ACTIVITY OF SPINAL NEURONS DURING CONTROLLED LOCOMOTION

by

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A B S T R A C T

Studies in the early 1900's demonstrated that the cat lumbar spinal cord contains a pattern generator which is capable of producing co-ordinated hindlimb stepping. The neuronal identity of this generator remains unknown but it is clear that interconnections between Ia inhibitory interneurons (IaIN) motoneurons (Mn) and Renshaw cells (RC) could operate to assist the function of this spinal stepping generator. On the other hand, indirect evidence from other laboratories has suggested that RCs are depressed during locomotion and presumably do not contribute to stepping. This thesis has directly examined the activity of RCs during locomotion and has attempted to assess the contribution of these interneurons to locomotion.

Cats were anaesthetised with halothane, the spinal cord exposed by laminectomy, mounted in a stereotaxic frame and then decerebrated. Some cats were decerebrated at a premamillary (thalamic) level and induced to step in the air with either gentle cutaneous stimuli or dorsal root stimulation. In other animals the decerebration was postmamillary and midbrain locomotor region (MLR) stimulation was used to initiate locomotion. A fine ventral root filament was isolated which showed phasic activity during the step cycle. The cat was then paralyzed with gallamine triethiodide and persistence of the rythmic ventral root filament activity was used as an index of fictive locomotion. Microelectrode recordings were then obtained from RCs Mns and IaINs during fictive locomotion in thirty animals.

The data obtained revealed that RCs and IaINs were rythmically active during fictive locomotion. Excitability measurements showed that RCs were not generally depressed diving fictive locomotion. Intracellular records from MNs with stimulation of a ventral root often revealed recurrent inhibitory postsynaptic potentials (R-IPSPs) or recurrent facilitatory potentials (RFPs). Both R-IPSPs and RFPs persisted during fictive locomotion giving further evidence that RCs are not depressed during fictive locomotion. Thus it is possible that the interconnections between RCs, MNs, and IaINs operate to assist the locomotor generator.

The cholinergic blocking agents, atropine and mecamylamine, are known to block the activation of RCs from motor axon collaterals and should reduce locomotor capabilities if the RC-IaIN system is important for stepping. Seven animals were decerebrated and induced to walk on a motor driven treadmill with MLR stimulation. Atropine and or mecamylamine were administered intravenously and their effects on electromyographic activity (EMG) recorded. Neither atropine (1.5 mg/Kg) nor mecamylamine (4 mg/Kg) had effects upon EMG bursting or the threshold for initiation of MLR locomotion.

Two animals were deafferented in one hindlimb (L_3 - S_3) and allowed to recover for one week. On the day of the experiment silastic cuffs were placed around peripheral nerves in the deafferented hindlimb and locomotion induced with MLR stimulation. Stimulus trains (100-400 ms duration) were applied to combinations of the nerve cuffs in order to activate the RCs by way of motor acon collaterals. Examination of EMG records showed that strong stimulation of the RC system did not change the time of onset of rythmic EMG activity during treadmill locomotion.

This thesis has developed a preparation in which the activity of individual neurons can be assessed during fictive locomotion. The data obtained showed that contrary to prevailing opinion RCs are not depressed during fictive locomotion and are still able to produce R-IPSPs and RFPs in MNs. When the RC system was blocked pharmacologically or activated with electrical stimulation, changes in the step cycle could not be detected. Thus it seems that while the RC-IaIN system can operate in a manner consistent with assistance of the stepping generator, the RC IaIN system is not likely to be powerful enough to modify or disrupt the rythmicity of the generator in any major way.

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Consider the following two quotations:

"Before I studied neurophysiology, I thought that recurrent inhibition was the reason that Betty-Lou would never come across for me in the back seat of my '59 Chevy."

"Yesterday I couldn't spell neurophysiologist; today I are one."

Quote (1) was recorded from a speech by the author of this thesis back in the winter of '73 and shows the characteristic ignorance often associated with recent B.Sc. graduates. Quote (2) on the other hand was uttered in the spring of '79 by the same author and illustrates the characteristic ignorance of a recent Ph.D. graduate.

- P. Gumby; Sociologist

It is easy for one to laugh off a Ph.D. dissertation as a bad joke and to lose sight of the fact that if it were not for the support of many this thesis would never have been written. I express my sincere thanks to all for creating an environment that was both stimulating, and even more important, enjoyable.

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p.s. Thank you L.B. for opening my eyes and my heart.

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INTRODUCTION

Although the co-ordinated use of limbs to propel the body was an early evolutionary development, the neuronal nature of the mechanisms responsible for alternate flexion and extension of the limbs remains, with the exception of a few invertebrates, a subject of almost total mystery. In order that the series of joint movements within a particular limb and between limbs produces stable locomotion, it is intuitively obvious that a pattern generator exists to ensure a co-ordinated sequence of muscle contractions. The idea of a generator of locomotion intrigued Graham Brown during the early 1900's, and it is to this man that we largely owe our basic concepts of the nature and location of the stepping generator. Brown (1911) demonstrated that cutting a cat's mid-thoracic spinal cord with a pair of scissors resulted in rhythmic co-ordinated contractions in the muscles of the hind limbs which closely resembled activity occuring during stepping, and he suggested that the lumbar cord contained a stepping generator capable of co-ordinating activity both within a limb and between limbs. Brown also showed that the alternating series of contractions occured in the de-afferented preparation and was able to conclude that this generator does not depend upon a rhythmic peripheral input for its initiation or maintenance. More recent work further establishes the existence of a spinal generator for locomotion. Perret (1973) confirmed Brown's experiments using a curarized preparation by recording muscle nerve activity peripherally. Perret coined the term "fictive locomotion" to describe this preparation in

which the spinal cord is generating the appropriate signals for muscle activity in the absence of muscular contractions. If the spinal cord is transected at T_{12} in the decerebrate cat, locomotion on a treadmill can be induced by intravenous administration of either L-DOPA (L-dihydroxyphenylethylamine) (Grillner, 1969) or clonidine (Forssberg et al., 1973). In chronic spinal cats (T_{13}) treated with DOPA or clonidine and bilaterally deafferented, stimulation of the cut dorsal roots results in co-ordinated stepping movements (Grillner and Zangger, 1974). Paralyzation with curare does not abolish the rhythmic activity recorded in peripheral nerve filaments (Grillner and Zangger, 1974).

In order to further convince his contemporaries that walking was an inate function of the spinal cord, Brown (1914a) looked at the locomotor capabilities of unborn kittens. Fetuses were removed from the uterus of a decerebrate cat and placed in a saline bath while leaving the umbilical cord intact. These fetal kittens were able to exhibit spontaneous co-ordinated movements of all four limbs; the kittens could also "locomote" immediately after a decerebration. Thus, it was now clear that basic locomotion patterns are a "hard wired" capability of the nervous system and are not merely the result of an early learning process.

Further experimentation by Brown (1914b) examined the phenomenon of narcosis progression in which, under certain depths of anaesthesia, an animal (cat) will exhibit stepping movements of the limbs. By various procedures it was possible to obtain stepping in one hind limb or both depending upon the experimental manipulations, and in the

report on this work Graham Brown developed the concept of the "half centre" organization of the spinal stepping generator. Brown believed that for each limb there is one centre capable of generating either flexion or extension. Mutual interactions between two "half centres" for a single limb resulted in co-ordination of muscle activity and thus stepping in that particular limb. In quadrapedal locomotion eight such "half centres" would exist. Thus Graham Brown established that the capability for locomotion was resident in the spinal cord and his concept of "half centre" organization suggested that there should be populations of neurons interconnected such that activity in one group caused both a particular movement of a limb (e.g.flexion) and also influenced the activity of another neuronal group which would allow subsequent antagonistic movement of the limb (e.g.extension).

To use the words of Graham Brown (1914b); "...The efferent neurone (motoneurons) may be supposed not only to activate the effector organ (muscle) through the mediation of its axone but also, by means of some other branch-fibre or side channel, at the same time reciprocally to depress the activity of an antagonistic efferent neurone ..." Brown found support for this notion of motoneurons activity influencing the firing of other cells in the histological work of Cajal (1909), which demonstrated the presence of motoneuron collaterals which branched and ended in the ventral horn.

Birdsey Renshaw was intrigued with Brown's suggestion that activity in some motoneurons could influence the discharge of other motoneurons, and in 1941 Renshaw found that an antidromic volley in a

portion of a cut ventral root could inhibit the discharge of axons in another part of the same ventral root. Renshaw believed, as did Brown, that the motor axon collaterals of Cajal were responsible for mediating this antidromic inhibition.

Renshaw (1946) then searched for and found a population of interneurons which were activated by antidromic ventral root impulses. These interneurons responded to a single ventral root stimulus with a train of spikes, the number of spikes being proportional to the stimulus intensity. Renshaw cautiously suggested that perhaps these interneurons were excited by the motor axon collaterals and mediated effects upon other motoneurons. Eccles et al., (1954) supported this suggestion of Renshaw and asked that these interneurons be called "Renshaw cells". Eccles et al., (1954), using the then newly developed technique of intracellular recording, recorded from motoneurons and antidromically stimulated the ventral root. It was found that antidromic ventral root stimulation which was below threshold for spike initiation in the impaled motoneurons often caused an IPSP (inhibitory postsynaptic potential) that was 1-5mv in amplitude and about 30 msec in duration. The time course of the IPSP and the appearance of a number of small fluctuations during the IPSP correlated well with observed Renshaw cell firing in the same preparations (Eccles, 1954), thus supporting the notion that this "recurrent IPSP" is due to the activation of Renshaw cells and their subsequent actions on motoneurons. The latency to onset of this IPSP was about 1.5 msec after the volley reached the cord, suggesting that this IPSP is indeed only disynaptic; a motoneuron collateral to Renshaw

cell synapse and a Renshaw cell to motoneuron synapse.

Although Renshaw established the existence of these ventral horn interneurons which were excited from motor axon collaterals to the satisfaction of most investigators (see Willis 1971), others (see Schiebel and Schiebel, 1971) argued against their existence as distinct neurones, since they were unable to find the Renshaw cell in Golgi preparations (Schiebel and Schiebel 1966). The existence of Renshaw cells was demonstrated unequivolcally by Jankowska and Lindström (1971) when they recorded intracellularly from Renshaw cells and stained them with Procion yellow. The Procion yellow stained neurons were found to be located in the ventral horn just medial to the motoneuron nuclei (Jankowska and Lindström, 1971) and to be small cells 10 - 15 μ in diameter with dendrites that projected up to 150μ and even extended out into the white matter. The axons of the Renshaw cells were traced histologically up to 400μ, suggesting that these cells could project to other segments of the spinal cord. Thus the system of collaterals from motoneurons feeding back into the cord and influencing motor output as hypothesized by Brown in 1914 has gained sound anatomical support.

Projections of the Renshaw Cell

To further define the axonal projections of Renshaw cells, Jankowska and Smith (1973) used a stimulating microelectrode and a recording microelectrode to antidromically activate the Renshaw cell axon while recording extracellularly from the same cell. They found that the axonal projections were as long as 12 mm, ran in the ventral

funiculus, and ended in motoneuron nuclei as well as in more dorsal spinal areas. Most of the axons that terminated upon motoneuron nuclei were found within a millimeter of the Renshaw cell body, thus lending anatomical support to the finding (Eccles et al., 1954) that the inhibitory effects of Renshaw cells upon motoneurons are distributed within one segment of the cord. The Renshaw cell axons that end upon motoneurons end only on ipsilateral motoneurons; Willis and Willis (1966) failed to find any evidence of recurrent Renshaw effects upon motoneurons when a contralateral ventral root was stimulated.

The projection of Renshaw cells to cells other than motoneurons is suggested by the work of Jankowska's group. The longer Renshaw cell axons projected across a number of segments to areas in the ventral funiculus dorsal and medial to the motoneuron nuclei. Electrophysiological evidence is available which demonstrates Renshaw cell effects upon group Ia afferent inhibitory interneurons (IaIN), ventral spinocerebellar tract cells (VSCT), and other Renshaw cells as well as inhibitory effects upon certain motoneurons.

The IaINs are ventral horn interneurons located medially and dorsally to the motor nuclei. These cells are activated from the periphery by volleys in the lowest threshold muscle afferents (group Ia) and have inhibitory actions upon certain motoneurons. In general, if the Ia afferents from a particular muscle are stimulated, the IaINs to the antagonist muscle are excited and thereby mediate reciprocal inhibition between antagonist motoneuron pools (Eccles et al. 1956a, Hultborn et al. 1971c). The finding that these IaINs

were depressed from the motoneuron collaterals through Renshaw cells was first reported by Hultborn et al. (1971b), who also systematically defined the distribution of the depressant Renshaw cell effects upon IaIN IPSPs in motoneurons (Hultborn et al. 1971 b, c).

Cells of the VSCT convey information from the cord to higher centres, and Lindström and Schomburg (1973) have shown that antidromic stimulation of the ventral root produces IPSPs in some VSCT cells. Lundberg (1971) has suggested that the VSCT functions to relay information about the transmission in interneuronal inhibitory reflex pathways to motoneurons. It is even more interesting that Renshaw cells can influence the VSCT cells in view of the fact that VSCT is the only ascending tract known to be phasically active due to the operation of the spinal stepping generator in locomoting preparations without phasic afferent input (Arshavsky et al. 1972).

The axonal projections of one Renshaw cell to another have been most thoroughly investigated by Ryall (1970). In these experiments various motor nerves were mounted for stimulation in a deafferented anaesthetized preparation and the effects of antidromic stimulation of these nerves upon Renshaw cells were assessed. It was found that in addition to the characteristic excitation of Renshaw cells usually seen by the earliest investigators (Renshaw, 1946), antidromic ventral root stimulation could also produce inhibition of spontaneous Renshaw cell firing or firing which had been induced by either antidromic stimulation or microiontophoretic drug application. Although these authors could not conclusively show that there was a direct Renshaw/Renshaw inhibitory pathway, the short latency of the inhibition elicited from the ventral

root tends to support the notion that Renshaw cells inhibit other Renshaw cells directly. Renshaw cells thus constitute an inhibitory spinal system that serves to inhibit IaINs, VSCT cells, motoneurons and other Renshaw cells.

The data discussed so far has concerned the motor axon collateral endings upon Renshaw cells and the subsequent consequences of Renshaw cell firing. For the sake of completeness we must address two other questions: 1) do motoneuron collaterals end upon cells other than Renshaw cells ? 2) do axons other than those of motoneurons end upon Renshaw cells ?

