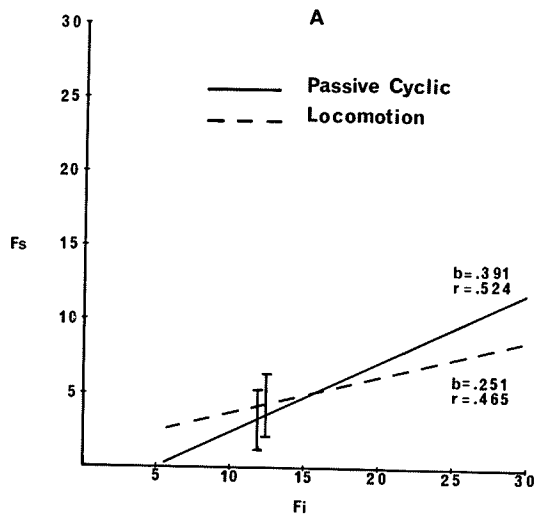
Figure 10: Motoneuron discharge in an L7 ventral root filament during static muscle stretch (10A and B), passive cyclic limb movement (10C) and controlled treadmill locomotion (10D) which exhibits a greater incidence of doublet firing during antidromic stimulation of the L7 ventral root. The dots above each trace indicates the period of ventral root stimulation. Record B is an expanded version of 10A; traces A, C and D were all filmed at the same speed. The larger amplitude spike is motoneuron 1 and the smaller one is motoneuron 2 from Table 1.

A

B

250 ms

C

D

500 ms
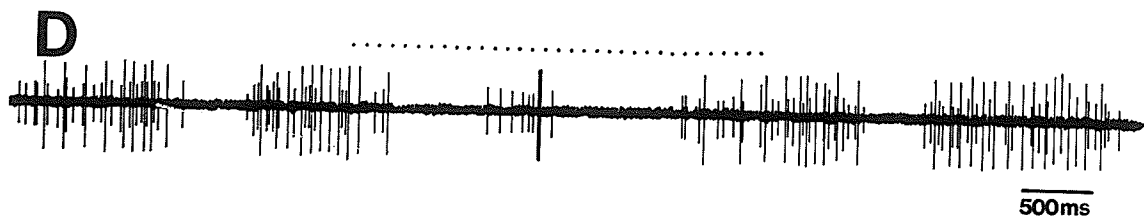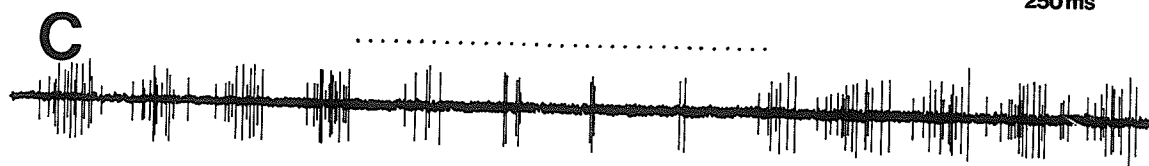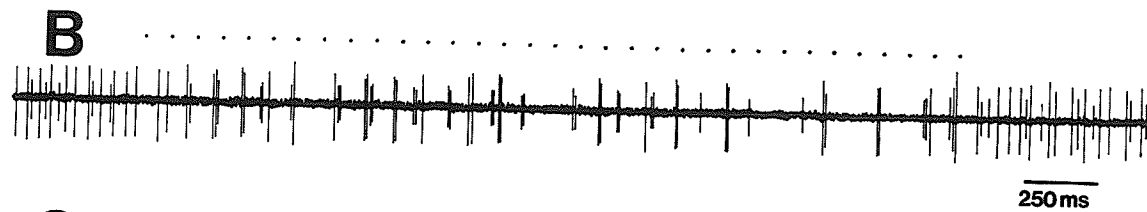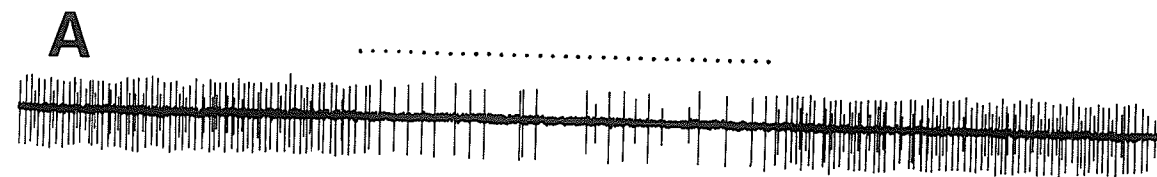
Figure 11: Mean interspike interval (ISI) duration for the first 6 ISIs observed in 5 motoneurons recorded in ventral root filaments during controlled treadmill locomotion (solid line) and in 11 motoneurons recorded intracellularly or from ventral root filaments during MLR-evoked fictive locomotion (broken line).
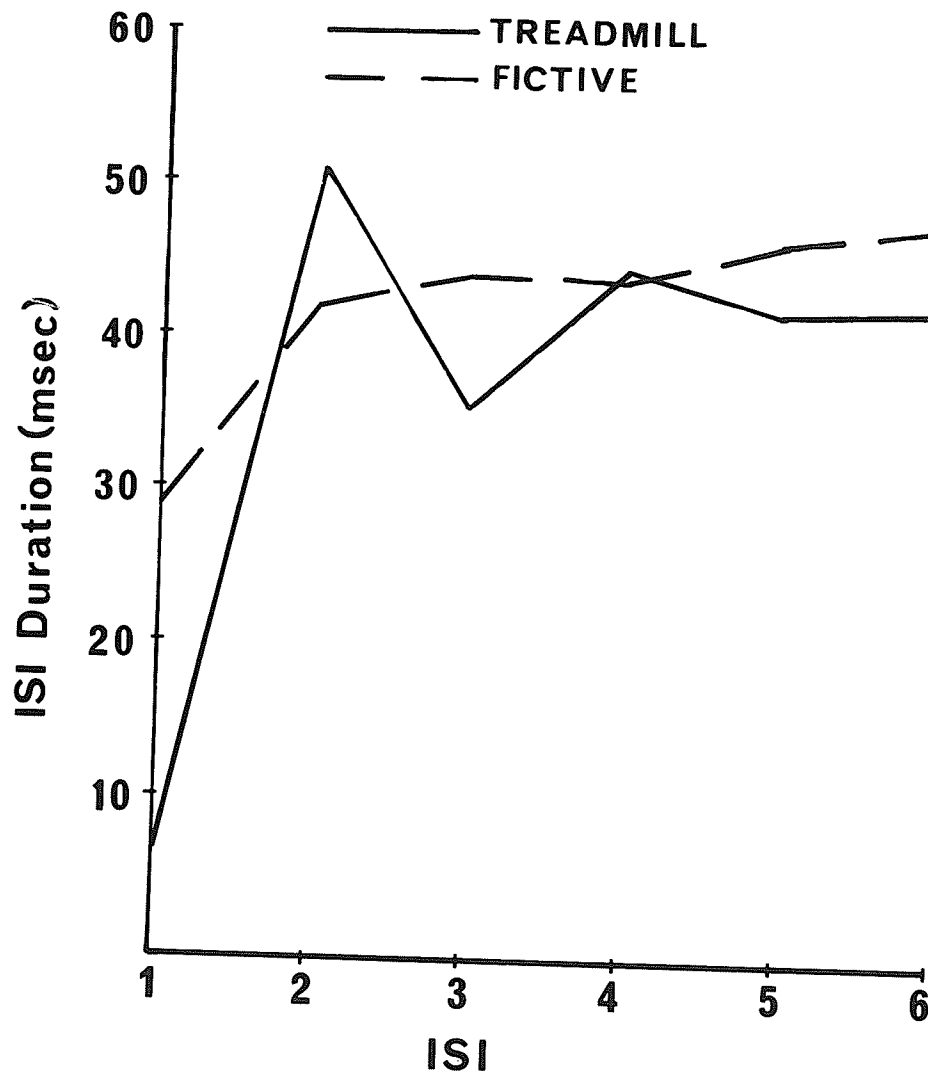
Figure 12: Identification of IaINs. The upper trace in A - E is the microelectrode recording of a quadriceps-coupled IaIN (Ia-10#1). The bottom trace in panels A - D is the record from the cord dorsum electrode situated at the L6 dorsal root entry zone. The bottom trace in E is the recording of an L6 ventral root filament. The calibration shown in panel B applies to panels A - D. Panel A shows the monosynaptic (0.8 msec) response of the IaIN to stimulation of the Ia afferents in the nerve to Q quadriceps. Panel B shows the faithful response of the IaIN to 250 Hz stimulation. The microelectrode slipped outside of the cell after high frequency stimulation. The traces shown in panel C are several superimposed sweeps showing the repeated activation of the IaIN at short latency to low threshold stimulation of the Q nerve. In D a stimulus pulse delivered to the cut L6 ventral root 10 msec prior to the stimulation of the Q nerve blocked the activation of the IaIN. The record in E shows the tonic spontaneous activity of the IaIN at rest (note the absence of activity in the filament). The small vertical lines are the stimulus artifacts marking the period of antidromic ventral root stimulation. It can be seen that the activity of the IaIN is completely abolished by the train of ventral root stimulation and by the single spurrious stimulus which preceded each train by about 250 msec.

A

B

$\rfloor$1mv

2ms

C

D

E

$\overline{\text{250 ms}}$

293
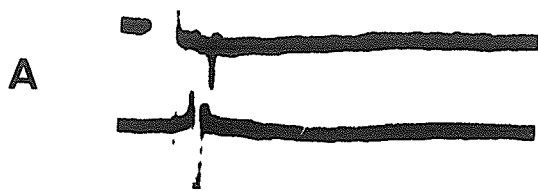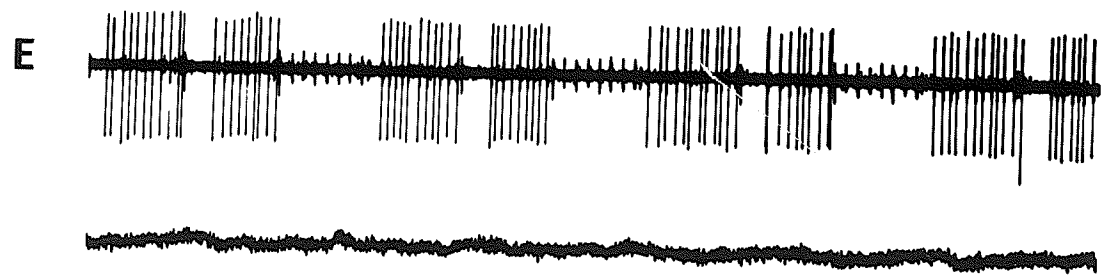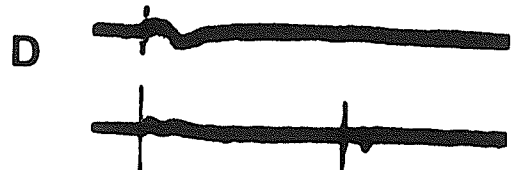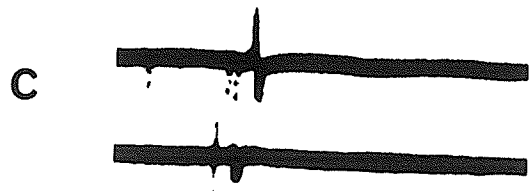
Figure 13: Effects of antidromic ventral root stimulation on spontaneous IaIN activity (A1, B1) and IaIN discharge during fictive locomotion (A2, B2). The cell illustrated in A1 and A2 is IaIN #1 and the one shown in B1 and B2 is IaIN #5 from Table 7. The periods of ventral root stimulation are indicated by the stimulus artifact in A1, B1 and B2 and by the horizontal lines drawn above the record in A2. The bottom trace in each record is the recording from an L6 ventral root filament. IaINs #1 and #5 were recorded in different experiments.