Motoneuron axon collateral termination

The early histology of fetal cat spinal cord showed extensive branching of the motor axon collaterals (Cajal 1909) throughout the grey matter of the anterior horn. More recent work using horseradish peroxidase in adult cats (Cullheim et al, 1977) has demonstrated direct connections between α motoneurons and α motoneuron collaterals. These synaptic endings contain vesicles and presumably could release acetylcholine during firing of the parent motoneuron. The physiological role of these motoneuron synapses remains speculative, but it is worth mentioning that there has never been any report in the literature of monosynaptic recurrent effects recorded in a motoneuron.

Synaptic Input to the Renshaw Cell

Curtis and Ryall (1966c) found that some Renshaw cells were fired in response to low threshold muscle afferents with a delay of about 1 msec after the monosynaptic reflex. This would probably indicate an activation of Renshaw cells from motoneurons. Ross et al (1972)

examined more closely the relationship between motoneuron discharge and Renshaw cell excitation. They found that about 20% of the Renshaw cell population studied responded to group Ia threshold dorsal root stimulation with a latency suggesting activation secondary to motoneuron firing; the remainder of the Renshaw cells were not influenced by the low threshold afferents. Ross et al (1972) showed a quantitative relationship between the number of Renshaw cell spikes in response to the dorsal root and the monosynaptic reflex size (i.e. the number of motoneurons recruited to fire). Ryall and Piercey (1971) in a systematic survey of Renshaw cell activation from afferent stimulation, found that for the low threshold afferents the minimum latency between the entering dorsal root volley and the Renshaw cell firing was 1.3 msec. Thus there is no evidence, other than a brief mention by Frank and Fuortes (1956) which has not been confirmed, of direct monosynaptic Renshaw cell activation from group 1 afferents.

Curtis and Ryall (1966c) and Curtis et al (1961) also reported Renshaw cell response from ipsilateral dorsal root stimulation which appeared unrelated to monosynaptic motoneuron discharge. Curtis and Ryall (1966c) state that the most common type of Renshaw cell response to dorsal root stimulation was a burst of 4 or 5 spikes with a latency of around 5 msec. This burst is followed by a period of up to .5 sec during which the spontaneous firing rate of the cell was reduced (Curtis and Ryall, 1966; Ryall and Piercey, 1971). This pause phase after dorsal root stimulation was of greater duration than the pause phase seen after ventral root stimulation. The late excitation seen to

ventral root stimulation was not present after dorsal root stimulation in the barbiturate anaesthesized cats of Curtis and Ryall (1966), but a late long lasting increase above spontaneous discharge frequency after dorsal root stimulation was noted by Frank and Fuortes (1956) using decerebrate cats. The use of higher threshold afferents of course elicits polysynaptic motoneuron reflexes, and it becomes difficult to differentiate between polysynaptic activation of motoneurons causing Renshaw cell firing and direct activation of Renshaw cells from the higher threshold afferents. Curtis and Ryall (1966c) favoured the view that higher threshold afferents had direct actions on Renshaw cells since dihydro-β-erythroidin (DBE) (Curtis et al, 1961) or atropine (Curtis and Ryall, 1966c) failed to affect high threshold dorsal root activation, whereas DBE (Curtis and Ryall, 1966) and mecamylamine (Ryall et al, 1971) reduced the low threshold evoked disynaptic responses. Piercey and Goldfarb(1973) stimulated various ipsilateral hindlimb nerves using very high stimulus intensities and recorded the Renshaw responses. The activation of these high threshold afferents (the flexor reflex afferents, FRA; Holmquist and Lundberg, 1961) caused three types of Renshaw discharges; discharge concomittant with the polysynaptic motoneurone reflexes, discharge only after the end of the polysynaptic reflexes, and biphasic discharge occuring during and after motoneuron excitations. The existence of some Renshaw dicharges clearly after the activation of motoneurons makes it likely that there are excitatory polysynaptic pathways from high threshold muscle and cutaneous afferents directly to Renshaw cells. Ryall et al (1971), Curtis and Ryall (1966c), Piercey and Goldfarb (1973), Frank and Fuortes (1956), and Eccles et al

(1954) all reported excitations of Renshaw cells by electrical stimulation of ipsilateral skin and muscle afferents. With natural (i.e. touch, pressure and noxious) stimulation and electrical stimulation of the contralateral limb and its nerves, Wilson et al (1964) reported profound inhibition of the Renshaw cell burst elicited by ipsilateral antidromic ventral root stimulation. Natural stimulation of any part of the body surface could often depress Renshaw activity, and noxious stimulation exerted the most powerful inhibition. It is interesting to note that the natural stimulation of the ipsilateral hindlimb apparently caused inhibition of Renshaw activity whereas reports using ipsilateral electrical stimulation reveal Renshaw cell excitation, but it is unclear from Wilson et al (1964) whether this phenomenon was thoroughly investigated. Wilson et al (1964) demonstrated that on rare occasions contralateral electrical stimulation caused weak discharge of Renshaw cells, but the preponderance of contralateral effects were inhibitory and the result of group II and III muscle afferent activation. Contralateral cutaneous nerve stimulation caused Renshaw cell inhibition which was dependent upon the larger alpha fibers, with little increase in inhibition when the delta afferents were also activated. More recently, Fromm et al (1977) have shown that activation of group II afferents causes a reduction in the efficacy of recurrent inhibition of motoneurons made to fire by group I afferent activity. Pompeiano et al (1975) noted a reduction in Renshaw cell discharge when repetitive group II stimulation was added to group I stimulation. It is probable, then, that group II afferents have a depressant effect upon Renshaw cells.

In summary, of the effects of electrical stimulation of peripheral nerves on Renshaw cell discharges, it seems that group I muscle afferents have no direct effects (not mediated via motoneurons) on Renshaw cells; the ipsilateral FRAs cause excitation, and contralateral FRAs inhibition, of Renshaw cell discharges.

In addition to effects on Renshaw cells from peripheral afferents, there is good evidence for a variety of supraspinal centers capable of affecting the Renshaw cell system. Granit et al (1960) plotted the tonic frequency of firing of motoneurons against the motoneuron firing rate during recurrent inhibition. This curve could be shifted to the left or the right depending upon the site of cerebellar stimulation. One of the problems with such studies is the differentiation between direct effects of stimulation upon the Renshaw cells and indirect effects mediated by changes in the motoneurons. However, the study by Haase and Vogel (1971) clearly shows a direct supraspinal effect on Renshaw cells. They found that stimulation in the nucleus interpositus caused a reduction in the test monosynaptic reflex and a concomitant increase in the number of Renshaw cell spikes produced by dorsal root stimulation. Other investigators have presented evidence suggestive of supraspinal connections to the Renshaw cell system, for example the reticular formation (Haase and Van Der Meulen, 1961), the fields of Forel, globus pallidus, and pericruciate cortex (MacLean and Leffman, 1967).

It is clear that any discussion of the function of the Renshaw cell system must ultimately take into account the possibility of modulation by both segmental afferent and supraspinal pathways.

Effects of Ventral Root Stimulation on Renshaw Cells

The most studied pathway for activation of the Renshaw cells has been the α motoneuron axon collateral system using antidromic ventral root stimulation (Renshaw 1946; Eccles et al. 1956b, 1961; Curtis, 1966b,c). Antidromic stimulation of the whole ventral root results in a very high frequency ($\leq$1500 Hz) discharge of the Renshaw cell for about 20 msec, followed by a decline in frequency until the spikes occur every 20 msec or so (Eccles 1956b). The actual number of spikes seen is a function of the antidromic stimulus strength (Renshaw 1946, Eccles et al, 1961a), but the pattern of initial high frequency discharge which slows in a few milliseconds is always seen. Renshaw cells are also "spontaneously" active (i.e., active in an animal in which there is no electrical stimulation (Curtis and Ryall 1966).

When one examines the effect of ventral root activation against a background of spontaneous Renshaw cell activity, the Renshaw cell response to a single antidromic pulse often consists of 3 parts; the initial high frequency discharge that slows quickly (0 - 50 msec after the stimulus), a period of reduced activity beneath the spontaneous rate (50 - 250 msec), and a period (up to 3 seconds) of activity above the spontaneous rate (Curtis and Ryall, 1966).

Since one antidromic ventral root pulse can elicit a large number of spikes in the Renshaw cell, one wonders whether the repetitive discharge is due to convergence upon the cell from a large number of motoneuron collaterals or whether the Renshaw cell has a membrane that behaves

differently to a single stimulus than do other neurons. Recently Ross et al (1975) found that if instead of stimulating the entire ventral root a fine ventral root filament was used, the Renshaw cell would respond to a single stimulus with a single spike. It seems likely then that the repetitive discharge seen when stimulating a number of motor axons is due to a large number of excitatory motoneuron collaterals converging upon a single Renshaw cell. The high frequency discharge rate (up to 1500 Hz) may therefore be a consequence of prolonged transmitter action and may be limited only by the absolute refractory period of the Renshaw cell. Intracellular records from a Renshaw cell responding to whole ventral root stimulation reveal an EPSP of about 40 msec duration, probably composed of a number of excitatory impulses on the cell (Eccles, 1961a).

The pause in firing that follows the initial high frequency discharge has been examined by Curtis and Ryall, (1966c). These authors suggested that the pause was due to membrane desensitization to transmitter and was not due to a lack of synaptic input which was later enhanced to produce the prolonged low level excitation. They showed that during the period of decreased firing the Renshaw cell showed decreased excitation to micro-iontophoretically applied excitatory amino acid and ACh. More recently Ryall (1970) has demonstrated that Renshaw cells can inhibit the firing of other Renshaw cells, and he proposed that the pause in firing is due to an active inhibitory process mediated by inhibitory interactions between Renshaw cells. The Renshaw cell/Renshaw cell inhibition was long lasting (up to .5 sec) and could be

seen with a minimum latency of 2.2 msec. It is possible that the time course of inhibition is long enough and the latency short enough to adequately account for the pause phase of Renshaw firing. This Renshaw cell/Renshaw cell inhibition could only be seen if certain muscle nerves were antidromically activated; for example, tibialis anterior never caused inhibition of Renshaw cell firing. In addition to demonstrated inhibition of spontaneous and late discharges of Renshaw cells, as a result of muscle nerve stimulation, Ryall also demonstrated a slight inhibition of the high frequency early discharge. As will be discussed later, the activation of Renshaw cells from motor axon collaterals is cholinergic (Eccles et al 1954, 1956). Thus a drug which blocks ACh excitation from motoneurons to Renshaw cells should not interfere with Renshaw cell/Renshaw cell inhibition. Curtis and Ryall (1966) found that the pause phase was not appreciably shortened by iontophoretic application of dihydro-β-erythroidine, a drug which blocks motoneuron collateral activation of Renshaw cells (Eccles, 1954).

The Renshaw cell/Renshaw cell inhibition is therefore pharmacologically different from the motoneuron/Renshaw cell activation, and the simplest explanation of the pause phase of firing is mutual Renshaw cell inhibition.

The cause of the late, long-lasting excitation following ventral root stimulation remains a mystery. This excitation is often seen 2 or 3 seconds after a single antidromic pulse (Curtis and Ryall, 1966, Ryall 1970). This is long after the high frequency discharge and occurs after the pause in firing. One possible explanation is that the Renshaw cell membrane does not hydrolyse the ACh very quickly and thus the

continued presence of ACh is responsible for this late excitation. This late excitation has only been adequately described in barbiturate preparations and the possibility of anaesthetic effects should not be ruled out.

Renshaw Cell Pharmacology

The pharmacology of Renshaw cell excitation has been examined in some detail. Dale's principle (Dale, 1934) suggests that if the motoneuron releases ACh at the motor end plate it should also release ACh at the motor axon collateral. Eccles (1954, 1956b) and Curtis (1966a, b) have investigated the excitation of Renshaw cells and found it indeed to be cholinergic. Dale (1914) divided ACh receptors into two classes, nicotinic and muscarinic, on the basis of the differential abilities of nicotine or muscarine to mimic the action of ACh at different sites in the autonomic nervous system. This concept of different types of ACh receptors has been extended to the central nervous system primarily by pharmacological investigations upon Renshaw cells. Eccles (1954) showed that intra-arterial injections of minimal amounts of ACh (8 µg) caused an excitation of Renshaw cells. This effect was enhanced by pretreatment with an anticholinesterase (eserine) but was not present on all Renshaw cells (Eccles, 1954). Anticholinesterase also increased Renshaw cell responses to an antidromic ventral root pulse, the effect being to increase the frequency of the later discharge but to leave the initial high frequency discharge unchanged. This finding supports the notion that the limiting factor in the high frequency discharge is the refractory period of the Renshaw cell membrane. The application of the anticholinesterases eserine, dimethyl carbonate of 3-hydroxy-2-dimethyl-

aminomethyl pyridine dihydrochloride, tetraethyl-pyrophosphate, and diisopropylfluorophosphate prolonged the Renshaw cell discharge in each case, but prostigmine was relatively ineffective (Eccles 1956b). Eccles et al (1956b) suggested that the ineffectiveness of prostigmine was due to the presence of a diffusion barrier around central nervous system blood vessels. Using the recording electrode as a cannula, Eccles et al (1956b) injected prostigmine onto a Renshaw cell directly and found that the drug was indeed effective as an anticholinesterase (Eccles 1956b). This was the first observation that the presence of the blood-brain barrier could alter the synaptic activity of a neurone in response to a drug.

When blockage of the motoneuron/Renshaw cell synapse was attempted, Eccles et al found that intravenous atropine, gallamine triethiodide and curare had no effect, but the dihydro-β-erythroidine (DBE) blocked almost all of the Renshaw cell activation from the ventral root (Eccles 1954, 1956b). DBE is a drug with curare-like action; it causes paralysis of skeletal muscle without affecting impulse transmission in the motor nerve (Unna et al, 1944) and, according to the terminology of Dale (1914), is a nicotinic blocker. The failure of gallamine triethiodide and curare, also nicotinic blockers, to affect Renshaw cell activity may be due to the diffusion barrier into the central nervous system (Eccles, 1956b); intra arterial injection of nicotine itself (Eccles et al, 1956b) excited Renshaw cells, suggesting the presence of nicotine receptors on the Renshaw cell.