500 ms

295
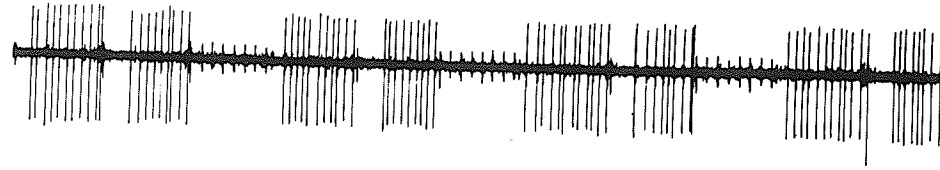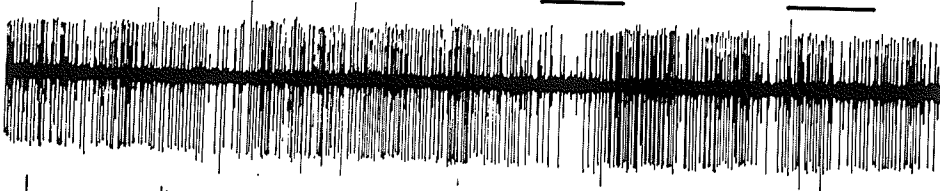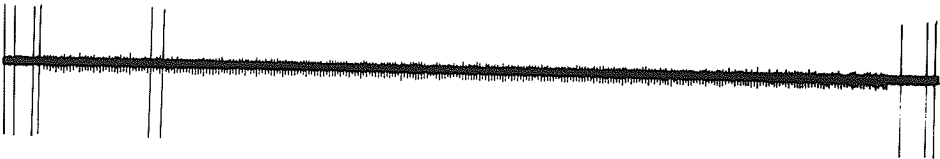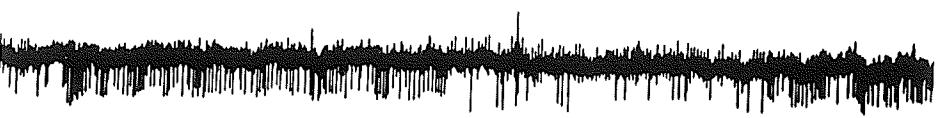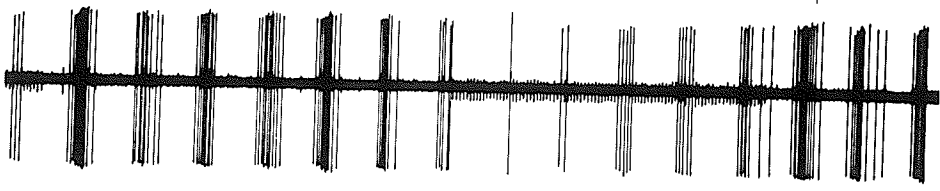
Figure 14: Relationship between IaIN discharge before
(Fi) and during (Fs) antidromic ventral root stimulation
(solid line) and between Fi and the amount of recurrent
inhibition ($\Delta F = Fi - Fs$) (broken line). The correlation
coefficients (r) and regression coefficients (b) are
presented for each line. The standard error of the
estimate is drawn in at the means for each regression
line.

297
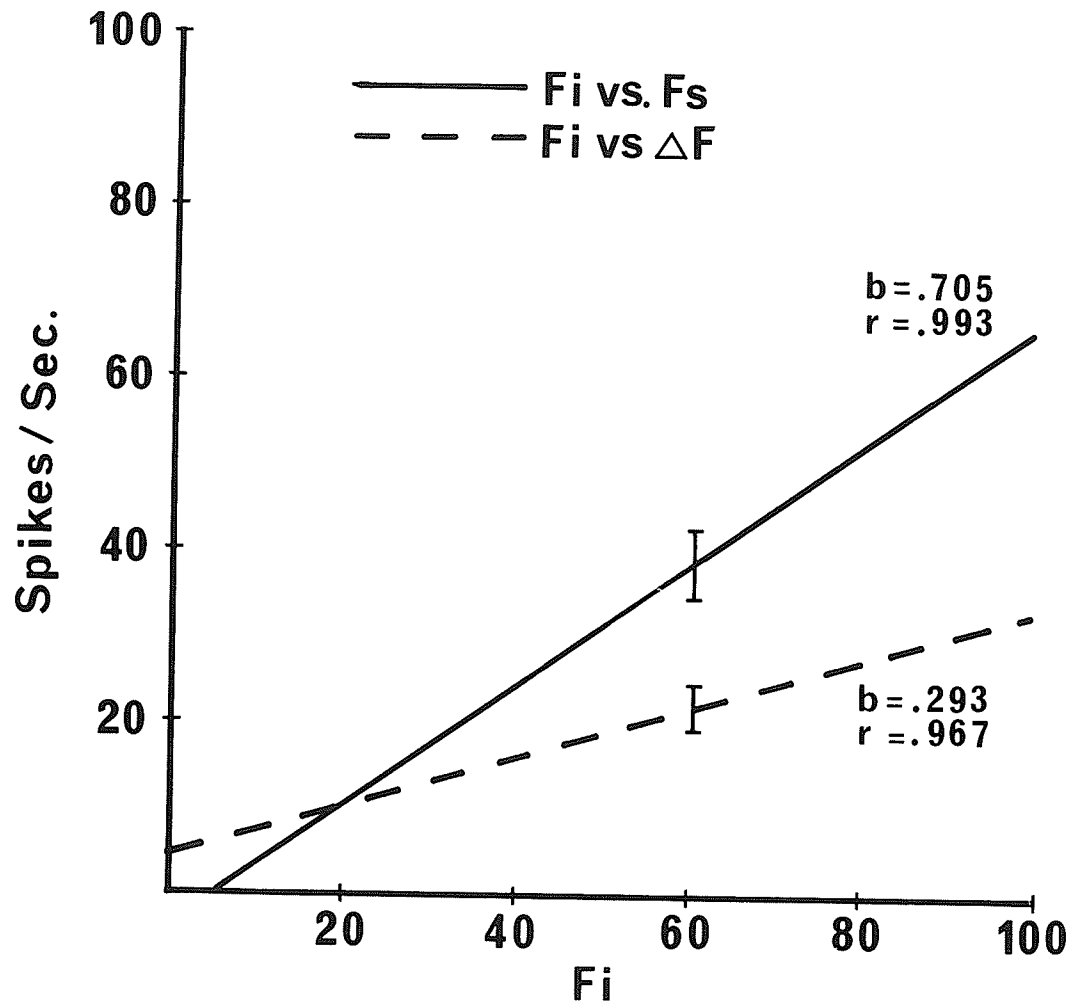
Figure 15: Monosynpatic EPSPs evoked in 2 motoneurons.

Ia-25 #5 (14A) and Ia-8 #3 (14B) during fictive locomotion.

The arrowheads mark the evoked EPSPs. The time scale

shown in B applies to both records. In both A and B,

membrane potential shifts in an upward direction indicate

depolarization. In B, 4 msec pulses of hyperpolarizing

current were injected through the recording microelectrode

just prior to the generation of the EPSP to measure membrane

resistance. The amplitude of these pulses (downward

deflections) is inversely related to changes in membrane

conductance.

299
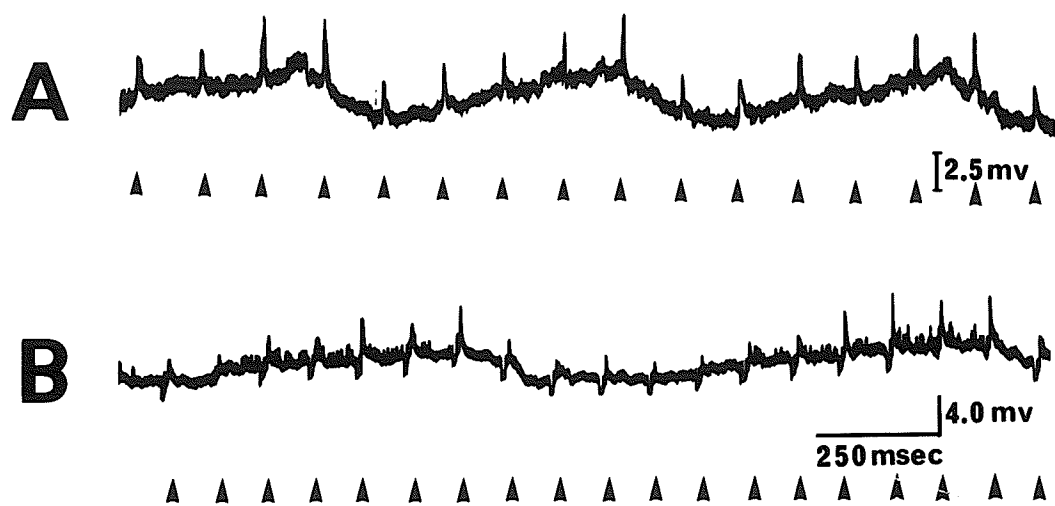
Figure 16:   Relationship between the amplitude of Ia

EPSPs and motoneuron membrane potential (E_M) for two

motoneurons, Ia-8 #3 (solid line) and Ia-26 #5 (broken

line).   The regression (b) and correlation (4)

coefficients are presented for each regression line.
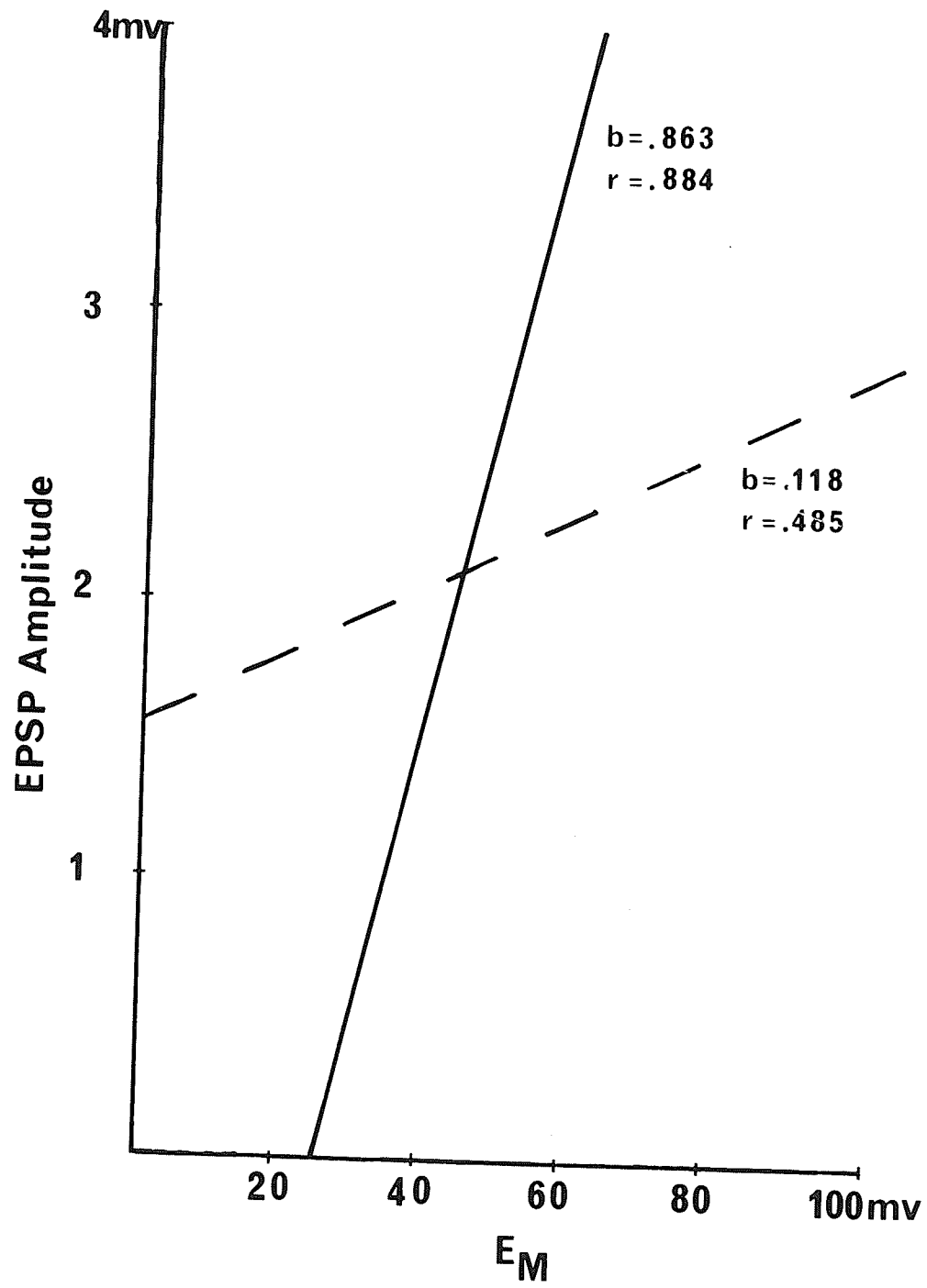
Figure 17: TA Ia IPSPs evoked in a Q motoneuron (Ia-2 #6)
during fictive locomotion. A. Monosynaptic EPSP evoked at
Ia strength. The upper trace is the AC coupled and the
bottom trace the DC coupled microelectrode records. The
calibration refers to the AC trace. The middle trace is
the cord dorsum potential which shows that the stimulus
strength was at group I strength. B. Antidromically evoked
action potential produced by stimulation of the proximal
portion of the severed L5 ventral root. C. TA IPSP evoked
by stimulation of the nerve to TA at Ia strength and at a
latency of 2 msec. D. The continuous record is the DC
coupled record of the motoneuron during fictive locomotion.
The trace above it shows the A gate pulses which were evoked
simultaneously with the stimulus pulse to the nerve to TA.
The IPSPs that occur at A gates numbered 1 - 4 are presented
again at higher gain and a slower sweep speed in the bottom
of the figure. IPSPs 1 and 2 were evoked when the
motoneuron membrane potential was more depolarized and are
substantially larger than IPSPs 3 and 4 which were evoked
when the motoneuron was inactive.

303
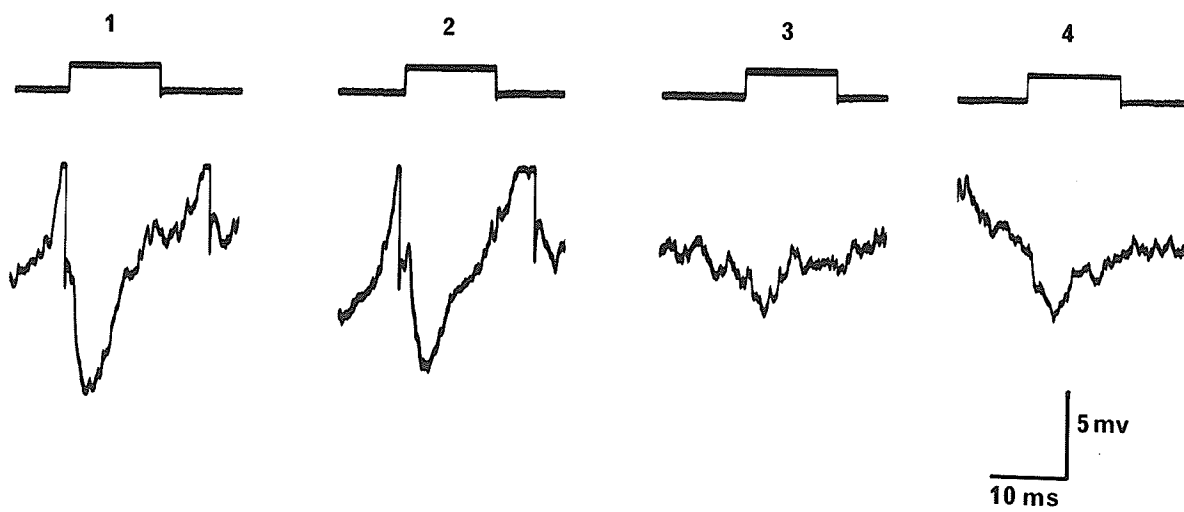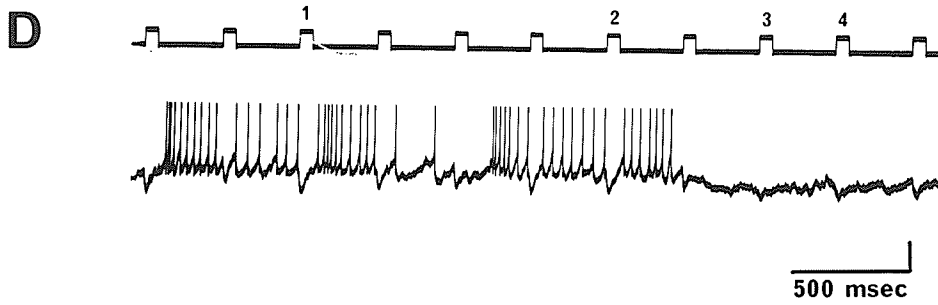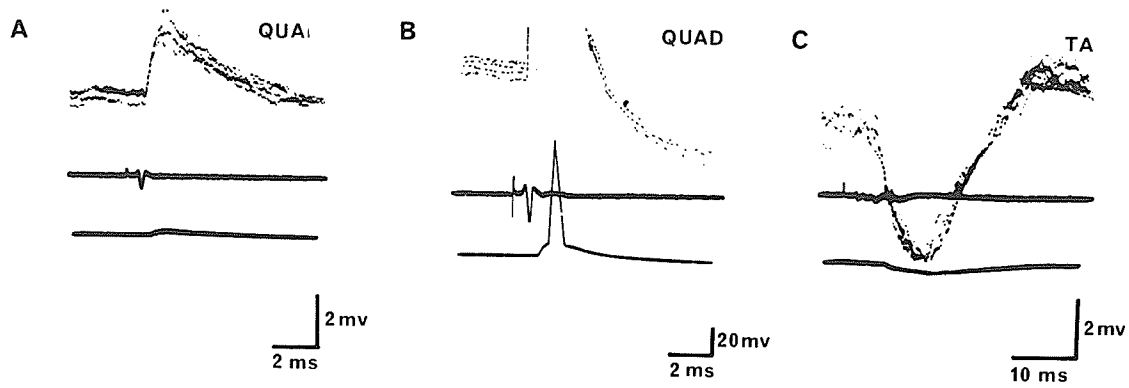
Figure 18: Relationship between Ia IPSP amplitude and motoneuron membrane potential for two Q motoneurons, Ia-2 #6 (solid line) and I1-27-6-78 #10 (broken line). The regression (b) and correlation (r) coefficients are presented for each regression line, and the standard error of the estimate is drawn at the means for each line. The lower X axis is associated with the solid regression line and the upper X axis with the broken line. The resting membrane potential (E_R) is indicated by an arrow for each motoneuron. In both motoneurons, the IPSP was evoked by stimulation of the nerve to the TA muscle at Ia strength and at latencies that were clearly disynaptic ($\leq$ 2 msec).

305
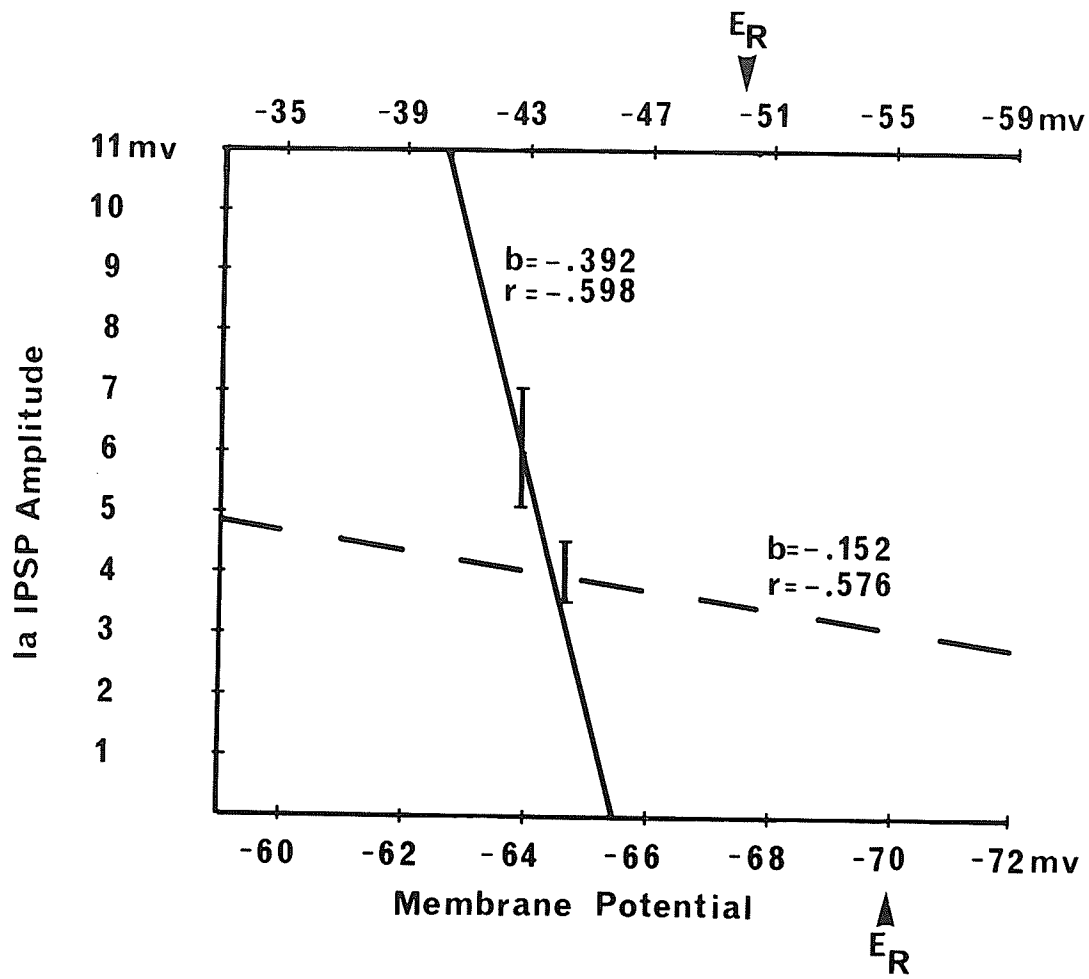
Figure 19: Membrane potentials taken from the DC record of 4 Q motoneurons recorded during fictive locomotion. The membrane potential plots represent the oscillation about the resting membrane potential (E_R). The abscissa represents the normalized step cycle. Each measurement point is the mean measured over 10 step cycles. The motoneurons illustrated are, from top to bottom, motoneurons 7, 1, 2 and 3 from Table 11.
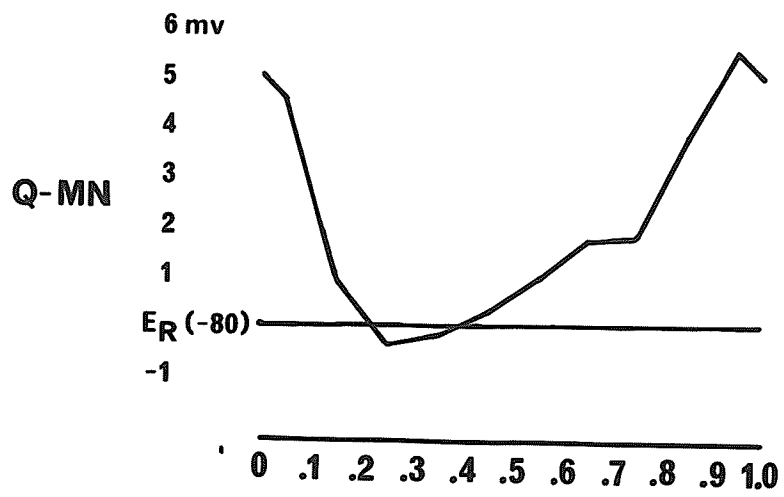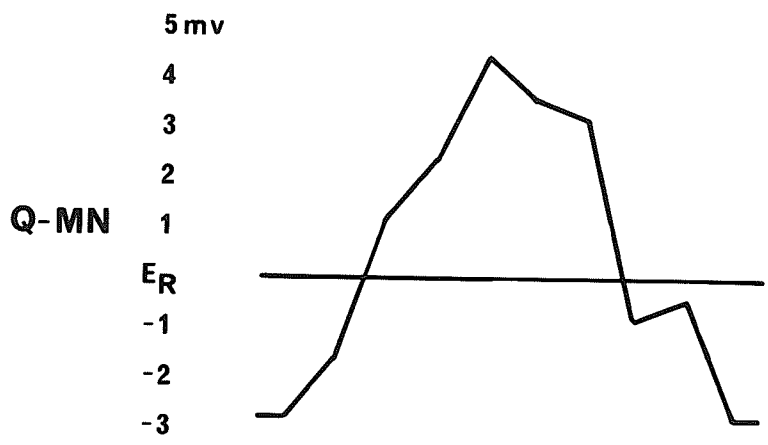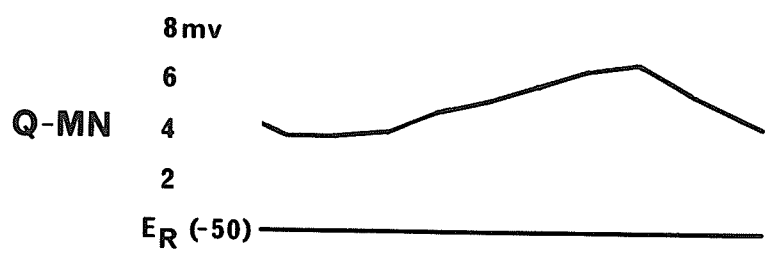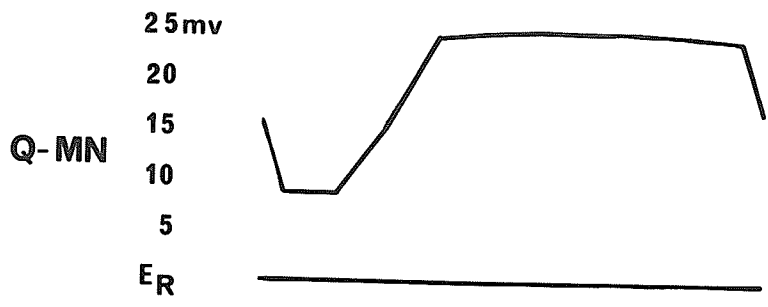
Figure 20: Membrane potentials taken from the DC record of 3 flexor motoneurons recorded during fictive locomotion. The membrane potential plots represent the oscillation about the resting membrane potential (E_R). The abscissa represents the normalized step cycle. Each measurement point is the mean measured over 10 step cycles. The motoneurons illustrated are, from top to bottom, motoneurons 13, 14 and 15 from Table 11.