To clarify the type of ACh receptor on the Renshaw cell, Curtis and Eccles (1958) and Curtis and Ryall (1966a) used local application of drugs onto individual Renshaw cells by using iontophoresis and thus

avoiding the problem of drug accessibility to the Renshaw cell. In testing a whole series of nicotinic and muscarinic compounds, Curtis and Ryall (1966a) found that nicotinic substances were more powerful in exciting Renshaw cells than were muscarinic compounds. The same paper shows that, of all the drugs tested, ACh had the fastest rate of increase and of decrease in changing the Renshaw cell firing rate, but the greatest firing rate was obtained with nicotine or carbamylcholine. The excitations seen due to the iontophoretic application of muscarinic compounds were usually not as great as those due to nicotinic compounds, and the duration of action was prolonged. Curtis and Ryall (1966) suggested that there might be both nicotinic and muscarinic receptors on the Renshaw cell, and they tested the efficacies of various iontophoretically applied cholinergic blocking agents. The nicotinic blocker DBE reduced Renshaw cell excitation by iontophoretic ACh application but did not reduce the excitation seen to application of an excitatory amino acid, D-L-homocysteric acid, or the muscarinic agent acetyl-β-methylcholine (Curtis and Ryall, 1966b). In an attempt to reveal the muscarinic nature of Renshaw cell receptors, atropine was tested for its ability to block both nicotinic and muscarinic excitations. Atropine blocked acetyl-β-methylcholine effects to some extent and caused very little blockage of nicotinic or carbamycholine. The atropine results suggest the presence of a muscarinic Renshaw cell receptor, but atropine had a depressant action of its own, making interpretation difficult. These authors concluded that Renshaw cells possess both types of ACh receptors and that the predominant response to ventral root stimulation was a nicotinic one.

Curtis and Ryall (1966c) went on to discuss the role of nicotinic and muscarinic receptors on the Renshaw cell response to a single antidromic ventral root volley which, as mentioned previously, consists of phases of initial high frequency discharge, a pause, and then a late, long-lasting, low level excitation. Because of the ability of DBE to reduce the initial discharge, the activation of nicotinic receptors was suggested. The pause, as noted above, is probably due to active inhibition of Renshaw cells from other Renshaw cells. The late excitation was explained as muscarinic receptor activation. Curtis and Ryall (1966c) suggested that when ACh was released by ventral root stimulation the nicotinic action was powerful but of short duration, whereas the muscarinic receptor activation was slower in onset and much longer in duration. Thus, after the critical rapid nicotinic effects and the pause phase, one would see the muscarinic response as the late, long-lasting, low level excitation.

The initial high frequency discharge can be reduced by DBE but not abolished (Eccles 1954, Curtis and Ryall 1966c). In all cases the first few spikes elicited from ventral root stimulation are still present after intravenous or iontophoretic DBE application. Eccles (1954) showed that DBE could not alter the presence or the timing of these first few spikes. The possibility arises, then, that the first few spikes are not produced by the same mechanism as most of the spikes in the initial discharge (Werman, 1972). In an attempt to completely abolish cholinergic transmission, Quastel and Curtis (1965) applied hemicholinium -3 iontophoretically to Renshaw cells. Hemicholinium-3 has been shown to cause failure of impulse transmission by inhibiting ACh synthesis (Birks and

MacIntosh, 1961). Although hemicholinium was able to abolish totally the Renshaw cell response to ventral root stimulation, interpretation of these results is complex, since it also has a direct depressant action on nerve propagation (Frazier, 1968) and a procaine-like action on muscle end plate potential (Martin and Orkand, 1961). Thus, the action of hemicholinium may not be due to ACh blockade (Krnjevic, 1974), but the slow onset of hemicholinium action and its ability to delay the first spike in the Renshaw discharge (Quastel and Curtis 1965) makes it seem likely that hemicholinium acts via blocking ACh transmission and that the first few spikes in the Renshaw cell discharge, although resistant to pharmacological manipulations, are due to chemical synaptic activity.

Another drug which has Renshaw cell blocking properties similar to DBE is the ganglionic blocking agent mecamylamine. Mecamylamine in a secondary amine and thus penetrates the blood barrier easily. Its ability to reduce the activation of Renshaw cells from motor axon collaterals was first described by Ueki et al (1961), who demonstrated that mecamylamine blocked all but the first few Renshaw cell spikes when given intravenously in doses of 2.5 mg/kg. Mecamylamine also blocked Renshaw cell responses to intravenous nicotine and physotigmine, and its effects lasted for as long as these investigators were able to record from the Renshaw cell (at least one hour). Ryall, Piercey and Polosa (1971) applied mecamylamine iontophoretically onto Renshaw cells and showed that the response to ventral root stimulation was greatly reduced. Mecamylamine is then very similar in action to DBE, and since it is

readily available commercially, it has been used for investigation of Renshaw cell function.

Natural Renshaw Cell Activation

The preceeding discussion of Renshaw cell pharmacology has for the most part relied upon the stimulation of an entire ventral root antidromically to produce Renshaw cell excitation. Such a massive discharge of motoneurons is very unphysiological, and it can safely be said that it never occurs during the normal functioning of the cat nervous system. A word of caution may be introduced here to prevent one from relying too heavily on the classification of ACh receptors as nicotinic and muscarinic, when these responses have been observed during abnormal neuronal excitation in anaesthetized cats. Although there is considerable convergence from many motoneurons onto one Renshaw cell, as evidenced by the excitatory effect of increasing the stimulus strength to the ventral root (Renshaw 1946, Eccles et al, 1954), it may be that during normal physiological processes there is not this great amount of excitation upon the Renshaw cell, and, in fact, the Renshaw cells may always respond with just a few spikes, not the 20 or so seen in the initial high frequency discharge due to ventral root stimulation. If this were the case then the initial few spikes resistent to the administration of DBE or mecamylamine may be the ones that are physiologically important.

In the one pharmacological experiment to date which has activated Renshaw cells physiologically in decerebrate cat, Haas and Ryall (1975) have shown that the Renshaw cell response to stretching of the Achilles tendon could be abolished by small amounts of atropine (.5 mg/kg) but

not by mecamylamine administered intravenously or iontophoretically. These results stress the need for caution when trying to apply the Renshaw pharmacology to normally behaving Renshaw cells.

Stimulation of a whole ventral root of course excites many motoneurons simultaneously, and the resultant Renshaw cell burst is due to coincident motoneuron convergence. To examine the response of the Renshaw cell to the firing of a single motoneuron, Ross et al. (1975) stimulated a fine ventral filament and recorded a single monosynaptic spike from the Renshaw cell. The Renshaw cell followed the filament stimulation up to about 10 Hz and then the discharge probability fell below one at frequencies above 10 Hz. This work suggests two points about physiological firing of the Renshaw cell. Firstly, it is likely that a single motoneuron can fire a Renshaw cell with a single spike, and secondly, during normal movement which involves a recruitment of motoneurons the Renshaw cell would probably fire with a series of several spikes in an attempt to follow motoneuron firing. Thus, it is probable that the fast bursting type of firing seen with stimulation of the whole ventral root would not occur during natural movement of the animal.

Consequence of Renshaw cell firing

The functional role of Renshaw cell activity during movement remains to be elucidated, but several suggestions for the consequences of Renshaw cell firing on motoneurons have been offered. Eccles, et al, (1961) suggested that since there seem to be more recurrent effects upon tonic rather than phasic motoneurons, during rapid movements which involve phasic motoneurons such as running or jumping Renshaw cells could

function to suppress discharges from tonic motoneurons. Granit and Rutledge (1960) showed that recurrent inhibition was most effective in silencing the activity of weakly excited motoneurons and proposed that Renshaw cells stop feebly supported discharges and would focus motoneuron activity during reflex actions. Brooks and Wilson (1959) pointed out that the suppression of weakly driven motoneuron pools would tend to reduce the possibility of inducing reflex activation of a motoneuron group by synergistic muscles and thereby localize reflexes, which in turn might optimize the performance of fine movements. Granit et al (1957) noted that whereas the γ-motoneurons receive very little recurrent Renshaw cell control (see also Brown et al., 1968) the motoneuron output of γ loop activation is under strong recurrent control, and, therefore, the Renshaw cell pathway can function as the physiological antagonist of the gamma driven system. These several authors suggest that the Renshaw cell's function is to apply negative feedback to the motoneurons and thereby stabilize or limit the motoneuron firing frequency. In all of these studies, the Renshaw cells have been activated by the highly unphysiological stimulation of the ventral root, resulting in maximal Renshaw cell activity and presumably maximal effects upon the motoneurons. One wonders about the ability of Renshaw cells to effect such large cells as motoneurons if the Renshaw cell discharges with only a few spikes as a result of natural motoneuron activity.

The functions of Renshaw cells mentioned above concentrated upon the effects of inhibitory actions of these cells on motoneurons. The inhibition of IaINs by Renshaw cells and the subsequent facilitation of

motoneurons raises the possibility for other roles for Renshaw cell behavior.

The phenomenon of recurrent facilitation of motoneurons was first discovered by Renshaw (1941), and its distribution, as described by Wilson et al, (1960), is generally confined to antagonist motoneuron pools with recurrent inhibition occurring in the agonist motoneurons. Hultborn et al (1971a) and Jankowska and Roberts (1972) have shown that Renshaw cell mediated recurrent facilitation of motoneurons operates via inhibition of IaINs by Renshaw cells resulting in concomitant dis-inhibition of the motoneurons. Hultborn and Lundberg (1972) demonstrated that the level of reciprocal inhibition that exists between antagonist motoneuron pools in the resting decerebrate cat is reduced during certain types of motor output. Specifically, during the co-activation of α and γ motoneurons in the stretch reflex reciprocal inhibition is reduced. Lundberg (1975) has suggested that the Renshaw cells, via their actions on the IaINs, decrease transmission in the reciprocal inhibitory pathway between antagonist motoneuron pools.

Within any given motoneuron pool there exists both IaIN inhibition and recurrent inhibition. Cleveland et al (1972) have investigated the interactions of these two types of inhibitions on monosynaptic reflexes. They found that the amount of inhibition of a reflex subjected to concomitant reciprocal and recurrent inhibition was smaller than the sum of the separate inhibitions. The most likely explanation for this result is that there is convergence of both types of inhibition onto the same motoneurons. Thus a motoneuron that was already inhibited reciprocally could not further contribute to the reduction of the monosynaptic reflex

when antidromic inhibition was added. These authors were able to demonstrate that the distribution of recurrent and reciprocal inhibition was uniform throughout the motoneuron pool and also that reciprocal and recurrent effects were of about equal magnitude upon the monosynaptic reflex.

The idea that Renshaw cells function to reduce reciprocal inhibition is interesting in view of the fact that locomotion involves co-activation of the α and γ motoneurons (Severin et al, 1967 ; Sjöström and Zangger, 1975). Presumably Renshaw cell mediated facilitation and inhibition could be operative during locomotion.

Fig. 1 is a schematic diagram of some of the known interconnections between IaINs, motoneurons and Renshaw cells. These interconnections have been presented previously by Hultborn et al. (1971c) and Cleveland et al. (1972). The limitation of inhibitory Renshaw cell effects to agonist motoneurons in the decerebrate preparation has been suggested by Brooks and Wilson (1959) (but see also Eccles et al. 1961b).

The confinement of Renshaw cell-IaIN connections to IaINs of the antagonist motoneuron pool is suggested by the work of Cleveland et al. (1972). The reciprocal connections between the antagonist IaINs have been defined by Hultborn et al. (1976). As Feldman and Orlovsky (1975) have shown, the IaINs are phasically active in locomoting preparations which have no phasic afferent activity and are thus presumably associated in some way with the spinal locomotor generator.

With reference to Fig. 1, consider the effects of an extensor motoneuron firing. The extensor activity excites the extensor Renshaw

cell, which in turn has two inhibitory effects: a) the Renshaw cell-motoneuron pathway reduces the excitability of the extensor motoneuron, and b) the Renshaw cell-IaIN pathway reduces the excitability of the IaIN which terminates upon the flexor motoneuron. Since the IaINs are phasically active during locomotion, this reduction in flexor IaIN firing would have at least two effects, a) the flexor motoneuron would become relatively disinhibited and hence more likely to initiate firing, and b) the IaIN to the extensor motoneuron would also be disinhibited via the reciprocal connections between the flexor and extensor IaINs. This in turn would tend to inhibit the extensor motoneuron as well as to further reduce the activity in the flexor IaIN via their reciprocal connections. Thus, extensor motoneuron firing would tend to reduce the excitability of the extensor motoneuron pool by both Renshaw cell and IaIN pathways, as well as to increase the excitability of the flexor motoneuron pool. This scheme suggests that the IaINs and the Renshaw cells may be functionally connected during locomotion so as to facilitate the switching by the spinal generator between flexion and extension phases of locomotion. If Renshaw cells operate during locomotion this scheme represents a new and possibly very important role for the Renshaw cell in modifying motor output.

Recurrent Inhibition During Locomotion

While no one has directly examined the behavior of Renshaw cells during locomotion, there are three studies (Severin et al., 1968; Bergmans et al. 1969; Feldman and Orlovsky, 1975) that have been quoted (Grillner, 1975; Shik and Orlovsky, 1976; Hultborn, 1976)

in support of the idea that Renshaw cells are depressed and presumably not functioning during locomotion. Severin et al. (1968) observed that antidromic stimulation of a cut ventral root was not as effective in inhibiting motoneuron discharge during locomotion as compared to inhibiting the discharge elicited by stretch of the muscle. These authors (1967, 1976) and Grillner (1975) have proposed, then, that Renshaw cells are depressed during locomotion.

Another possible explanation for the reduction in recurrent inhibition during locomotion seen by Severin et al. (1968) is that there was a change in the excitability of the motoneurons in which attempts to measure recurrent inhibition were made. In fact, the data which they presented do show that the motoneuron firing rates during locomotion were often greater than the rates resulting from stretch of the muscle, and it may be that motoneurons in this condition of excitation are more difficult to inhibit. The occurrence of recurrent facilitation during locomotion of one of the motoneurons seen in their data (Filament number 6 in their Table 1) would seem to be strong evidence in favour of the ability of the Renshaw cell pathway to perform during locomotion and would also lend support to the notion that Renshaw cells are not depressed during locomotion.

The second study quoted in support of the inhibition of Renshaw cells during locomotion is that of Bergmans et al. (1969). These authors demonstrated that after DOPA (dihydroxyphenylalamine) injection, conditioning stimuli to the flexor reflex afferents (FRA) causes a reduction in the Renshaw cell discharge elicited by antidromic ventral

root stimulation. Since acute spinal cats will step after DOPA (Grillner, 1969), it is reasonable to draw comparisons between DOPA preparations and actual walking preparations. In other studies in DOPA treated cats without FRA stimulation, recurrent inhibition in motoneurons is either unchanged or enhanced after DOPA (Andén et al. 1966). In addition, Fu et al (1975) have shown that, after DOPA treatment, the recurrent inhibition of IaINs is still effective. Nevertheless, Grillner (1975) and Shik and Orlovsky (1976) have interpreted the Bergmans et al. (1969) study as suggesting that Renshaw cells are generally depressed in the two preparations.