TA-MN

10mv
5
E_R(-60)
-5
-10
-15

SART-MN

12mv
10
8
6
4
2
E_R(-50)
-2

SART-MN

4mv
3
2
1
E_R(-62)
-1
-2
-3
-4
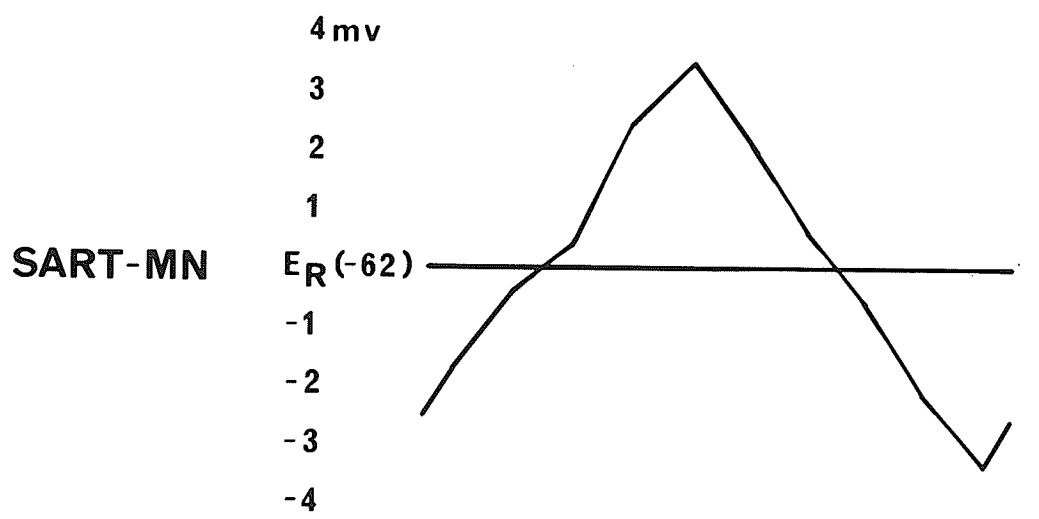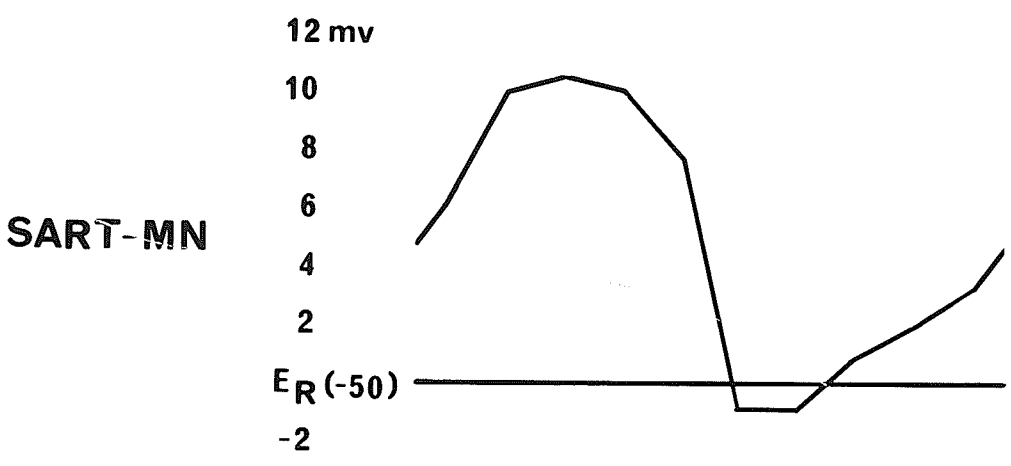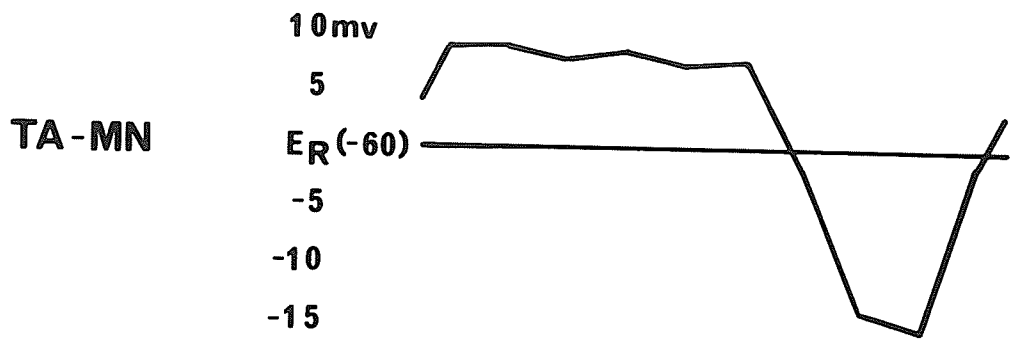
0   .1   .2   .3   .4   .5   .6   .7   .8   .9   1.0

Figure 21: Normalized data from experiment Ia-10 showing

the phase relationships among the fictive locomotor activity

of 2 quadriceps-coupled IaINs (A and B), a quadriceps-

coupled RC (C), a flexor-coupled RC (D) and an interneuron

which was monosynaptically excited by low threshold

stimulation of the nerve to quadriceps but which was not

inhibited by ventral root stimulation (E). The data

represent the means of measurements made during 20, 10, 8,

10 and 10 step cycles for neurons A through E, respectively.

The motoneurons in the ventral root filament were extensor

motoneurons; the vertical hashed line represents the

division between the flexion and extension phases of the

step cycle as based upon the activity in the filament.

Records of the non-normalized fictive locomotor activity of

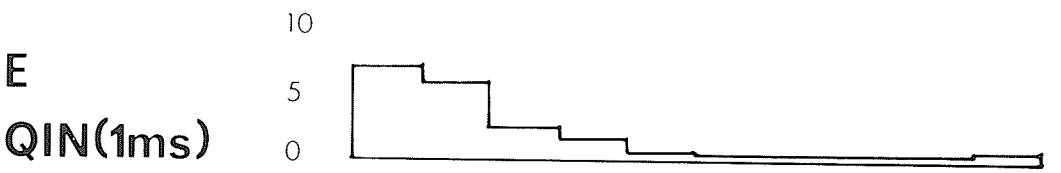the neurons illustrated in this figure are shown in Figure

27.

Frequency

A
QIaIN

B
QIaIN

C
QRC

D
RC

E
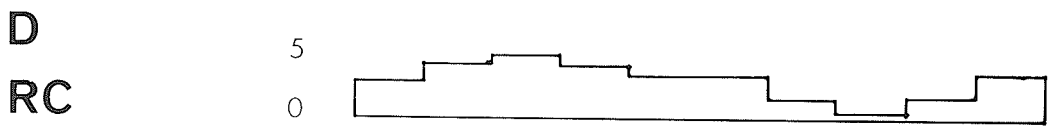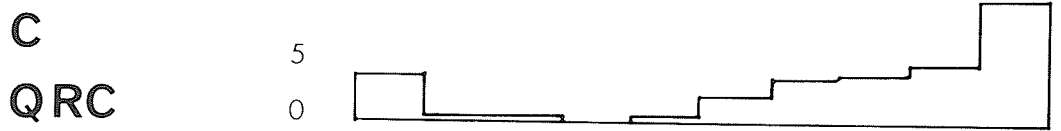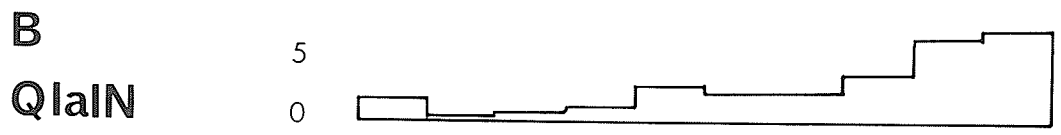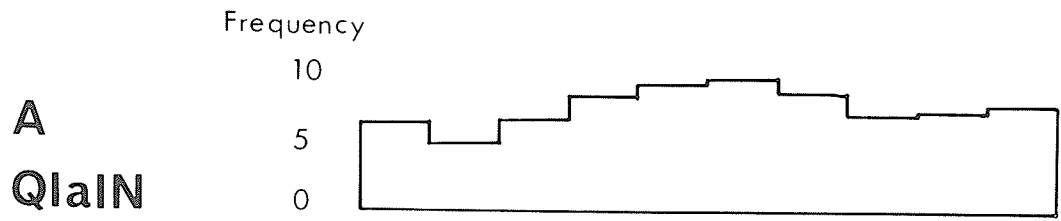QIN(1ms)