Lastly, the work of Feldman and Orlovsky (1975) has been used as further evidenced for depression of Renshaw cells during locomotion (Hultborn, 1976). Feldman and Orlovsky recorded from IaINs during locomotion and tried to antidromically inhibit the firing by stimulating a ventral root. In two of the four cells tested the efficacy of recurrent inhibition was unchanged during locomotion and in the other two it was reduced. An examination of their Figure 8 shows that recurrent inhibition reduces the firing of the IaIN during locomotion. The IaIN is not totally silenced during locomotion, but it is apparent that its pattern of firing is quite different during locomotion compared to the non-locomoting state. Since there is a tremendous amount of convergence from supraspinal, segmental, and intraspinal sources upon IaINs (for a review see Hultborn, 1976) it is possible that the reduction in recurrent inhibition of IaINs during locomotion seen by Feldman and Orlovsky (1975) is a result of a change in IaIN activation and not a

depression of Renshaw cells.

Since the question of Renshaw cell depression during locomotion has yet to be resolved, one aspect of this thesis is to directly examine the excitability of Renshaw cells during locomotion.

The Fictive Locomotion Preparation

Perret (1973) showed that the activity pattern recorded in muscle nerves during locomotion was essentially unchanged after the animal was paralyzed with curare. Perret described this rhythmic efferent activity without muscle activation as "fictive locomotion", a term which will be used throughout this thesis. Feldman and Orlovsky (1975) have also used the locution "fictive locomotion" to describe rhythmic ventral root filament activity in a cat with the ventral roots cut. Evidence has been presented earlier in this introduction which shows that the spinal cord contains a locomotor pattern generation. Since stimulation of the subthalamic nucleus (Orlovsky, 1969) the pontine locomotor area (Mori et al, 1977), the dorsal roots (Budakova, 1971), the midbrain locomotor region (Shik, Severin, and Orlovsky, 1966), the dorsal columns (Grillner and Zangger, 1974) as well as the administration of L-DOPA (Jankowska et al, 1967) and clonidine (Forssberg and Grillner, 1973) to spinal animals all induce locomotion on a treadmill, the most parismonious explanation is that all of these act upon a common stepping generation. The use of any of these stimuli should then produce a fictively locomoting preparation, the activity of which can be monitored by observing the rhythmic activity in a ventral root filament before and after paralyzation.

The experiments to be conducted here will, for the most part, use a fictively locomoting preparation which "walks" spontaneously, or by stimulation of either the midbrain locomotor region (MLR) or a cut dorsal root. The use of L-DOPA or clonidine was considered imprudent because of the possibility that the drug may be interfering with the system under study as well as inducing locomotion. The spontaneously walking preparation has the advantage of avoiding even electrical stimulation and differs from the MLR preparation in the amount of brain removed during the decerebration (See Figure 2 and Methods).

RESEARCH OUTLINE

This introduction has pointed out the existence of a spinal mechanism capable of producing co-ordinated stepping movements. The Renshaw cell is in a position in which it could assist the operation of this generator, and a schematic model was presented earlier (Figure 1) which utilized some of the known connections of Renshaw cells to facilitate the switching between flexor and extensor motoneurons.

Indirect evidence has been used by some authors (Grillner, 1975; Shik and Orlovsky, 1976) to suggest that Renshaw cells are depressed and presumably of relatively little importance in stepping. As mentioned earlier there are serious objections to the hypothesis of Renshaw cell depression during locomotion, and this thesis will direct itself to the activation and activity of Renshaw cells during fictive locomotion. A summary of the research strategies follows.

a) Renshaw Cell Depression:

The ability of the motoneuron collaterals to activate Renshaw cells will be measured before and during fictive locomotion. Single stimuli will be applied to the ventral roots and the number of Renshaw cell spikes elicited will be counted. This test is hereby defined as Renshaw cell excitability testing, and will detect the presence of an inhibitory process on the Renshaw cell during locomotion.

b) Renshaw Cell Activity During Locomotion:

The spontaneous activity of Renshaw cells will be examined to see if they are activated naturally during locomotion and also to see if

a Renshaw cell is activated at the same time as its motoneuron pool is active. The scheme of Figure 1 is operational only if Renshaw cells are phasically active and fire with the appropriate motoneuron pool.

c) The Recurrent Inhibitory Pathway:

The ability of the Renshaw Cell pathway to produce IPSPs in motoneurons during locomotion will be investigated using intracellular recordings from motoneurons.

d) Pharmacological Manipulations:

If the scheme in Figure 1 is an essential part of the spinal stepping generator, then drugs that block Renshaw cell activation from motoneuron axon collaterals should reduce the ability of the stepping generator to produce locomotion. To this end mecamylamine and atropine will be administered to cats walking on a treadmill and locomotor ability will be assessed.

e) Stimulation of the Renshaw Cell System During Locomotion

If the Renshaw cell system is an important part of the locomotor generator, then one should be able to alter the locomotor generator by electrically activating this system. Nerve cuffs will be placed on muscle nerves in deafferented decerebrate cats walking on a treadmill and used to antidromically activate the Renshaw cell population. Examination of the patterns of EMG activity will detect any changes in the locomotor rhythm.

METHODS

Surgical and Anaesthesia Procedures

All data was collected from decerebrate adult cats of either sex (2.0 - 4.5 kg). Microelectrode recordings were obtained in 30 successful preparations from approximately 125 attempts. Electromyographic data from decerebrate animals walking on a treadmill was recorded in 9 cats during 13 attempts.

Surgery was performed under halothane anaesthesia administered in a mixture of nitrous oxide and oxygen using a Dragger halothane vaporizer. Anaesthesia was induced in an airtight box and maintained using first a face mask and then a tracheal catheter.

A midline incision of the throat allowed exposure of the trachea and common carotid arteries. The trachea was cut open and a metal "T" catheter inserted. The common carotid arteries were tied off and the right carotid artery cannulated proximal to the tie with a silastic catheter filled with a heparinized lactated Ringer's solution. The arterial catheter was connected to a Statham pressure transducer which was used in conjunction with a Grass polygraph for continuous blood pressure recording. A venous cannula was placed in either the right femoral or left saphenous vein for administration of fluids or drugs. When required a dorsal lumbar laminectomy was performed. The skin was incised along the midline of the back from approximately the T_{10} to the S_5 level. A cut along the tops of the vertebral spines gave exposure of the spinalis dorsi muscles which were then removed. The spinal canal was entered at the L_7 S_1 junction, and bone cutters

and rongeurs were used to remove the dorsal portions of the L_7 to L_4 vertebræ. Any muscle bleeding was controlled with cautery, and bone wax was used to stop bleeding from bone edges.

The animal was then placed in a spinal frame (a modified version of a Transvertex frame). The L_3 and S_1 spines were rigidly clamped and sharp pins were pushed through the skin just under the iliac crests in order to secure the pelvis. A back pool was constructed using the skin flaps and filled with warm paraffin oil to prevent the cord from drying out. Specially constructed clamps were placed against the lateral edge of the lumbar vertebra where the microelectrode was to be inserted.

The spinal frame had been modified so that the cat's legs were freely suspended above the table. Animal temperature was maintained using a heat lamp above the back pool. End tidal CO_2 and arterial blood pressure were continuously monitored.

The spinal dura was opened, and with the aid of a dissecting microscope, the appropriate dorsal and ventral roots were cut as they exited from the spinal canal. They were then mounted on platinum bipolar electrodes for stimulation or recording as required. In most experiments only the L_7 dorsal and ventral roots were cut on one side.

Some of the experiments were performed on animals which were walking on a treadmill. The treadmill was a variable speed motor driven design with a stereotaxic headholder positioned above one end. The rear of the cat was suspended loosely above the treadmill with a small foam rubber belt placed around the belly and adjusted to an appropriate height which allowed proper placement of the rear limbs on the treadmill (see also Figure 4).

All animals received lactated Ringer's solution intravenously in a slow drip throughout the surgery. Dextran 70 was given as a rapid i.v. push (5-15 cc) immediately after decerebration to compensate for blood volume loss. In a few animals, recovery after decerebration was accompanied by very high blood pressures and Mayer waves. In such cases a small amount of meperidine hydrochloride (1 mg/kg i.v.) was administered, resulting in a normal blood pressure record.

Decerebration

All data was obtained from decerebrate animals. The animal's head was mounted in a stereotaxic headholder and the scalp cut along the midline. An extensive craniotomy was performed, and all exposed bone edges were sealed with bone wax to prevent possible air embolism formation. The cerebral cortices were removed with a blunt spatula, and the superior saggital sinus was cut and tied. The transection for the decerebration was performed at the appropriate level using a stereotaxically guided blunt spatula. Halothane anaesthesia was then discontinued and bleeding controlled by packing the skull with Surgical^{R}. The skull cavity was then covered with a 4% agar solution to prevent the remaining brain from drying out.

The level of transection of the decerebration was either pre- or post mammillary. In the premammillary (i.e. thalamic) cats the transection extended from 1 mm rostral to the rostral border of the superior colliculus to approximately the caudal border of the optic chiasm.

In successful preparations, within 1 to 2 hours of termination of anaesthesia, locomotion either occurred spontaneously or could be induced by a brief stimulus train to the cut dorsal root (30 Hz; .5 msec

pulses; up to 5 volts; train duration less than one second). Locomotion induced by a brief dorsal root stimulation persisted for several seconds after termination of the stimulus; therefore, it was considered to be due to activation of the locomotion generating centers and not to be a reflex phenomenon (see Budakova, 1971). The thalamic cats were often very responsive to external stimuli and sometimes could be made to initiate locomotion by simply gently stroking or tickling the animal's ribs or perianal region.

Some of the animals used were decerebrated at a more caudal level; the postmammillary preparation. In the postmammillary (i.e. mesencephalic) cats the transection extended from the rostral border of the superior colliculus dorsally to the caudal border of the mammillary bodies ventrally (see Fig. 2). Such animals do not walk spontaneously, but can be induced to walk with electrical stimulation of an area in the mesencephalon, the midbrain locomotor region (MLR).

In order to induce controlled locomotion in postmammillary animals, a monopolar steel electrode was lowered to a depth of 4 mm from the brain surface at a point 4 mm lateral to the midline and 1.5 mm caudal to the inferior/superior colliculi border. Constant current pulses of .5 msec duration, 30 Hz, 40-200 μA were used to stimulate the MLR region. The electrode was lowered in .5 mm increments until stable locomotion occurred. This mesencephalic preparation was used to produce controlled walking on the treadmill as well as to provide a preparation for fictive locomotion.

Nerve Cuffs

Since in some experiments it was desirable to stimulate individual muscle nerves without destroying the muscle innervation, small nerve cuffs were placed around the muscle nerve just proximal to the entrance of the nerve into the muscle. These nerve cuffs consisted of .058" (or smaller) diameter silastic tubing into which insulated 40 A.W.G. enamel coated wire was placed. The wire was inserted into a 30 gauge needle and the needle was pushed through the tubing to form a line parallel with the tubing diameter. The tubing (4 - 8 mm in length) was slit open longitudinally and the wire inside the tubing bared of insulation with a scalpel. There were two such wires in each cuff, at opposite ends. The cuff was opened longitudinally, placed around the appropriate nerve and gently tied in place. The wire leads were taken out through the incision in the skin and attached for stimulation or recording as required. Electrical stimulation of the cuff electrode allowed visual identification of the particular muscle contracting before the skin in the leg was closed. The same type of cuff used was used sometimes to stimulate a dorsal or ventral root in the spinal canal so as to maintain the peripheral innervation.

Stimuli to peripheral nerves or nerve roots consisted of constant current pulses of .1 ms duration delivered from Grass S88 stimulators.

Electromyogram Recording

Besides direct visual examination of the treadmill locomoting animal, electromyograms (EMG) were employed to record the contractions of various hindlimb muscles. EMG records were obtained by inserting two 36 gauge wires directly into the muscle in question and recording the

EMG activity using differential amplifiers. About 1 mm of the copper wire was stripped of insulation and placed in a 25 gauge needle. The bared end protruded from the point of the needle and was bent back towards the hub of the needle to form a hook. The needle and wire could be inserted into a muscle and the needle withdrawn, leaving the wire in the muscle. For muscles which lay immediately against the skin (e.g., semitendinous, vastus lateralis) the needle was inserted through the skin into the muscle. For deeper muscles (e.g., iliopsoas, adductor femoris) it was necessary to open the skin and expose the muscle before inserting the electrodes.

The EMG electrodes were fed into differential amplifiers, amplified, and displayed on a pen recorder. The EMG records were then analyzed in order to determine changes in locomotor patterns produced by experimental manipulations.

Fictive Locomotion

In order to monitor the occurrence of fictive locomotion, a filament of the L_7 ventral root was isolated which showed rhythmic activity coincident with movement of the cat's legs in the air corresponding to a particular phase of the step cycle. Filament activity was recorded while the cat's legs were moving in the air, then the animal was paralyzed with 5 mg/kg gallamine triethiodide (i.v.). Brooks and Wilson (1959) previously demonstrated that this drug exerts no effect on Renshaw cell discharge or recurrent inhibition. The animal was artificially respired, and end tidal CO_2 was monitored and maintained between 3.5 and 5%. Ventral root filament activity after paralyzation

was very similar to that before administration of the paralyzing drug, and this type of locomotion has been termed "fictive locomotion" (see Introduction).

Sometimes it was necessary to rhythmically swing a forelimb of the paralyzed thalamic animal to initiate the rhythmic ventral root activity. The rhythmic filament activity persisted for a number of fictive "steps" after the forelimb swinging stopped, demonstrating that the forelimb swinging facilitated activation of the spinal locomotor generator and did not merely initiate propriospinal reflex activity. In the cats that required dorsal root stimulation to initiate fictive locomotion, swinging the forelimb alone did not induce rhythmic ventral root discharge. During the course of the experiment, recovery from the effects of the paralyzing agent would periodically occur, and the correspondance of ventral root filament activity to actual locomotion could again be ascertained.

In preparations which had no nerve cuffs it was possible to assign the terms "flexor" or "extensor" to the ventral root filament discharge by noting the correspondance of filament activity to actual movements of the limb before paralyzation. When nerve cuffs were employed, the activity of the filament could be compared to electrical activity recorded in the nerve cuff to identified muscles as well as actual limb movements.

Microelectrode Recording

Conventional glass microelectrodes were used to record from single cells in the spinal cord. The electrodes were pulled on a Narashige electrode puller from either glass capillary tubing or

fiber filled electrode blanks (A.M. Systems) and filled with 3M NaCl or 3M Potassium acetate (KAc). Electrodes were bevelled to tip diameters of $\leq$1 micron and had DC resistances of 5 to 15 megohms.

The glass microelectrodes were mounted on a mechanical microdrive (Transvertex) and lowered into the cord by a remote control. The electrodes were fed into a high impedance voltage follower circuit (W.P.I. model M4A). The output of the electrometer was taken to both DC and AC coupled vertical amplifiers in an oscilloscope (Tektronix 565, Tektronix DIO).

Film records of the oscilloscope traces, (Grass C4, Kymography camera, Kodak 2495 RAR film or Kodak Linagraph 1930 paper) were developed (Kodak D-19) and analyzed. The acquisition of a tape recorder (Hewlett Packard, 3960, 2 channels FM, 2 channels direct) for the latter experiments allowed continuous data collection which could be played back and displayed for filming or for reproduction with an ultraviolet oscillograph recorder (SE labs, 3006/DL, 4 channel, -3db at 9KHz; Kodak Linagraph Direct Print Paper, #1895).

In the earlier experiments the Grass S88 stimulators were used to stimulate the preparations and also to trigger the horizontal sweep section of the oscilloscopes. In latter experiments a Digitimer was used to program control of the stimulators and the oscilloscopes. An audio monitor was used as an aid in locating neurons. Figure 3 shows a schematic diagram of the recording set up.

A variety of spinal cord interneurons were recorded during fictive locomotion. The data presented here were obtained from Renshaw cells, IaINs, and motoneurons. Renshaw cells were identified by their

characteristic high frequency discharge caused by ventral root stimulation (Renshaw, 1946). Motoneurons were impaled intracellularly and identified by their single response to single ventral root stimuli. IaINs were distinguished by their monosynaptic (less than .5 msec) activation from dorsal root stimulation at a threshold equal to the appearance of the first deflection recorded in the cord dorsum electrode. In addition IaINs followed dorsal root stimulation at up to 300 Hz and were depressed by stimuli to the ventral roots. All IaINs recorded from were in the ventral horn and often were close to Renshaw cells.

Renshaw Cell Activity During Fictive Locomotion

Renshaw cells were located and their extracellular activity recorded during fictive locomotion. Renshaw cell excitability testing consisted of applying a single stimulus to the ventral root and counting the number of Renshaw cell spikes elicited before commencement of and during fictive locomotion. Spontaneous activity of these cells was also recorded during fictive locomotion.

Recurrent Potentials During Fictive Locomotion

Motoneurons were impaled with 3M KAc electrodes and single stimuli applied to the ventral root. Such stimuli produce recurrent inhibitory potentials (RIPSPs) or recurrent facilitatory potentials (RFPs) in non-locomoting animals (Wilson and Burgess, 1962), and these phenomena are mediated by activity of Renshaw cells (Eccles et al 1956, Hultborn et al 1971). The ability of Renshaw cells to produce recurrent effects in motoneurons was examined during fictive locomotion.

Blocking Activation of Renshaw Cells

As pointed out in the Introduction, mecamylamine and atropine can

block activation of Renshaw cells from the motor axon collaterals. Seven animals were decerebrated and induced to walk on an motor driven treadmill. Injections of mecamylamine and then atropine (4 cats) or atropine and then mecamylamine (3 cats) were given i.v. and effects upon treadmill locomotion assessed using EMG records.

Stimulation of Renshaw Cells During Treadmill Locomotion

In 2 animals a unilateral dorsal root transection was performed using aseptic precautions and Nembutal anaesthesia (35 mg/kg i.p.). The L_3 to S_3 dorsal roots were transected as they entered the cord after removing a small channel of bone from the dorsal vertebral surfaces just lateral to the spiny processes. A recovery period of about one week was allowed during which systemic ampicillin administration (50 mg/kg/day; i.m.) was used as prophylaxis against infection. On the day of the experiment nerve cuffs were placed around hindlimbs nerves under halothane anaesthesia before the animal was decerebrated and induced to walk on the treadmill with MLR stimulation.

RESULTS

The results described here were all taken from unanaesthetized, decerebrate cats which were engaged in either locomotion on a motor-driven treadmill or in what has been termed "fictive-locomotion". For the purposes of this thesis we have defined fictive locomotion as rhythmic discharges recorded in ventral root filaments after paralyzation of the animal with gallamine triethiodide. The fictively locomoting animal is thus a preparation without muscle activity or cyclic afferent activity, and the rhythmic motoneuron discharges are the result of co-ordinated neuronal activity of central origin.

One of the major contributions of the work presented in this thesis has been the development of a fictively locomoting preparation which permits the use of microelectrode recording in order to further understand the phenomenon of rhythmic motoneuron activity. The fictively locomoting cats were either decerebrated at the pre-mammillary or post-mammillary level (see Methods). The post-mammillary animals were induced to "walk" by stimulation of the MLR. The stimulus required to initiate locomotion varied for each of the premammillary animals but consisted of one or more of the following; a brief dorsal root stimulus, swinging of a forelimb, or gentle cutaneous stimulation in the perneal region. In some of the premammillary animals fictive locomotion occurred spontaneously.

The Transvertex stereotaxic frame with the modified baseplate allowed the animal's legs to swing freely in the air above the table. With the appropriate stimulus the animal would commence co-ordinated stepping movements of the limbs. The isolation of a fine ventral

root filament with rhythmic activity coincident with stepping was used as the monitor of locomotion, and its activity was photographed. If the rhythmic activity of the ventral root filament persisted after paralyzation with gallamine triethiodide, the preparation was said to be fictively locomoting.

Figure 5 is an example of ventral root filament discharge before and after paralyzation. The trace in 5A shows filament activity during actual stepping of the animal in the air. The leftmost "step" in 5A is an example of the burst beginning with doublet firing, a phenomenon previously described in cats induced to walk on a treadmill by MLR stimulation (Zajac and Young, 1975). As the traces in 5B show, rhythmic filament activity was very similar both before and after paralyzation. In this case the doublet activity was still present after paralyzation.

In some of the experiments, the use of nerve cuffs allowed more precise assessment of the periodicity of the filament discharge during the step cycle. Figure 8A shows the activity of a ventral root filament along with electrical activity in the nerve to quadraceps. Since quadraceps is a knee extensor, and filament activity occurs after the nerve cuff activity, this filament was labelled as belonging to a flexor motoneuron pool (see Discussion). In the animals in which nerve cuffs were not employed, the portion of the step cycle in which the filament was active was identified by visually noting the position of the leg while listening to the filament discharge on the audio monitor.

During the course of microelectrode recording in the fictively locomoting preparation, many interneurons were found to be phasically active. Of particular interest were the IaINs which were identified

according to standard criteria (Hultborn et al, 1971); activated from lowest threshold afferents, capable of following peripheral stimulation at high rates, and subject to inhibition from the ventral root. Figure 6 shows the activity of a IaIN recorded during fictive locomotion. This cell and the four others studied showed phasic activity in the absence of cyclic afferent input in the paralyzed animal and confirms a similar finding obtained by Feldman and Orlovsky (1975) in the de-efferented "locomoting" preparation.

In the three cells obtained in preparations with dorsal roots intact and a cuff stimulating electrode placed around hindlimb muscle nerves, it was possible to identify the source of their monosynaptic excitation from group Ia afferents. All three were coupled to extensor muscles of the hindlimb and all displayed maximal rates of discharge during the "stance" phase of fictive locomotion. These results demonstrate that IaINs are modulated centrally during fictive locomotion, since there was no cyclic afferent activity in these paralyzed animals.

The close correspondance of the activities of motoneurons and IaINs in these experiments to those reported in other walking preparations suggests that the phenomena reported here may also occur in locomoting preparations used in other laboratories (Zajac and Young, 1975; Feldman and Orlovsky, 1975; Severin et al, 1968).

Renshaw Cell Activity During Fictive Locomotion

Extracellular microelectrode recordings from Renshaw cells allowed assessment of their activity during fictive locomotion. Examples of Renshaw cell activity are presented in Figures 7, 8, and 9,

which show that Renshaw cells display bursts of activity dependent upon the phase of fictive locomotion. Some Renshaw cells were silent before fictive locomotion and then became rhythmic once locomotion commenced; other Renshaw cells were active before locomotion and became phasically active during fictive locomotion. The activity of a Renshaw cell which was spontaneously active before locomotion was sometimes of a lower frequency during than before locomotion (see Discussion).

As Figure 7, 8 and 9 demonstrate, the discharge rates of the Renshaw cells during their active phase of fictive locomotion are much lower than the rates obtained by single stimuli to the cut ventral root (Renshaw 1946, Eccles et al, 1954). Analysis of the rates of discharge during fictive locomotion of 17 Renshaw cells shows that the cells were usually silent between active phases (16 out of 17 cells), and that they fired between 1 and 22 spikes during their active phase of the fictive step. The duration of Renshaw cell firing was up to 1.25 sec., and the maximum rate of firing during the active period was 45 Hz.

A total of 17 Renshaw cells were examined and found to be rhythmically active during locomotion. In 11 of these, it was possible to relate their period of firing with that of a ventral root filament known to fire in a specific phase of locomotion. In this way, 6 cells were shown to be active during the swing phase, and 5 cells during the stance phase of locomotion.

Although there is a great deal of excitatory convergence onto a

Renshaw cell, it is well established that the convergence follows the general rule that synergists about a joint may send motoneuron collaterals to a given Renshaw cell, whereas antagonist muscles have virtually no input to the Renshaw cell (Brooks and Wilson, 1959). If Renshaw cell firing during fictive locomotion is determined by excitatory input from motoneuron axon collaterals, it would be expected that Renshaw cells primarily activated from flexor motoneurons (flexor-coupled Renshaw cells)would be active during the stance phase of fictive locomotion. The use of nerve cuffs on individual muscle nerves allowed identification of the Renshaw cell and determination of the activity phase of the cell.

Figure 8 shows a Renshaw cell which was coupled to the posterior biceps motoneuron pool (8b). This cell did not respond to either quadraceps or lateral gastrocnemius stimulation. This flexor coupled Renshaw cell was active during the same phase as the isolated ventral root filament. As Figure 8a shows, the active units in the filament were identified on the basis of their firing relative to recordings made in the nerve cuff on the knee extensor quadraceps prior to paralyzation. Thus this flexor coupled Renshaw cell fires in phase with the ventral root filament which is active during actual flexion of the limb.

Figure 9 is another example of Renshaw cell identification and activity during fictive locomotion. This cell was driven maximally by lateral gastrocnemius stimulation (an ankle extensor) but not by the ankle flexor tibialis anterior. The knee flexors posterior biceps and semitendinosis did not excite this cell and the quadraceps nerve

produced a low level, long latency excitation of this Renshaw cell. As shown in Figure 9d, this cell fires out of phase with the ventral root filament during fictive locomotion. This filament was identified as being active during the swing phase of locomotion prior to paralyzation. The stimulus artifacts in Figure 9d result from MLR stimulation.

This pattern of extensor coupled Renshaw cells active during the stance phase of fictive locomotion and flexor coupled Renshaw cells active during fictive swing was observed in each of the three extensor coupled and the one flexor coupled Renshaw cells studied in this manner.

Renshaw Cell Excitability During Fictive Locomotion

As pointed out in the introduction, some authors have suggested that Renshaw cells are depressed during locomotion. In order to test this hypothesis we have excited Renshaw cells by single ventral root stimuli and compared the number of action potentials elicited during locomoting and non-locomoting states. This test is operationally defined as Renshaw cell "excitability testing".

Figure 10 shows excitability testing in one of Renshaw cells studied. The upper traces in Figure 10 show the resulting discharge of the cell after a single stimulus to the cut ventral root. The lower traces in Figure 10 a, b, c are records of ventral root filament activity. The dot near the centers of Figure 10 a, b, c is an oscilloscope derived pulse which occurs during the period when the upper trace sweeps across the face of the oscilloscope. Note that the upper traces in Figure 10 and 50 ms in duration and the lower traces are 5 sec long.

Figure 10A is the Renshaw cell excitability in the non-locomoting state and shows 19 spikes elicited by a single ventral root stimulus.

Figure 10B shows the Renshaw cell excitability when the cell was induced to fire at a time coincident with ventral root filament discharge. 17 Renshaw cell spikes were produced, whereas 18 spikes were elicited by the antidromic ventral root stimulus during the period out of phase with ventral root filament discharge (Figure 10C). The filmed records were examined, and each frame was placed into one of the following categories: no locomotion, fictive locomotion in phase with ventral root discharge, and fictive locomotion out of phase with ventral root discharge. The lower portion of Figure 10 shows the average excitabilities of this Renshaw cell during various phases of fictive locomotion. The excitability of the cell was virtually the same during the non-locomoting and the fictively locomoting states and is typical of the Renshaw cells studied.

Excitability testing was carried out in 10 Renshaw cells and these results are shown in Table 1. The data show that the Renshaw cells are not depressed during fictive locomotion, with the exception of cells number 2 and 9 in Table 1. Both of these cells showed a small but significant reduction in the number of spikes elicited by ventral root stimulation during fictive locomotion. Figure 11 is taken from the records of cell number 2, Table 1. Fictive locomotion was induced in this cat by brief train of stimuli to the dorsal root (Figure 1, panel 2).

As indicated in Table 1, Renshaw cell excitability was assessed during locomotion for periods both "in phase" and "out of phase" relative to ventral root discharge. Since the motoneuron filament could not always be identified as belonging to a specific motoneuron pool, it was

usually possible to assign the terms "out of phase" and "in phase" to a particular part of the step cycle only by observing the correspondance between the filament discharge and the movement of the cat's leg before paralyzation. There was no significant difference in the mean levels of excitability as determined by ventral root stimulation between either the phases of locomotion or between non-locomoting and locomoting states when the entire population of Renshaw cells was considered. Cell 9 was the only case observed in which there was a significant difference between phases of locomotion in the number of spikes evoked. In other words, Renshaw cell responsiveness to ventral root stimulation only rarely changed cyclically during locomotion.