B
C
D
E
Q-MN
Filament

STEP CYCLE

311
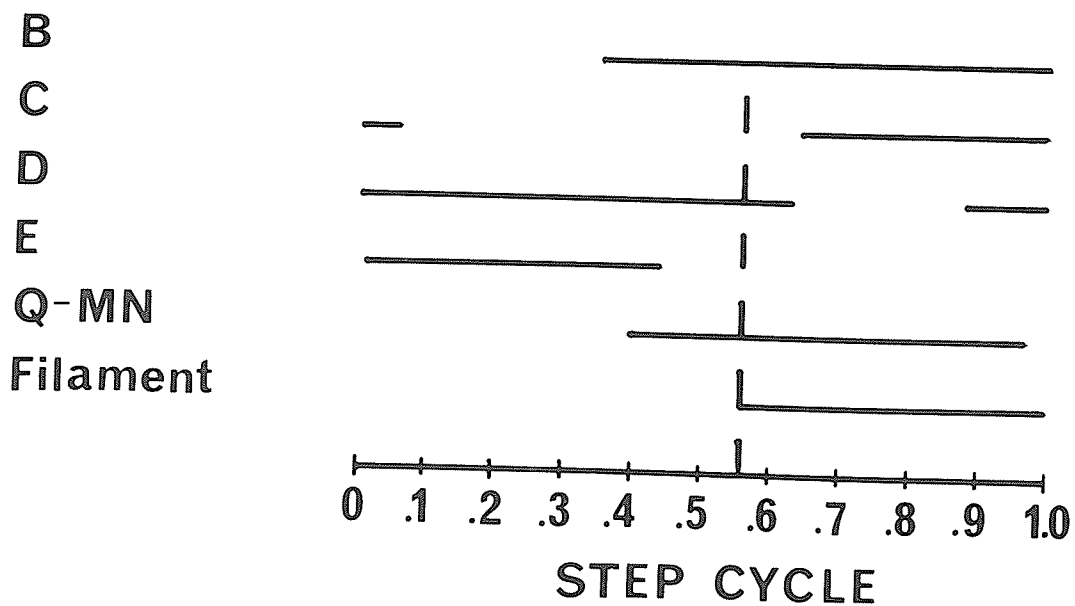
Figure 22: Normalized data from experiment Ia-24 showing

the phase relationships among the activity of an

intracellularly recorded quadriceps motoneuron (A), an

extensor-coupled RC (B) and a flexor-coupled RC (C) during

fictive locomotion. The motoneurons in the ventral root

filament were knee flexor motoneurons. The data represent

the means of measurements made during 5, 6 and 11 step

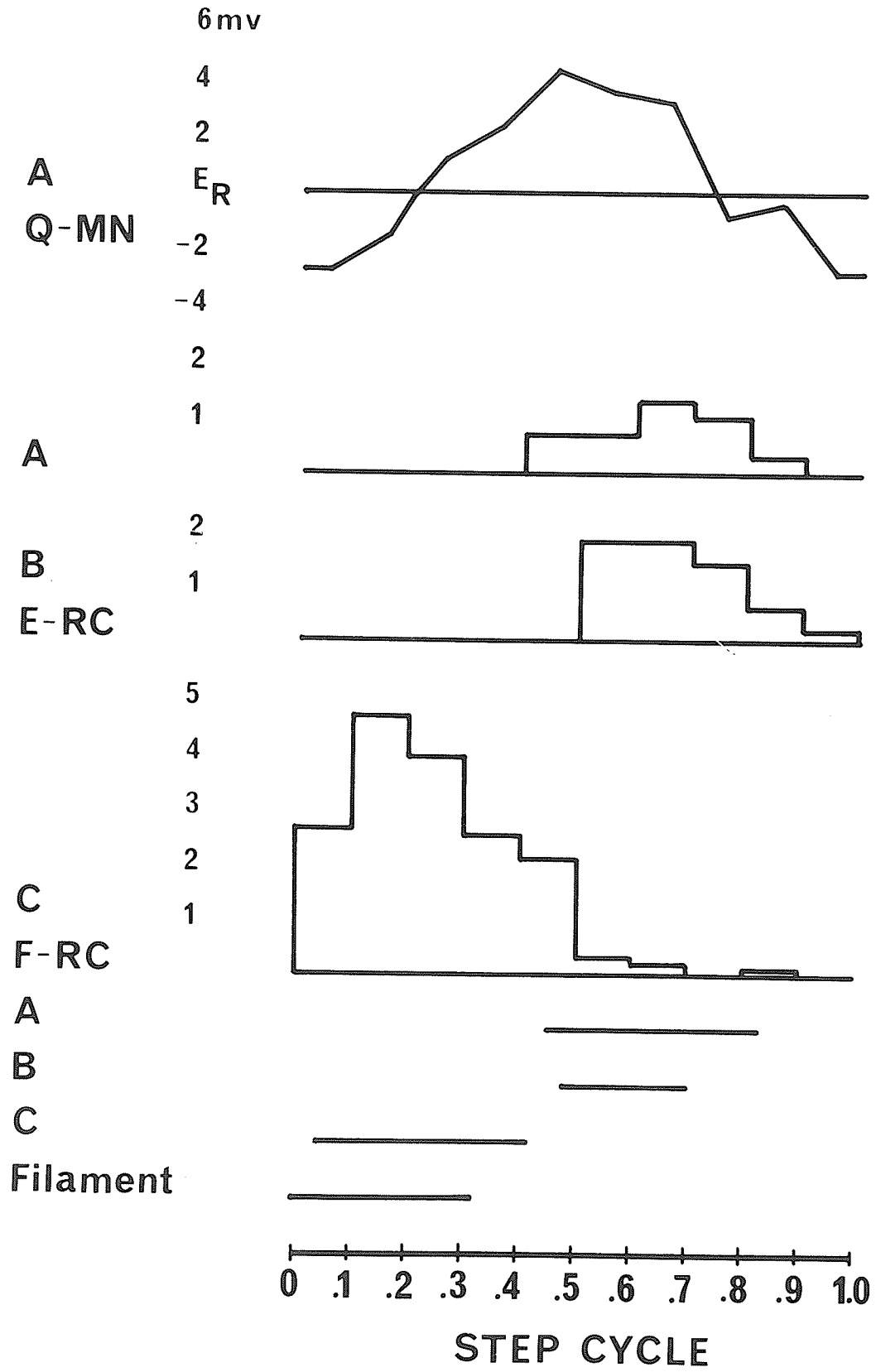cycles for neurons A through C, respectively.

STEP CYCLE

313

Figure 23: Normalized data from experiment Ia-3 showing

the phase relationships among the fictive locomotor

activity of 2 quadriceps motoneurons (A and B), an

extensor-coupled RC (C) and a quadriceps-coupled IaIN

(D). The motoneurons in the ventral root filament were

knee extensors. The data represent the means of

measurements made during 10, 10, 10 and 7 step cycles
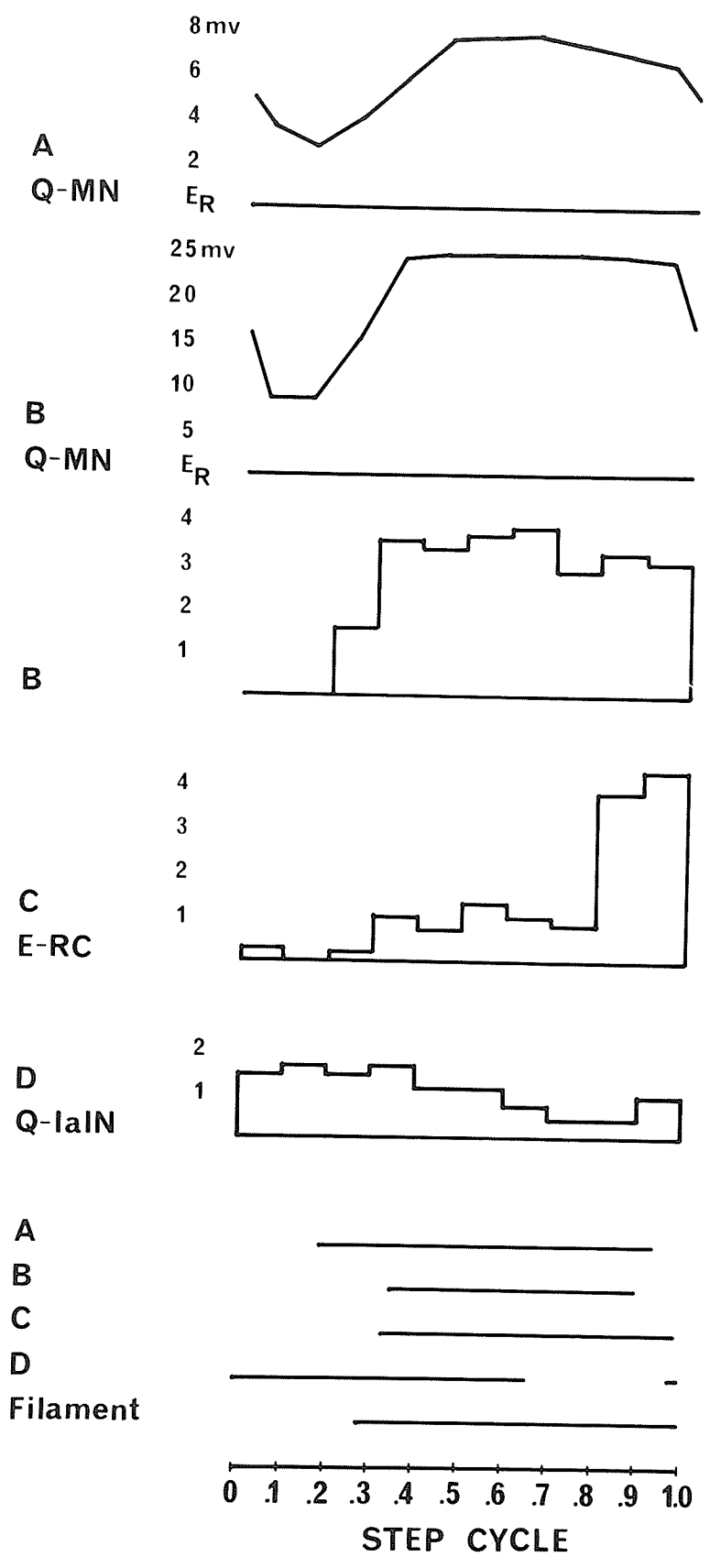
for neurons A through D, respectively.