In the Renshaw cell excitability determinations, usually only the first 50 ms after the stimulus are reported. Observation of such data on moving film or magnetic tape revealed that the high frequency discharge is over by this time and what follows is a period of no activity similar to the pause phase described by Curtis and Ryall (1966b). The late, long-lasting, low frequency discharge described in barbiturate anaesthetized cats and attributed to the activation of muscarinic receptors by Curtis and Ryall (1966) was not observed in these decerebrate preparations. The stimuli to the ventral root usually occurred every 5.6 seconds during our excitability testing.

Recurrent Events in Motoneurons During Fictive Locomotion

The results presented in the preceeding sections of this thesis have shown that Renshaw cells are rhythmically active during fictive locomotion and that excitability testing does not demonstrate a gen-

eralized depression of Renshaw cells during fictive locomotion. In order to determine whether the inhibitory and facilitatory pathways from motor axon collaterals to motoneurons are intact during fictive locomotion, intracellular recordings were obtained from motoneurons using potassium acetate filled microelectrodes.

Stimulation of the same ventral root in which the motoneuron was located at strengths less than that required for production of the antidromic action potential often revealed the recurrent inhibitory postsynaptic potential (R-IPSP) (Eccles et al, 1954). As illustrated in Figure 12, R-IPSPs can be recorded in α-motoneurons during fictive locomotion. The amplitudes of the R-IPSPs varied during fictive locomotion and were maximal when the motoneuron was in a depolarized phase of locomotion (Figure 12 c,d) and minimal during hyperpolarization (Figure 12 b,e). In all motoneurons having R-IPSPs studied (a total of 8), the R-IPSPs observed prior to the commencement of fictive locomotion persisted during locomotion.

Figure 13b shows the R-IPSPs produced in another motoneuron on a continuous record. Note the smaller amplitude of the R-IPSP during hyperpolarization (Figure 13b, left arrow). Figure 13c and d show the distributions of R-IPSP amplitude versus motoneuron membrane potential for the cells illustrated in Figure 13a and b respectively. The high correlation coefficients obtained (Figure 13c, $r = .95$; Figure 13b, $r = .75$) suggest that most of the variation in R-IPSP magnitude is associated with variations of the motoneuron DC potential.

Recurrent facilitatory potentials (RFPs) were recorded in five motoneurons during fictive locomotion and were obtained by stimulating an

adjacent ventral root. Figure 14 shows that the RFP is still present during fictive locomotion (Figure 14 c). In Figure 14 b the RFP is greatly reduced in size but, as in the case of $_{R}$-IPSPs, it is possible that this reduction could be a result of the DC potential of the cell approaching the reversal potential for the RFP. These results indicate that the recurrent facilitatory pathway, consisting of motoneuron to Renshaw cell to IaIN to another motoneuron, may still be operational during fictive locomotion.

Blocking the Motoneuron to Renshaw Cell Pathway During Locomotion

The scheme presented in Figure 1 suggests that the activity of Renshaw cells during locomotion could contribute to the switching between flexion and extension phases of stepping. Since the data from the fictively locomoting animals shows that Renshaw cells and IaINs are spontaneously active and that, in general, the Renshaw cells are not depressed, the question arises as to the importance of the interconnections in Figure 1 for the locomoting animal.

As discussed in the introduction, the drugs atropine and mecamylamine have both been employed to block activation of Renshaw cells from motor axon collaterals (Curtis and Ryall 1966, Ryall and Haas, 1975). These agents were administered to cats which were induced to walk on a motor-driven treadmill by stimulation of the midbrain locomotor region. Locomotion was observed, the stimulus for MLR locomotion noted, and EMG electrode implanted to assess the effects of these drugs.

One of the difficulties associated with mecamylamine is that it can greatly lower blood pressure. In fact, in some of the animals a fall in blood pressure to 50 mm Hg was seen. Nonetheless, mecamylamine

up to 4.0 mg/kg I.V.) atropine (up to 2.4 mg/kg I.V.), or combinations thereof in seven animals, never abolished MLR evoked locomotion activity. In some experiments the threshold for effective MLR stimulation was raised, and in some the co-ordination of front and rear limbs was not optimal, but in two animals the MLR threshold was decreased. In three animals no effects upon stepping could be seen. The fall in blood pressure was reversed in one animal with i.v. adrenaline, and this cat regained his ability to spontaneously locomote with a blood pressure of 85 mm.

Figure 15 is an example of EMG activity recorded in the knee flexor semitendinosis and knee extensor vastis lateralis in both hindlimbs. Note that after 1.5 mg/kg atropine and 4.0 mg/kg mecamylamine the EMG patterns still retain this co-ordinated activity.

Stimulation of the Renshaw System During Locomotion

If the scheme present in Figure 1 contributes strongly to the generation of flexion and extension, then stimulation of the system should cause alterations in the step cycle. To test this possibility, 2 cats were deafferented in the left hindlimb under pentabarbital anaesthesia and allowed to recover for several days. The animals were then anaesthetized with halothane and silastic nerve cuffs placed around the quadraceps, abductor femoris, posterior biceps, semitendinosis, tibialis anterior and lateral gastrocnemius nerves for stimulation. EMG electrodes were placed in iliopsoas, adductor femoris, quadraceps and posterior biceps muscles. The leads for stimulation and recording were taken out through the skin which was then sutured. The animal was decerebrated and induced to walk on the treadmill with MLR stimulation.

Stimuli were presented as pulse trains (100-400 ms duration; .1msec width; 200 Hz) to various combinations of the muscle nerve cuffs. The effects of stimulation on the EMG recorded in the same hindlimb were examined by stimulation of each nerve separately and then in combinations of all extensors and all flexors. The intervals between onset of EMG bursts during treadmill locomotion were then measured from the chart records.

Figure 16 shows an example of EMG activity recorded in the adductor femoris muscle during MLR evoked treadmill locomotion. The arrows indicate 100 ms pulse trains to the nerve of the same muscle. No changes in the time of onset of the subsequent EMG burst after stimulation could be detected. In both cats, for all the EMGs examined there were no consistent changes in the timing of EMG onset, even though the stimuli caused what seemed to be maximal muscle contraction.

Since the locomotion in the deafferented limb was often poorer than in the other three limbs, only trials which showed well co-ordinated EMG patterns prior to stimulation were analyzed. At the end of the experiment the extent of the deafferentation was verified by removal and examination of the spinal cord.

DISCUSSION

Fictive Locomotion

The realization that the isolated cat lumbar spinal cord contains circuitry which can be activated to produce co-ordinated stepping (Grillner and Zangger, 1974) presents an approachable challenge to the current state of the art in neurophysiology. The fact that several methods involving electrical or pharmacological manipulations all produce locomotor activity (see Introduction) makes it tempting to suggest that there is one common spinal stepping generator susceptible to activation by a number of mechanisms.

The recent developments of fictively locomoting preparations by Shik and Orlovsky (1966), Grillner and Zangger (1974), and Perret (1968) allows investigation of this stepping generator but eliminates such extremely important variables in natural locomotion as cyclic afferent input and conscious control of locomotion by the animal. Any of the preparations involving non-spinal decerebrate cats are plagued with the problem that the great increase in extensor tone makes interpretations of resting activity in both motoneurons and interneurons difficult. MLR stimulation is often associated with urination, increased blood pressure, and on occasion postural activity which precedes treadmill locomotion. The use of DOPA and nialamide, or clonidine may of course alter the activity of various spinal pathways as well as produce stepping.

The results presented here have for the most part been obtained in thalamic cats (see Figure 2) which were induced to step by gentle

stimuli such as tickling or stroking, or by brief dorsal root stimulation. This preparation was chosen at the outset because it involved neither chemical nor prolonged electrical stimulation. Our experiences with the thalamic fictive locomotion preparation have included very high blood pressures, Mayer waves and excessive bleeding from the decerebrated brainstem. All of these factors contribute to a very low yield of successful preparations and for this reason the mesencephalic preparation using MLR stimulation was utilized for some of the latter animals, resulting in a much more reliable preparation.

Gallamine triethiodide (Flaxedil, 5 mg/kg, i.v.) was used as the paralyzing agent; Brooks and Wilson (1959) have shown that neither recurrent inhibition nor Renshaw cell discharge are affected by this drug. Perret (1976) reported that ..."flaxedil paralysis seemed little favourable to development of rhythmic activities" ... recorded in the peripheral nerves, but in our cats rhythmic ventral root filament activity almost always persisted after gallamine triethiode. One of the problems associated with using any particular ventral root filament as a monitor of locomotion is that paralyzation and the subsequent reduction in afferent activity could result in the filament no longer being active during locomotion. A more important concern is the possibility that motoneuron pools may change their relative periods of activation during the step cycle as a result of no cyclic afferent input. Indeed, Perret (1976) has shown that the double burst of activity seen in semitendinosis (Perret and Cabelguen, 1976) becomes

a single burst after paralyzation.

Nevertheless, the general features of alternate activity between flexors and extensors persists after paralyzation, and if there are differences in the relative timing of motoneuron pool activity, the differences are likely to be slight (Edgerton et al, 1976; Perret, 1973). A useful study at this time would be to simultaneously record nerve cuff activity and EMG, paralyze the animal, and analyze the activity patterns in the nerve cuffs. Such data obtained using the various fictive locomotion preparations would yield valuable information about interpreparation and intrapreparation variables in motoneuron activity.

Another problem with fictive locomotion and natural locomotion is that walk trot and gallop show some differences in EMG patterns (Tokuriki, 1973b, 1974), and it is difficult to be certain of the locomotor mode being utilized during fictive locomotion. In spite of the probability of discrepancies existing between natural and fictive locomotion, fictive locomotion involves a highly complex patterning of motoneuron firing, and it seems likely that it utilizes much of the same circuitry as natural locomotion.

Problems Associated with the Use of Nerve Cuffs

Studies utilizing an isolated ventral root filament allow characterization of the fictive step cycle into periods of swing and stance, but this type of analysis can only be quantitative if the identity of the filament is certain.

The identification of the isolated filament becomes of great importance for fictive locomotion research, and to this end some of the latter preparations involved the use of nerve cuffs. As explained in the methods, the cuffs were placed around peripheral nerves close to the muscles innervated. The electrical activity recorded in such cuffs consisted of phasic bursts during stepping in the air (see Figure 8); the bursts were coincident with contraction of the muscle innervated.

Since the neural activity recorded in the nerve cuff will primarily be from the larger nerve fibres (Stein et al, 1977), the activity recorded in the cuff will largely result from activity in the α motoneuron efferents, the γ motoneuron efferents the Ia afferents and other large fibre afferents (eg. group I cutaneous). There is strong evidence for α, γ co-activation during treadmill and fictive locomotion; in the MLR preparation (Severin et al, 1967); in the deafferented, spinal, DOPA treated cat (Sjostrom and Zangger, 1975, 1976) and in the decorticate cat (Perret and Buser, 1972). Thus we know that both the α and γ efferents are active during shortening of the muscle. As a result of the α, γ co-activation, the Ia afferents reach their maximal discharge rates during muscle shortening in controlled locomotion[1] (Sjostrom and Zangger, 1976; Perret and Buser, 1972). In addition to the neural activity recorded by the cuff, there will be a large signal from the electrical activity in the nearby muscle (Stein et al, 1977). The available evidence therefore supports using maximal nerve

[1]This may not be the case during walking in the intact animal, where it appears that Ia afferent activity is reduced during certain voluntary contractions of the muscles (Prochaska et al, 1979).

cuff activity as an index of muscle shortening, and nerve cuff activity would seem to be a useful way of identifying the phase of activity of the isolated ventral root filament.

The use of a rhythmically active ventral root filament is adequate for qualitative assessment of fictive locomotion, but the use of several nerve cuffs would provide more quantitative information about the activation of motoneuron pools before and after paralyzation. Recordings from the cuffs after paralyzation would probably require more sophisticated cuff arrangements (Stein et al, 1977) but would avoid some of the problems associated with ventral root filaments discussed earlier.

Motoneuron Activity During Fictive Locomotion

The doublets seen in the filament discharge after paralyzation (Figure 5) are of interest since they have been reported in DOPA induced fictive locomotion (Sjöström and Zangger, 1976) as well as during MLR treadmill locomotion (Zajac and Young, 1975). Burke et al (1970) have suggested that this type of muscle activation, a short interspike interval followed by a slower more constant spike train results in a faster rise time in muscle tension then would result from activation with a steady stimulus rate. Doublet firing patterns have also been seen in anaesthetised cat motoneurons in response to intracellular current injection but are described as atypical events associated with the delayed depolarization often seen in motoneurons (Calvin and Schwindt, 1972). Thus it is not clear at this time whether or not the doublet firing seen during fictive locomotion is a result

of an intrinsic property of motoneurons or is due to the firing patterns of interneurons converging upon motoneurons during locomotion.

The rhythmic DC potential shifts seen in some of the motoneurons (see Figure 12a) before commencement of spiking indicates that the fictive locomotion generator can operate via a gradual change in motoneuron excitability. Also the presence of only rhythmic DC potentials and no spiking in some of the impaled motoneurons during fictive locomotion suggests that the fictive locomotion generator does not activate all motoneurons simultaneously and may in fact be able to recruit motoneurons. Measurements of the neural activity in peripheral nerve cuffs after paralyzation should be able to detect motoneuron recruitment and would allow for testing the effects of different systems on the output of the generator of fictive locomotion.

IaINs and Fictive Locomotion

The finding that IaINs are rhythmically active both in the paralyzed preparations in this thesis (Figure 6) and the de-efferented locomotor preparation (Feldman and Orlovsky, 1975) indicates that the IaINs are interposed in the neuronal circuitry involved in fictive locomotion. The three IaINs that could be identified by activation from the periphery all showed their maximal rates of discharge at a time when the motoneuron pools to which they were coupled were active. Thus these IaINs would be delivering their maximal inhibition to a motoneuron pool when the antagonist motoneurons are firing. Since the antagonist Renshaw cells would be inhibiting the IaINs during antagonist motoneuron activity (see Figure 1), there must be some kind of excitatory convergence

upon the IaINs during fictive locomotion. As Lundberg (1975) points out, there are at least eleven excitatory pathways known to converge upon IaINs and at the present time we can say nothing about the source of this excitation to IaINs during fictive locomotion.

Lindström et al (1973) reported that five out of two hundred VSCT cells recorded from showed convergence of group I excitation and recurrent inhibition. Since we did not employ a stimulating electrode in the cerebellum it is possible that some of the IaINs we recorded from were in fact part of this subpopulation of VSCT cells. However this possibility is not very likely since all of the IaINs were located in the L_7 segment, an area of the cat spinal cord with relatively few VSCT cells (Ha and Liu, 1968).