STEP CYCLE

Figure 24: Normalized data from experiment DA-3 showing the phase relationship between the fictive locomotor activity of a quadriceps-coupled RC (A) and flexor motoneuron activity in an L6 ventral root filament. The data represent the means of measurements made during 10 step cycles.
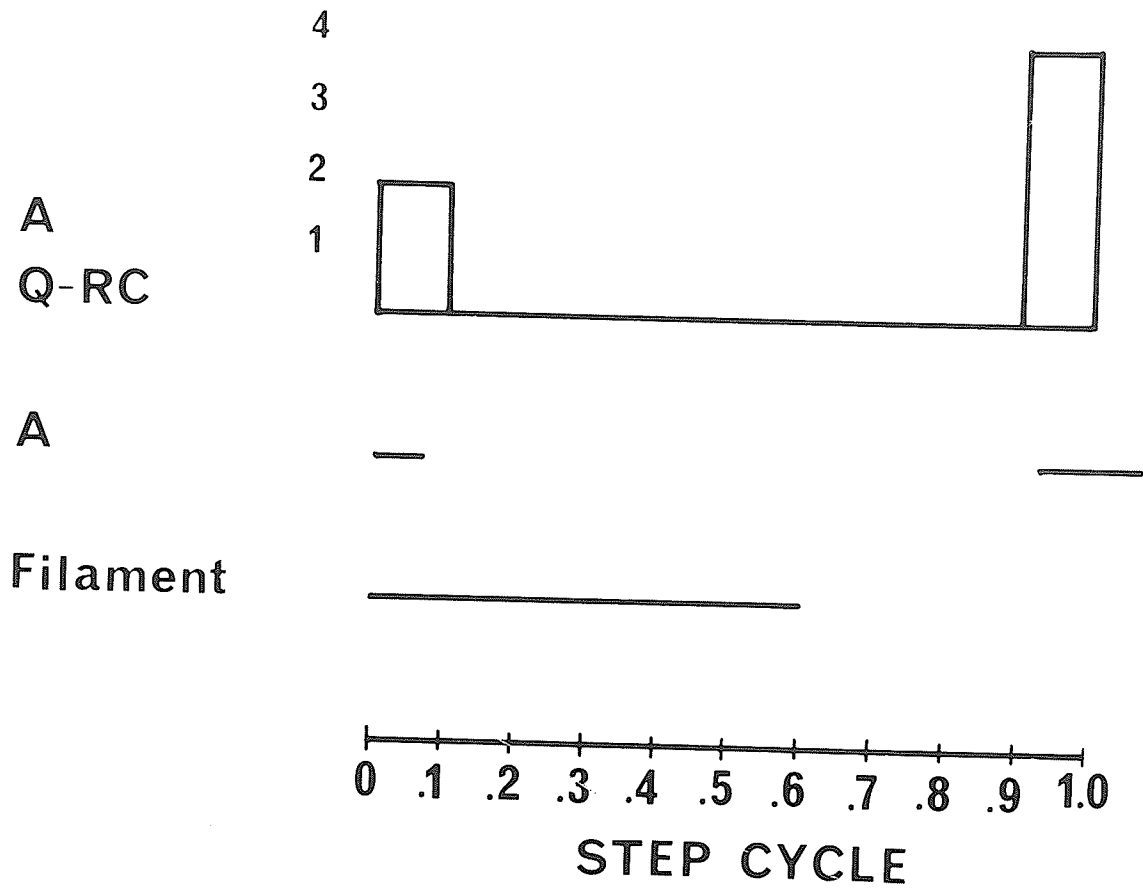
A
Q-RC

4
3
2
1

A

Filament

0 .1 .2 .3 .4 .5 .6 .7 .8 .9 1.0

STEP CYCLE

317

Figure 25: Normalized data from experiment I1-20-2-78

showing the phase relationships among the fictive

locomotor activity of an extensor motoneuron (A), a

lateral gastrocnemius-coupled RC (B), a semitendinosus

motoneuron (C) and a posterior biceps-coupled RC (D).

The data represent the means of measurements made during

9, 5, 5 and 9 step cycles for neurons A through D,

respectively. The motoneurons in the ventral root filament
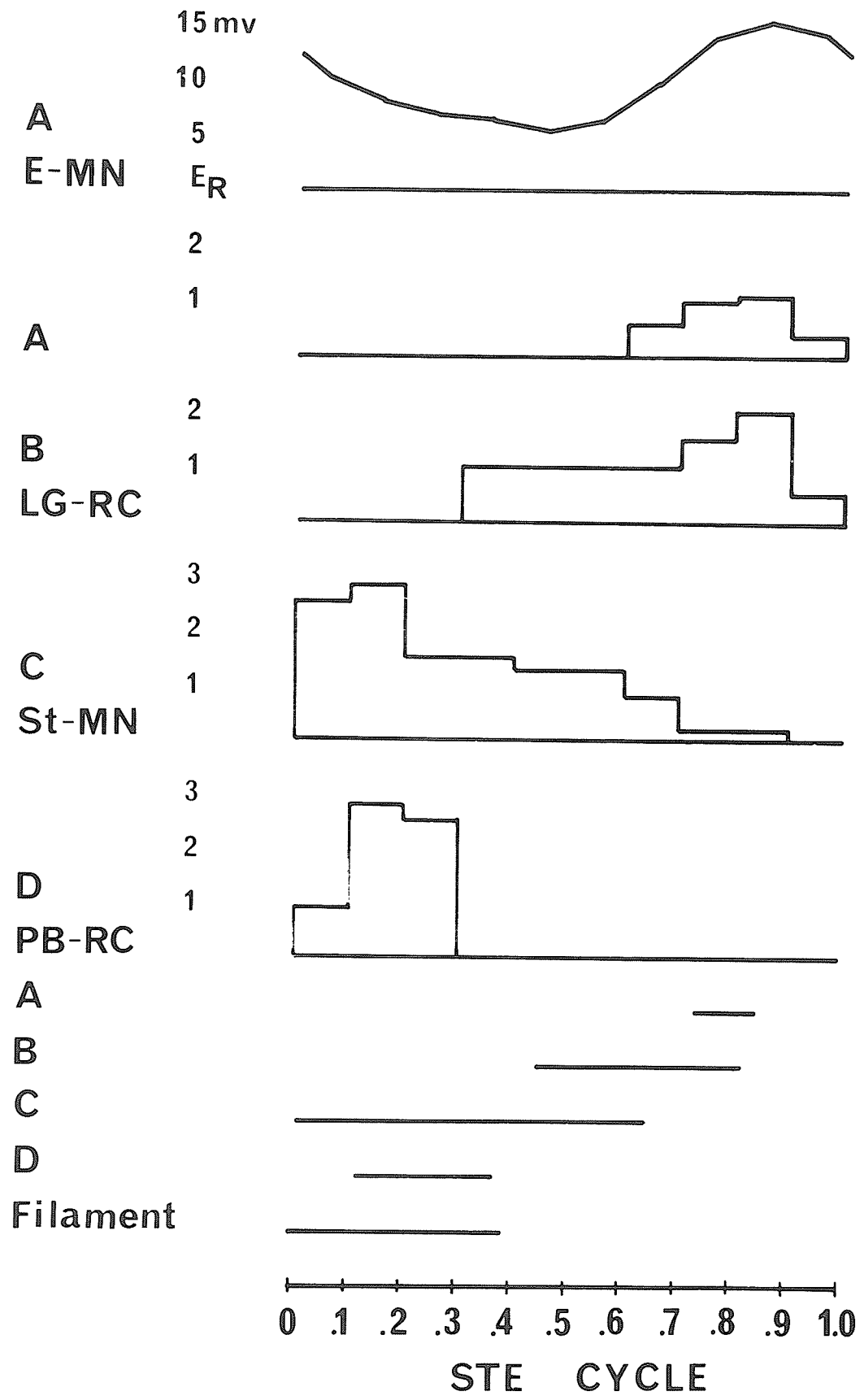
were flexor motoneurons.

319

Figure 26: Normalized data from experiment Ia-33 showing

the phase relationships among the fictive locomotor

activity of a sartorious motoneuron (A) and 3 quadriceps-

coupled IaINs (B,C,D). The motoneurons in the ventral

root filament were extensor motoneurons. The data

represent the means of measurements made during 6, 5, 5

and 7 step cycles for neurons A through D, respectively.