Renshaw Cell Activity During Fictive Locomotion

Extracellular microelectrode recordings revealed that Renshaw cells were active during fictive locomotion (Figures 7, 8 and 9). Some of the cells were silent before and commenced their rhythmic activity during fictive locomotion. Other Renshaw cells displayed a tonic firing rate before fictive locomotion which changed to a pattern of rhythmic activity during fictive locomotion. In some of the Renshaw cells, the rhythmic discharge rate during fictive locomotion was slower than the tonic discharge rate before locomotion.

There are two possible explanations for a lower firing rate during locomotion: 1) an active inhibition of the Renshaw cell during locomotion, and 2) removal of excitatory input to the Renshaw cell. The findings presented in Table I suggest that the phasic activity is due

to a change in excitatory input and is not due to active inhibition of tonic activity. It might be expected that since the animals were decerebrate, there would be a great deal of extensor motoneuron activity; therefore, some Renshaw cells would display spontaneous activity prior to initiation of locomotion, whereas others would not. Renshaw firing evoked from extensor motoneurons giving rise to decerebrate rigidity would be altered once locomotion began.

It was interesting to find that the maximum Renshaw cell discharge rate seen was 45 Hz during the active phase of fictive locomotion. This rate is very much slower than the rates obtained by antidromic ventral root stimulation (Curtis and Ryall, 1966) but is similar to that reported by Ryall and Haas (1975, their Figure 1), obtained using stretch of the Achilles tendon. Since 16 of the 17 Renshaw cells studied were silent during one phase of fictive locomotion, it is possible that there was either an active inhibition of the cell during this phase, or there was no excitatory input to the cell. Table 1 shows that Renshaw cell excitability was assessed for both phases of locomotion, and except for cell 9 there is no evidence for an cyclic active inhibition during one of the phases of fictive locomotion. The most likely explanation for the silence of Renshaw cells during one phase of fictive locomotion is a lack of excitatory drive.

There probably are synaptic inputs to Renshaw cells other than motoneuron axon collaterals (see Introduction). However the finding that the three extensor coupled cells were active during the stance phase of fictive locomotion and the one flexor coupled cell was active

during fictive swing makes it possible that the excitatory synaptic input to Renshaw cells during fictive locomotion is from motor axon collaterals. A more precise knowledge of interneuronal converge upon Renshaw cells along with data on the locomotor activity of these pathways is necessary before we can state that the rhythmic activity of Renshaw cells during fictive locomotion is due solely to motoneuron axon collaterals.

Peripheral nerve cuffs were used to identify the four Renshaw cells discussed above. In these experiments cuffs were placed around peripheral nerves as well as around the ventral roots and dorsal roots; both dorsal and ventral roots were left intact. Such an arrangement allowed locating the cells with ventral root stimulation and subsequent identification. We did not experience any difficulty in differentiating between dorsal root and ventral root evoked discharges because the response to ventral root always occurred at a lower threshold and shorter latency as measured from the cord dorsum. It was important however to identify the Renshaw cell using only a positive response to nerve stimulation. As Figure 9 shows, this Renshaw cell did not respond to the extensor quadraceps but was activated from the extensor lateral gastronemius. This cell was labelled an extensor and did not respond to semitendinosis, posterior biceps or tibialis anterior stimulation.

Renshaw Cell Excitability During Fictive Locomotion

As the data in Table 1 indicates, 8 out of the 10 Renshaw cells showed no evidence of depression during fictive locomotion. Such a finding is in direct conflict with the suggestions of Shik and Orlovsky

(1976), and Grillner (1975), that Renshaw cells are depressed during locomotion. The strongest basis for the depression of Renshaw cells claim is the Severin et al (1968) work on recurrent inhibition of motoneuron filament discharge during MLR evoked treadmill locomotion. As mentioned in the Introduction, there may be some problems with the interpretation of the Russian data. Recent work in this laboratory (Pratt and Jordan, 1978) using more detailed analysis has shown that recurrent inhibition is quite powerful during treadmill locomotion and was effective in reducing motoneuron firing rates in 71% of the motoneurons tested.

The two cases of Renshaw cell depression (cells 2 and 9, Table I) seen during fictive locomotion may in fact represent a subpopulation of Renshaw cells. Unfortunately in these cats peripheral nerve cuffs were not utilized for stimulation and their identity remains unknown. It is possible that the fictive locomotion generator regulates recurrent inhibition in selected motoneuron pools as an aid in the control of the fictive step cycle.

Nevertheless, the excitability testing data suggest that Renshaw cells cannot be neglected when considering operation of the fictive locomotion generator.

The possibility also exists that during locomotion in the non-paralyzed animal there is a modulation of Renshaw cell excitability by peripheral receptor organs. Wilson et al (1964) reported depression of ventral root elicited Renshaw cell discharge by a variety of cutaneous stimuli in the decerebrate cat. Microelectrode recordings

from unparalyzed animals could provide a solution to this question of afferent effects on the spontaneous activity of Renshaw cells.

Recurrent Effects on Motoneurons During Fictive Locomotion

The excitability testing data suggest that Renshaw cells are not generally depressed during fictive locomotion. The possibility exists however that the inhibitory pathway from the Renshaw cell to the motoneuron is depressed, possibly by presynaptic inhibition. To test this possibility, we examined the R-IPSP during fictive locomotion.

As shown in Figures 12 and 13 the R-IPSP is still present during fictive locomotion. The problem with making any judgements about the magnitude of the R-IPSP during fictive locomotion is that the motoneuron has a varying DC potential. As the classic work of Coombs et al (1955) demonstrated, the size of the Ia - IPSP as well as the R-IPSP vary with the DC potential of the motoneuron. One would expect the R-IPSP during fictive locomotion to vary in a similar manner. The data presented here do in fact show that when the DC potential of motoneuron is approaching the reversal potential for the R-IPSP (i.e. hyperpolarizing) the R-IPSP is smaller; when the motoneuron is depolarized the R-IPSP is largest.

As well as the R-IPSP varying with the DC level of the motoneuron, it will of course be altered by any conductance changes in the motoneuron membrane that occur between the site of Renshaw cell axon termination and the recording microelectrode. Burke et al (1970) have reasoned that since the R-IPSP is more difficult to reverse with

intracellular current injection then the Ia-IPSP, the Renshaw cell synapses are located more distal to the soma than are the IaIN synapses. Since there is activity in the IaIN system during fictive locomotion (Feldman and Orlovsky, 1975; also this thesis) one might predict some modifications of the R-IPSP during fictive locomotion.

In spite of the difficulties associated with analysis of the R-IPSP during fictive locomotion, the fact that in all 8 of the motoneurons which displayed R-IPSPs before fictive locomotion also displayed R-IPSPs during fictive locomotion strongly suggests that the recurrent inhibitory pathway is operable during fictive locomotion.

The persistence of RFPs during fictive locomotion demonstrates two points, 1) the IaINs must be exerting a tonic inhibitory drive during at least part of the fictive step cycle on some of the motoneurons since an EPSP can be demonstrated by stimulation of the ventral root (eg. Figure 14c), 2) Renshaw cells are still able to inhibit IaINs during fictive locomotion.

The recurrent facilitation of motoneuron firing seen during treadmill locomotion in the Severin et al work (1968, filament number 6, then Table 1) further suggests that the Renshaw cell system is not generally depressed during fictive or treadmill locomotion. The results presented here also do not support the claim that reduction in the inhibition of IaINs from ventral root stimulations (Feldman and Orlovsky, 1975) is due to inhibition of Renshaw cells during locomotion (Feldman and Orlovsky, 1975).

The finding that the recurrent pathways to IaINs and motoneurons can be activated during locomotion should be useful in future studies. By injecting current into motoneurons before locomotion the effect of motoneuron DC potential for the R-IPSPs and IaIPSPs could be examined. Differences between the plots obtained by current injection and during locomotion may provide some clues about the locations of synapses generating the changing DC levels in the motoneurons during locomotion.

Blocking the Motoneuron to Renshaw Cell Synapse

The experiments which utilized atropine and mecamylamine in an attempt to block the activation of Renshaw cells from the motor axon collateral did not reveal any changes in treadmill locomotion attributable to the use of these drugs. As Ryall and Haas (1975) have shown, atropine in smaller doses than the ones used here is quite effective in blocking Renshaw cell activation by stretch of the Achilles tendon. It seems reasonable to assume that the drugs used would actually block Renshaw cell discharge.

To test the blocking ability of these drugs during fictive locomotion, it would be necessary to locate a Renshaw cell and iontophoretically apply atropine, mecamylamine and perhaps DBE during fictive locomotion. Intravenous administration of mecamylamine was attempted in two animals during fictive locomotion but the fictive locomotion did not persist, possibly due to the fall in blood pressure.

These results suggest that even if the neuronal relationships presented in Figure 1 operate during locomotion, the contribution of Renshaw cells to the locomotor generator may be small. Recent unpublished observations from this laboratory (Menzies and Jordan) indicate that after intravenous strychnine, which acts to block the inhibitory synapses in Figure 1 (see Krnjevic, 1974), rhythmic motoneuron activity still persists. It would seem that the scheme of Figure 1 does not constitute the entire fictive locomotion generator.

Stimulation of the Renshaw System

The results of the deafferented limb experiments showed that no effects in the timing of muscle activation could be detected as a result of stimulation of the muscle nerve. Because of the de-afferentation, locomotion in that limb was not as strong as in the other limbs. The successful experiments occurred in animals which had been allowed to recover for several days after the dorsal root transection. Two other animals were transected on the day of the experiment; no locomotion in the de-afferented limb was observed.

These observations suggest that while there is a spinal stepping generator (eg. Grillner and Zangger, 1974) it is likely that peripheral receptors can contribute to support the operation of this generator.

Since stimulation of the Renshaw cells for periods of up to .4 sec did not alter the timing of muscle activation, there is now further evidence against the ability of the scheme of Figure 1 to contribute in a major way to the generation of locomotion.

Unpublished observations of Pratt and Jordan show that stimulation of a cut ventral root during MLR evoked treadmill locomotion fails to change the timing of the onset of subsequent ventral root filament bursting even though this type of stimulation is often effective in silencing the activity of the filament during stimulus application.

The possibility remains that other influences from proprio-spinal or supraspinal sources provide a large contribution to the generation of stepping in any particular limb. Perhaps the small perturbations produced by single nerve or ventral root stimulation are overridden by other forces.

The Scheme of Figure 1

During the initial development of this thesis it became apparent that the interconnections shown in Figure 1 could contribute or perhaps form the spinal stepping generator. Subsequent to this realization Miller and Scott (1977) developed an electronic model system based upon the connections of Figure 1 as an attempt to explain the operation of the locomotor generator. The Renshaw cells play a major role in such a scheme and if they were depressed during locomotion (see Introduction) such a scheme would not operate.

The data presented in this thesis has shown that most of the Renshaw cells studied showed no evidence of depression and that they were spontaneously active during fictive locomotion. In addition it seems probable that the recurrent facilitatory and inhibitory pathways are operating during locomotion.

There is now a substantial body of evidence, however, which suggests that such a scheme does not constitute the locomotor generator; 1) Mecamylamine and or atropine do not seem to impair locomotion (this thesis). 2) Strychnine blocks the inhibitory synapses in Figure 1 but motoneurons still retain their rhythmic activity (Menzies and Jordan unpublished). 3) Strychnine facilitates spinal stepping in dogs (Hart, 1971). 4) Activation of Renshaw cells does not alter the timing of muscle activation during locomotion (this thesis). 5) Conductance measurements of motoneurons during fictive locomotion show that there is an active excitatory process involved in motoneuron spiking; not just a disinhibition (Menzies et al, 1978).

None of the above studies rule out the possibility that the scheme of Figure 1 does not operate to assist the functioning of the locomotor generator. It may be that afferent systems or supraspinal systems in the intact animal operate to change the effectiveness of this system and thereby fine tune the locomotion generator. It does not seem likely at this time that the operation of the scheme of Figure 1 comprises the locomotor generator.

TABLE I: Mean Renshaw Cell Discharge Produced by Ventral Root Stimulation

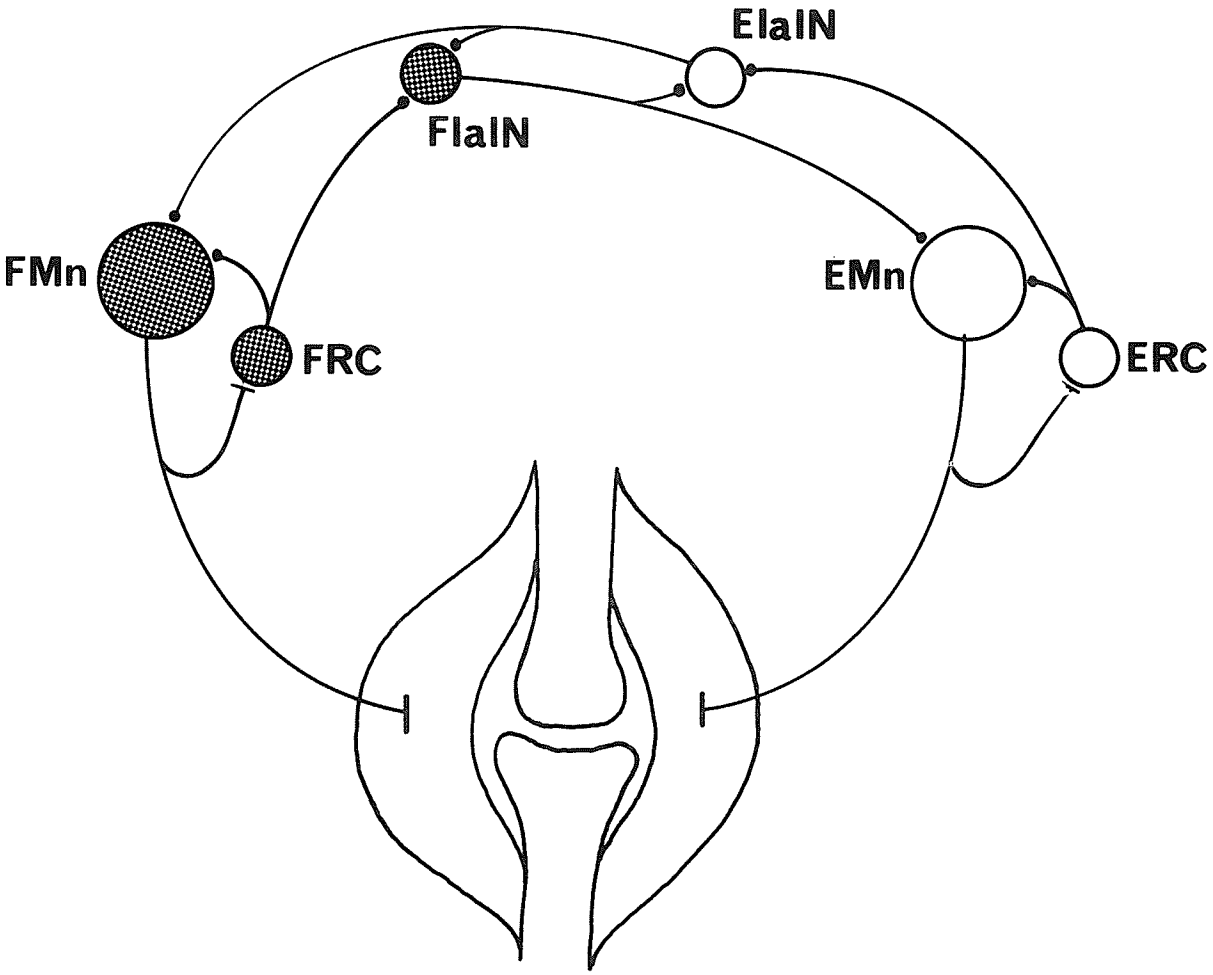
RENSHAW CELL	NO LOCOMOTION	LOCOMOTION IN PHASE WITH FILAMENT	LOCOMOTION OUT OF PHASE WITH FILAMENT
1	12.2*	12.1	11.5
2+	15.0	11.1	11.5
3	20.4	18.2	19.6
4	10.7	8.7	9.4
5	10.0	9.0	8.3
6	15.5	12.7	13.8
7	24.1	24.0	22.6
8	12	12	12
9+**	15	9.9	12.6
10	7.6	7.5	7.4
GROUP MEAN	14.3 (SD. 5.0)	12.7 (SD. 4.9)	

*Values determined from repeated single ventral root stimuli (N, 10-50).