4mv
3
2
1
E
R
-1
-2
-3

A
SART-MN

20
15
10
5

B
Q-IaIN

12
10
8
6
4
2

C
Q-IaIN

2
1

D
Q-IaIN
C
D
Filament

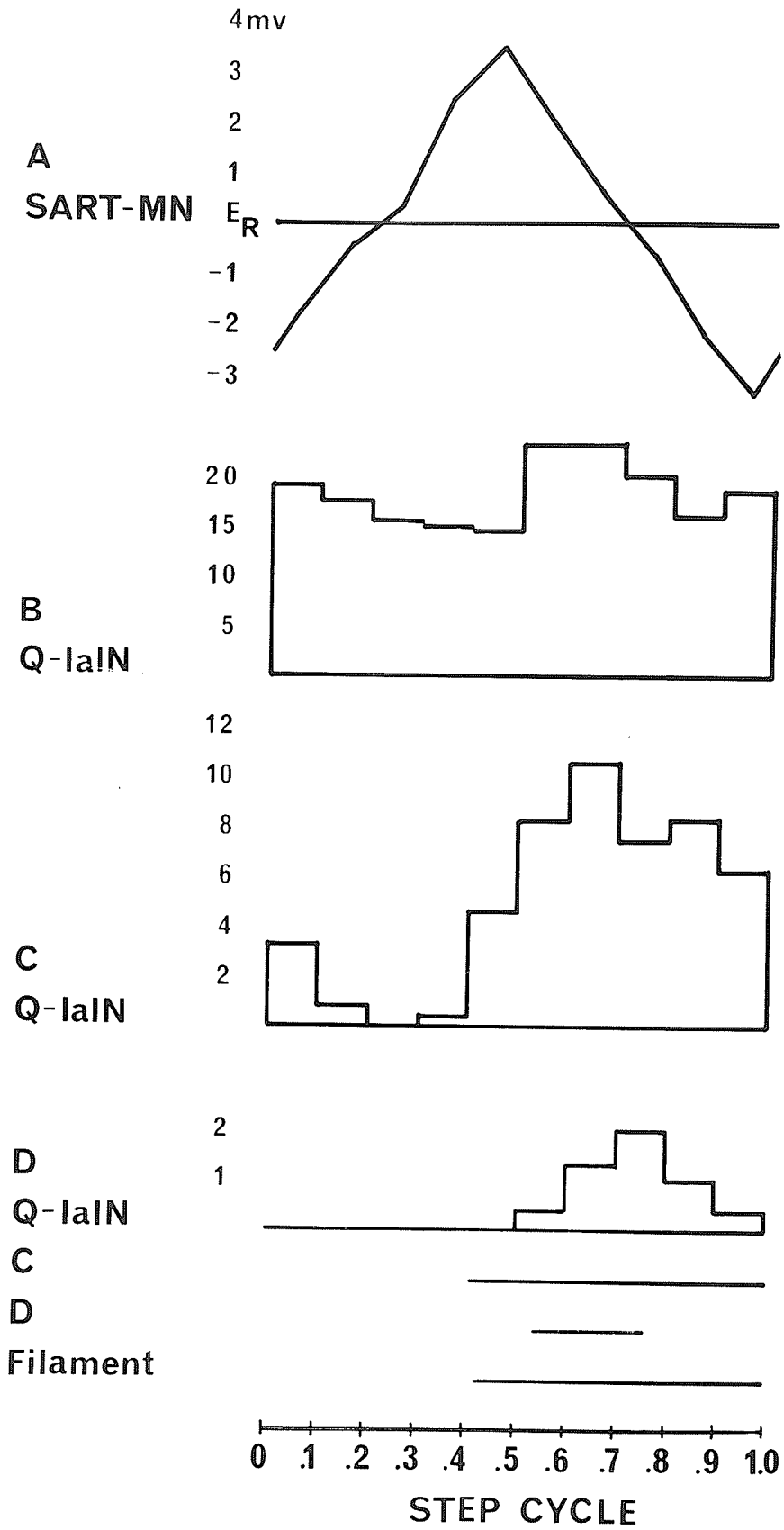0 .1 .2 .3 .4 .5 .6 .7 .8 .9 1.0

STEP CYCLE

321

Figure 27: Records of the activity of 5 neurons that were recorded during fictive locomotion at different times during experiment Ia-10. These records correspond to the normalized data presented in Figure 21. The top trace in each panel is the microelectrode recording and the bottom trace is the recording of extensor motoneuron activity in an L6 ventral root filament which was not changed throughout the experiment. The time calibration in each panel represents 500 msec. The regularly spaced vertical lines in the microelectrode trace in panels A, B, D and E are the stimulus artifacts associated with stimulation of the MLR. Interneuron E was the 13th cell recorded in this experiment, and it can be seen that the step cycle has increased in duration in correspondence with the elongation of the extension phase. Despite the reorganization of the locomotor rhythm, the tight coupling between interneuron and motoneuron activity is still apparent.
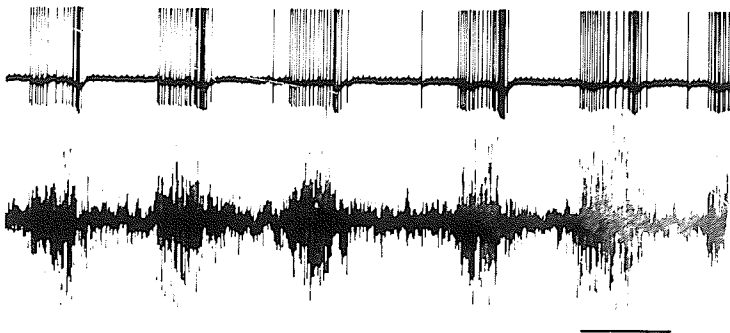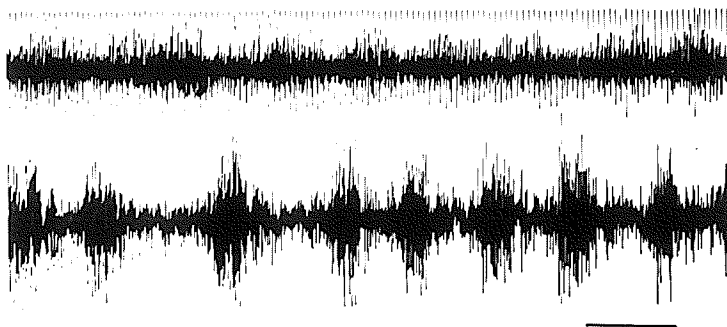
A

Q-IaIN
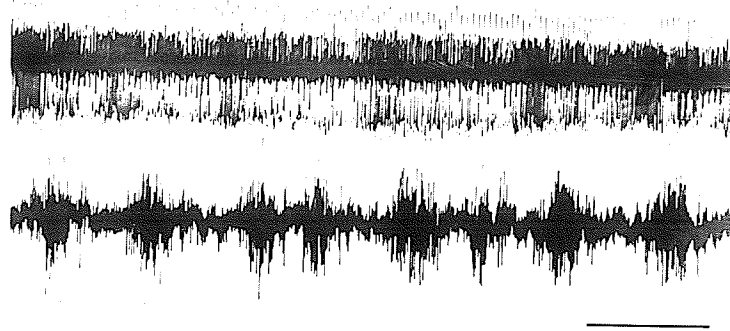
B

Q-IaIN
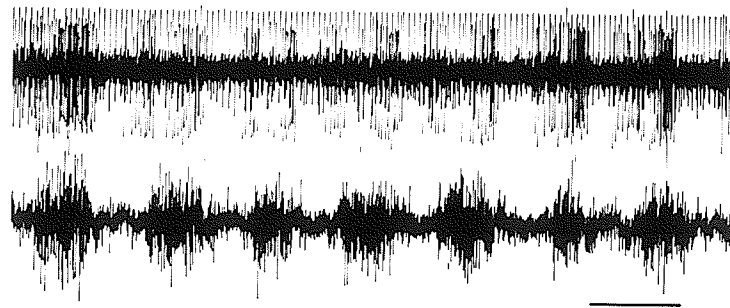
C

Q-RC

D

F-RC

E

Q-IN
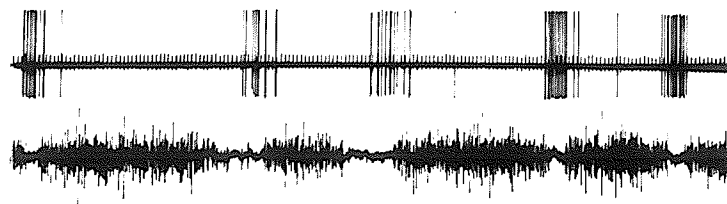
1 Second

Figure 28: Diagramatic summary of the pattern of discharge

and the activity periods of 7 extensor-coupled RCs (E-RCs)

and 3 flexor-coupled RCs (F-RCs) during fictive locomotion.

The mean X and Y values for each RC and the population

means which are diagramatically presented in this figure

are presented in Table 12. The solid horizontal lines

represent the mean duration of the normalized activity

period and the dashed lines represent 1 tail of the standard

deviation of the X and Y values of E-RCs and F-RCs and

extensor and flexor motoneuron activity recorded in ventral

root filaments. Since the beginning of flexor motoneuron

activity and the end of extensor motoneuron activity were

used as arbitrary definitions of the onset of the flexion

phase and the end of the extension phase of the step cycle,

respectively, there are no standard deviations associated

with these values.

E-RCs

F-RCs

E-RCs

E-MNs

F-RCs

F-MNs

0   .1   .2   .3   .4   .5   .6   .7   .8   .9   1.0

STEP CYCLE

325

Figure 29: The frequency histogram represents the average pattern of discharge of 6 quadriceps-coupled IaINs recorded during fictive locomotion. The solid horizontal lines represent the mean activity periods of 4 of these Q-IaINs which discharged in discrete bursts during fictive locomotion and of extensor motoneuron activity recorded in ventral root filaments. The mean X and Y values for each of these 4 Q-IaINs and the population means are presented in Table 13. The dashed horizontal lines represent 1 tail of the standard deviation associated with the X and Y values.

8
7
6
5
Q-IaINs 4
3
2
1

Q-IaINs

E-MNs

0 .1 .2 .3 .4 .5 .6 .7 .8 .9 1.0
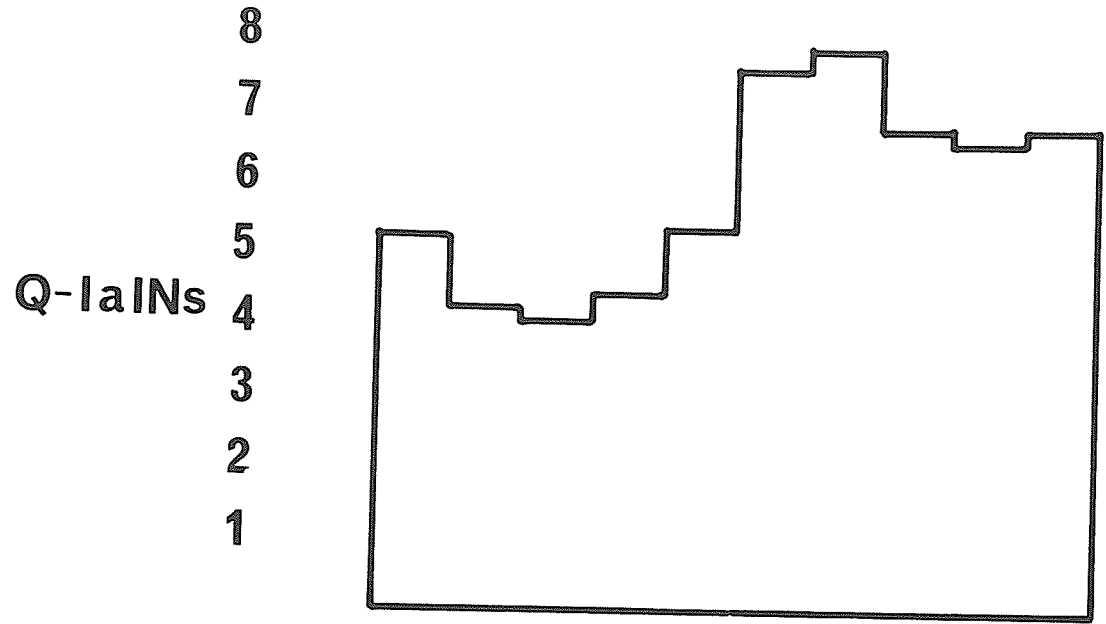
STEP CYCLE

Figure 30: The upper trace in A and B is the microelectrode recording of the activity of an unidentified but apparently flexor-coupled inhibitory interneuron during fictive locomotion, and the bottom trace is the recording of extensor motoneuron activity recorded in an L6 ventral root filament. This interneuron was recorded during the same experiment as the neurons illustrated in Figure 23. The interneuron fires just after the onset of extensor motoneuron activity in the filament in these records, although in earlier trials it was active primarily during flexion. There is a reduction in motoneuron activity coincidental with the discharge of the interneuron which can be seen more clearly in B which is an expanded illustration of the first step cycle shown in the far left in A. The motoneurons that discharge during the period of interneuron activity fire in doublets only while they fire in doublets and singly after the interneuron ceases firing.

The darkened section in the top trace in A is the stimulus artifact of a train of stimuli delivered to the L6 ventral root. Ventral root stimulation abolished the interneuron activity and reduced activity in the ventral root filament. Thus, this interneuron could be a RC or a IaIN.

A

1 Sec

B

125 ms
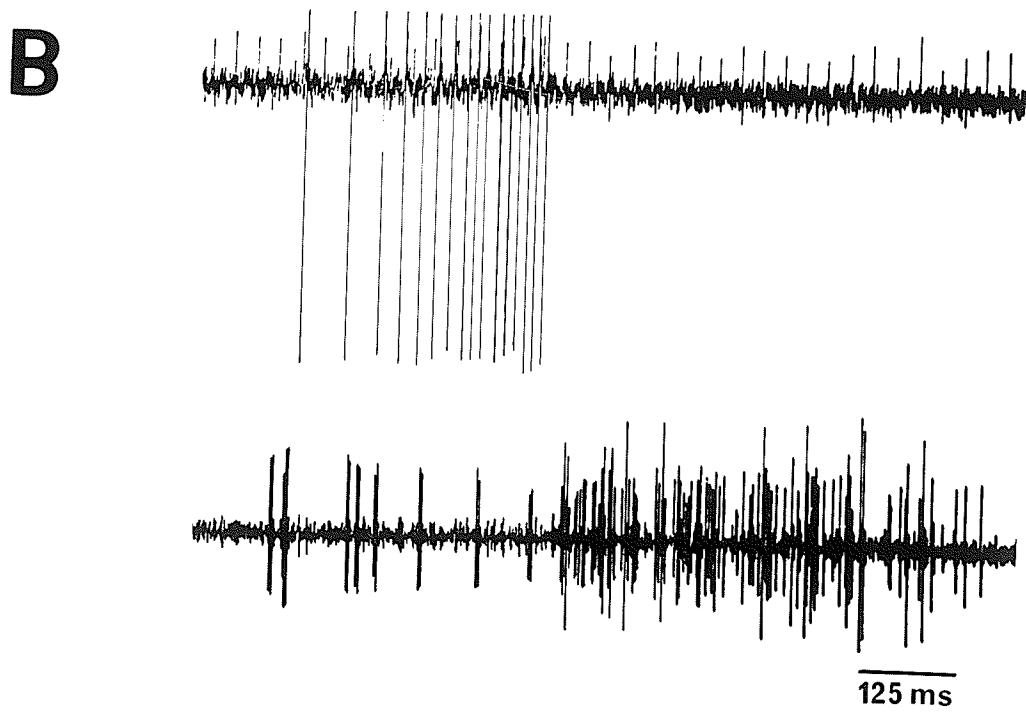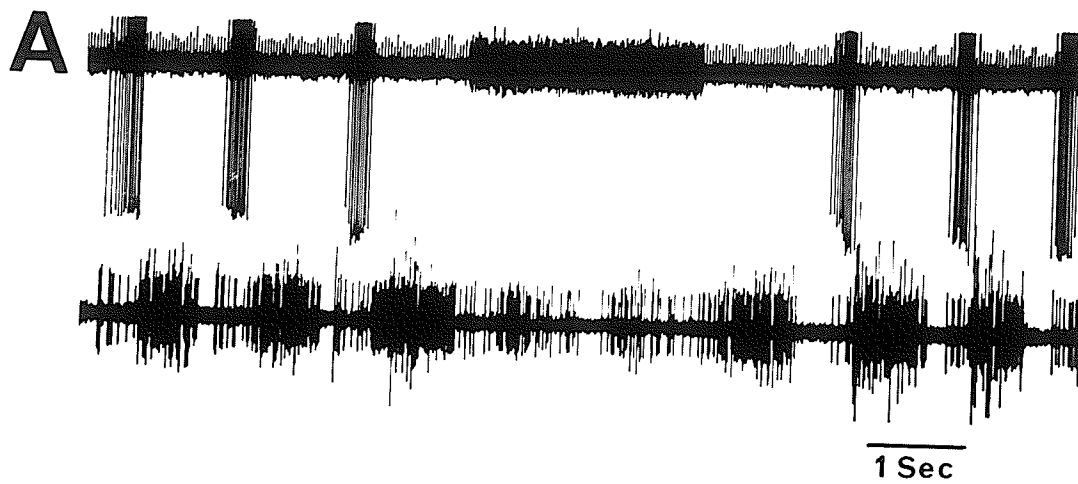
Figure 31: Antidromically evoked and spontaneous discharge of a quadriceps-coupled RC (RC #9 in Table 14; see also Figure 23) during fictive locomotion. The middle trace in A and the top trace in B are the intracellular recordings of the RC and the trace below this is a record of extensor motoneuron activity in an L6 ventral root filament. A. Single stimuli delivered to the L6 ventral root at a rate of 2.8 Hz (360 msec interpulse interval) during fictive locomotion evoked typical high frequency RC discharges which occurred concurrently wiht a reduction in motoneuron activity in the filament. The A gate pulses shown in the top trace in A were elicited simultaneously with the pulse from the stimulator to the ventral root. B shows the spontaneous activity of the RC during fictive locomotion in the absence of ventral root stimulation. The rhythmic activity of the RC was abolished by ventral root stimulation in A as virtually all of the RC activity is entrained to the ventral root stimulation.
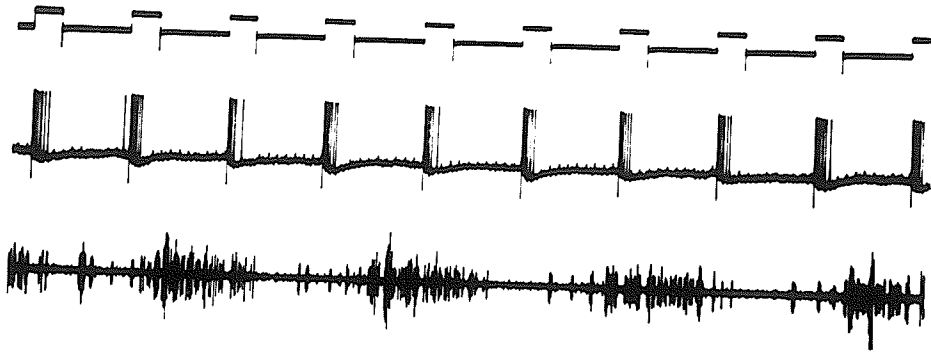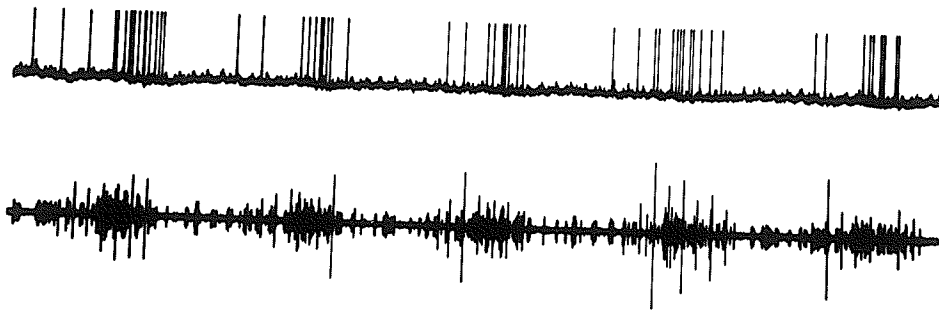
500 msec

Appendix A

Analysis of Covariance on Fs (Dependent Variable)

vs Fi (Covariate) During Cyclic Passive Limb Movement

(Group 1) and Controlled Treadmill Locomotion

(Group 2).

Group	Intercept	B	DF	SS	MS
1	-1.039	.393	17	275.527	16.207
2	.923	.251	17	272.291	16.017
Total			34	547.818	16.112
Pooled		.303	35	556.677	15.905
Pop	.070	.306	36	557.810	15.495

Tests of significance:

1. Equal slope: $F_{(1.34)} = .550$

2. Equal intercepts: $F_{(1.35)} = .071$

3. Equal treatment effects:

$$F = \frac{SS_{pop} - SS_{total}}{MS_{total}} = \frac{557.810 - 547.818}{16.112} = .620$$

$$DF = (2(k-1), total) = (2.34)$$

4. Test of whether the population regression line of Fi vs Fs has a slope significantly different from 1.

$$SS_T = SS_W + SS_B$$

$$SS_W = n_1 - 1\ (_1{}^2) + n_2 - 1\ (_2{}^2)$$

$$= 18(6.203^2) + 18(8.155^2) = 1826.66$$

$$SS_B = \frac{(n_1 - \bar{X}_1)^2}{n_1} + \frac{(n_2 - \bar{X}_2)^2}{n_2} - \frac{(n_1 - \bar{X}_1 + n_2 \bar{X}_2)^2}{N}$$

$$= \frac{(19 \times 10.758)^2}{19} + \frac{(19 \times 12.416)^2}{19} - \frac{(19 \times 10.758 + 19 \times 12.416)^2}{38}$$

$$= 2198.96 + 2928.98 - 5101.83 = 26.11$$

$$SS_T = 1826.66 + 26.11 = 1852.77$$

$$Var_b = \frac{MS_W}{MS_T} = \frac{15.495}{1852.77} = .008$$

$$\frac{1 - b}{Var_b} = \frac{1 - .306}{.008} = \frac{.694}{.091} = 7.63 \text{ ***}$$

$$t_{.999}(36) = 3.586$$

Appendix B

Factorial Analysis of Effect of Ventral Root
Stimulation Frequency (Factor A) and Mode of
Motoneuron Activation (Factor B) on Amount
of Recurrent Inhibition Expressed as Percent
Decrease in Motoneuron Discharge

Analysis of Variance

Source	DF	MS	F
A	3	684.408	17.02 (p ≤ .001)
B	1	120.431	3.00
AB	3	33.182	.83
Error	147	407.767	
Adjusted Error		40.210	
Total	154		

Appendix C

Analysis of Variance Test of Effect of Stimulation

Strength of Ventral Root Stimulation on Amount of

· Recurrent Inhibition Expressed as a Percent

Reduction in Motoneuron Discharge

1.   During cyclic passive limb movement:

Group	N	Mean	SD	SDM
1 (30uA)	35	83.99	20.53	3.47
2 (300uA)	39	71.21	22.43	3.59

Analysis of Variance

Source	DF	MS	F
Between	1	3015.86	6.49*
Within	72	· 464.54	
Total	73		

2.   During controlled treadmill locomotion

Group	N	Mean	SD	SDM
1 (30uA)	23	84.85	15.39	3.21
2 (300uA)	45	55.72	29.89	4.46

Analysis of Variance

Source	DF	MS	F
Between	1	12921.41	19.16***
Within	66	674.39	
Total	67		

Appendix D

Identification of Motoneurons Included in Table 6

Cell #	Motoneuron Identification
1	S8-3
2	S8-5
3	S8-8
4	S8-2
5	S8-4
6	I1-27-6 F
7	I1-20-2 #25
8	Ia-3 #9
9	I1-26-6 #10
10	Ia-5 F
11	Ia-2 #8
12	Ia-8 #2
13	Ia-2 #1
14	Ia-3 #10
15	Ia-2 #9

Appendix E

Two-way Analysis of Variance on the Duration of the

First 6 ISIs (Factor A) Recorded in Two

Different Locomotion Preparations, Controlled

Treadmill Locomotion and MLR-evoked Fictive

Locomotion (Factor B).

Analysis of Variance

Source	DF	MS	F
A	5	231.61	3.48 (p ≤ .01)
B	1	88.01	1.32
AB	5	52.52	.79
Error	84	457.80	
Adjusted Error	147	66.58	
Total	95		

Appendix F

Identification of IaINs Included in Table 7

IaIN #	Identification
1	Ia-10 #1
2	Ia-10 #5
3	Ia-11 #4
4	Ia-2 #7
5	Ia-33 #15
6	Ia-33 #11

Appendix G

Identification of Motoneurons Included in Table 11

Motoneuron #1	Identification
1	I1-27-6-78 #3
2	Ia-24 #9
3	I1-21-6-79 #6
4	Ia-2 #3
5	I1-20-2-78 #6
6	Ia-26 #5
7	Ia-3 #10
8	Ia-3 #4
9	I1-27-6-78 #10
10	I1-27-6-78 #2
11	Ia-2 #6
12	I1-21-6-79 #9
13	Ia-5 #2
14	Ia-8 #2
15	Ia-33 #9

Appendix H

Identification of Renshaw Cells Included in Table 12

Renshaw Cell #	Identification
1	Ia-10 #11
2	Ia-24 #8
3	Ia-3 #2
4	DA-3 #2
5	Sand1-30 #7
6	I1-28-2-78 #6
7	I1-20-2-78 #24
8	Ia-10 #2
9	Ia-24 #2
10	I1-20-2-78 #14

Appendix I

Identification of Renshaw Cells Included in Table 15

Renshaw Cell #	Identification
1	I1-15-2-78 #5
2	I1-15-2-78 #14
3	I1-28-2-78 #4
4	Ia-25 #1
5	I1-14-6-79 #9
6	I1-28-2-78 #6
7	Ia-33 #7
8	Ia-24 #2
9	Ia-3 #2
10	Ia-10 #2
11	Ia-10 #11
12	Ia-24 #8
13	DA-3 #2
14	I1-14-6-79 #8

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V I T A

Carol Ann Pratt was born in Philadelphia, Pennsylvania on December 27, 1948, the daughter of E. Joseph and Hazel M. Pratt. After graduating from Frontier Regional High School in South Deerfield, Massachusetts in 1966, she entered the Health and Physical Education program at Bridgewater State College in Massachusetts and was awarded the degree of Bachelor of Science in June of 1970. She attended the University of Massachusetts from September, 1970 to August, 1971 as a graduate student in the Department of Exercise Science in the School of Physical Education and was awarded the degree of Master of Science in June of 1973. Her research in the area of Motor Control was conducted under the direction of Dr. Walter Kroll. She taught and coached at Enrico Fermi High School in Enfield, Connecticut from Setpember 1971 to June 1974 and then at the University of Massachusetts in Amherst for the following two years. In September, 1976 she entered the University of Manitoba as a Ph.D. candidate in the Department of Physiology, Faculty of Medicine. Her research in partial fulfillment for the degree of Doctor of Philosophy

was conducted under the supervision of Dr. Larry M.

Jordan. She accepted an Assistant Professor's

position at Purdue University in Lafayette, Indiana

in January, 1980.

During the course of her research in the laboratory

of Dr. Larry M. Jordan at the University of Manitoba in

Winnipeg, she has been the author or co-author of the

following publications:

Albert, Carol Pratt and Larry M. Jordan. (1976). Efficiency
of recurrent inhibition in the mesencephalic locomoting
cat. Proc. Can. Fed. Biol. Sci., 21 (358).

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