+Analysis using student's t-test revealed a significant difference between states of locomotion and no locomotion ($p < .005$).

**Difference between the phases of locomotion significant ($p < .005$).

Figure 1: Schematic drawing of the synaptic connections between motoneurons to flexor muscles (FMn) and their associated Renshaw cells (FRC) and IaINs (FIaIN), and between extensor motoneurons (EMn), extensor-coupled Renshaw cells (ERC) and IaINs (EIaIN). Excitatory synapses are represented by bars, and filled circles represent inhibitory synapses. Illustrated below are hypothetical relationships between the firing of the various elements which would be expected to occur if RCs contribute to the control of switching between antagonist motoneuron groups during locomotion.


EIaIN
FIaIN
FMn
EMn
FRC
ERC

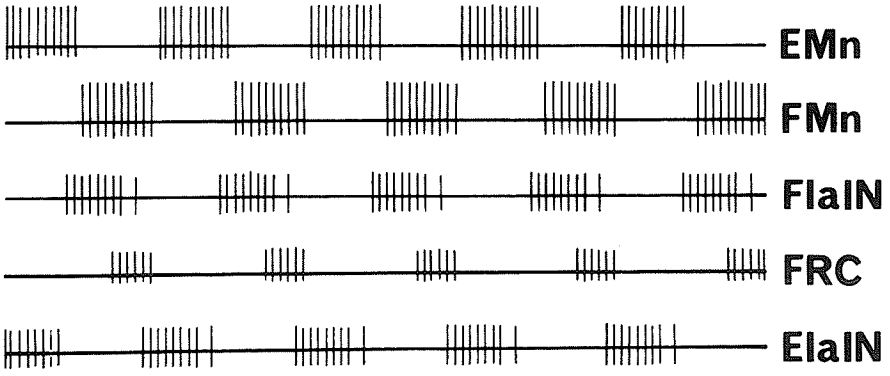

EMn
FMn
FIaIN
FRC
EIaIN

Figure 2: Schematic diagram of a saggital section of the cat brain 4.1 mm lateral to the midline. The dotted lines indicate the levels of transections used in these experiments. The transection with its inferior plane just caudal to the optic chiasm (OT) is termed the thalamic preparation. The more caudal transection is termed the mesencephalic preparation.

BC brachium conjunctivum

CNF cuneiform nucleus

IC inferior colliculus

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

The black dot shows the location of the midbrain locomotor region.

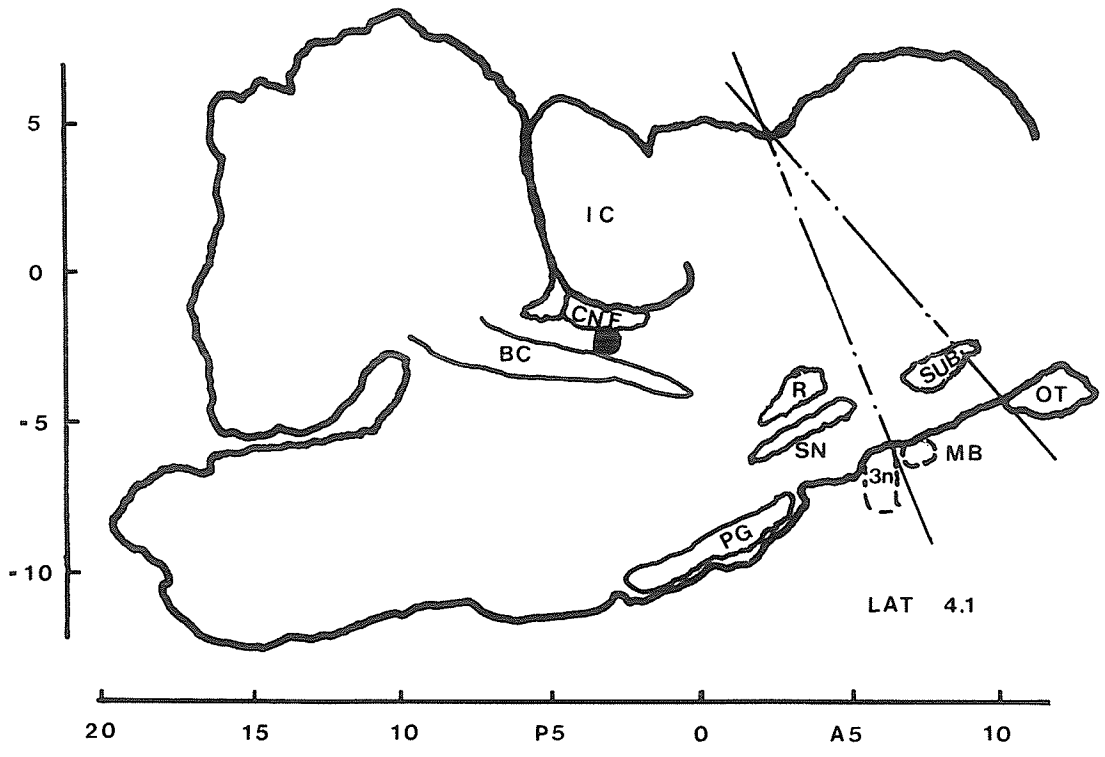

5
0
-5
-10
IC
CNF
BC
R
SUB
OT
SN
MB
3n
PG
LAT 4.1
20
15
10
P5
0
A5
10

Figure 3: Schematic diagram of the recording arrangement used for microelectrode recording.

A.C. Microelect. The signal from the electrometer was fed into the oscilloscope and amplified at high gain with an A.C. coupled amplifier

D.C. Microelect. Low gain DC coupled amplifier used for displaying the membrane potential an action potential of neurons

Mag Tape Hewlett Packard four channel FM taperecorder

STIM stimulation

ISOL stimulus isolation unit

CONST. CURR. constant current unit

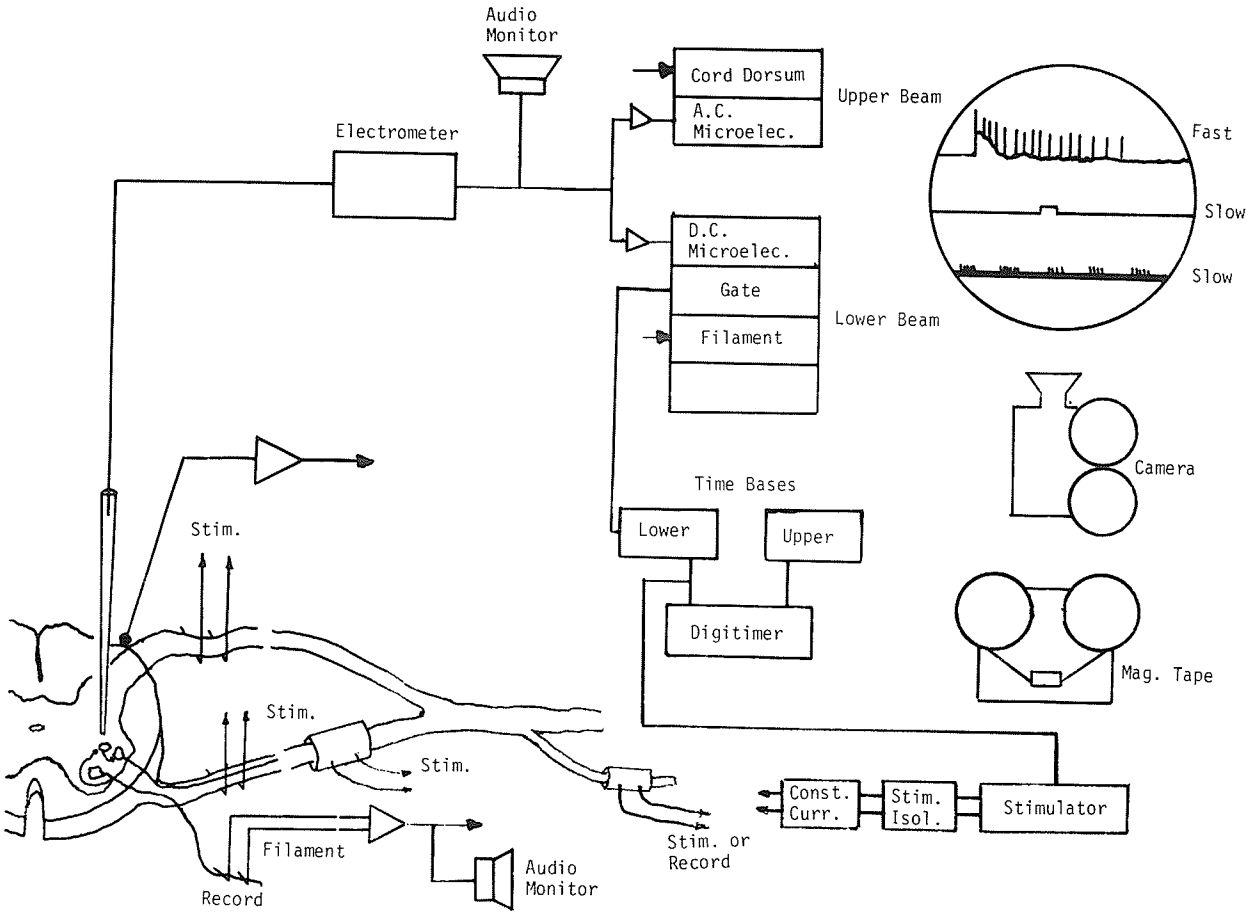

Audio
Monitor
Cord Dorsum
A.C.
Microelec.
Upper Beam
Electrometer
Fast
Slow
D.C.
Microelec.
Gate
Filament
Slow
Lower Beam
Camera
Time Bases
Lower
Upper
Stim.
Digitimer
Mag. Tape
Stim.
Stim.
Const.
Curr.
Stim.
Isol.
Stimulator
Filament
Stim. or
Record
Audio
Monitor
Record

Figure 4: Diagramatic representation of the experimental set-up for a controlled locomotor preparation on the treadmill.

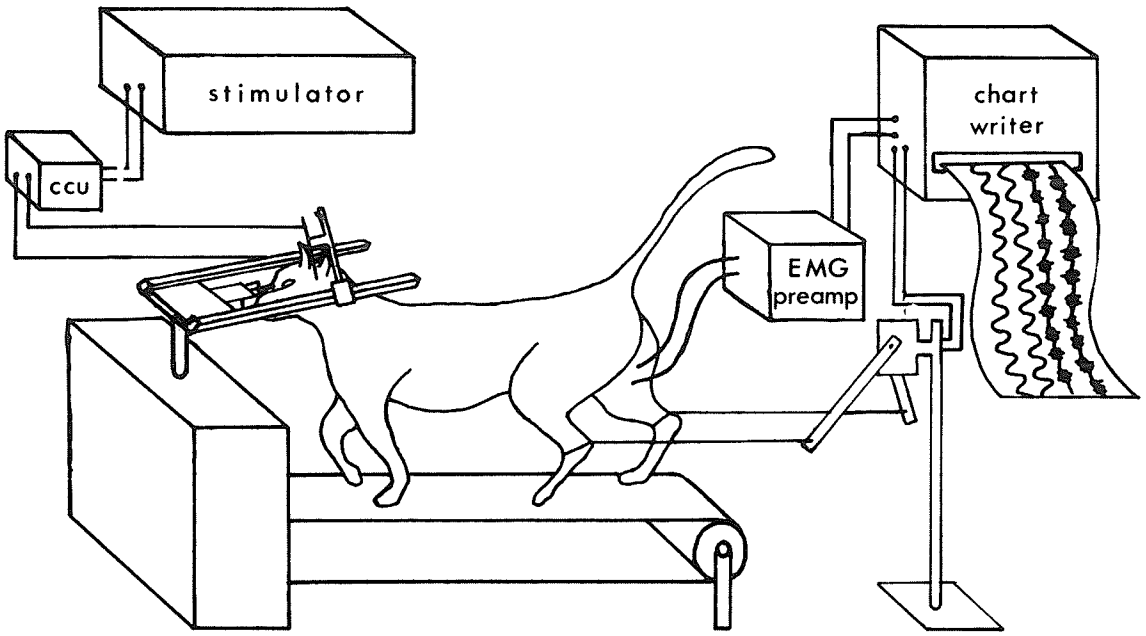

stimulator
ccu
chart
writer
EMG
preamp

Figure 5: Ventral root filament activity as a monitor of fictive locomotion. A. Rhythmic firing of a ventral root filament during spontaneous locomotion prior to administration of gallamine triethiodide. B. The same filament immediately before paralyzation and several minutes after administration of gallamine triethiodide, showing that locomotor-like activity is maintained in the paralyzed animal.

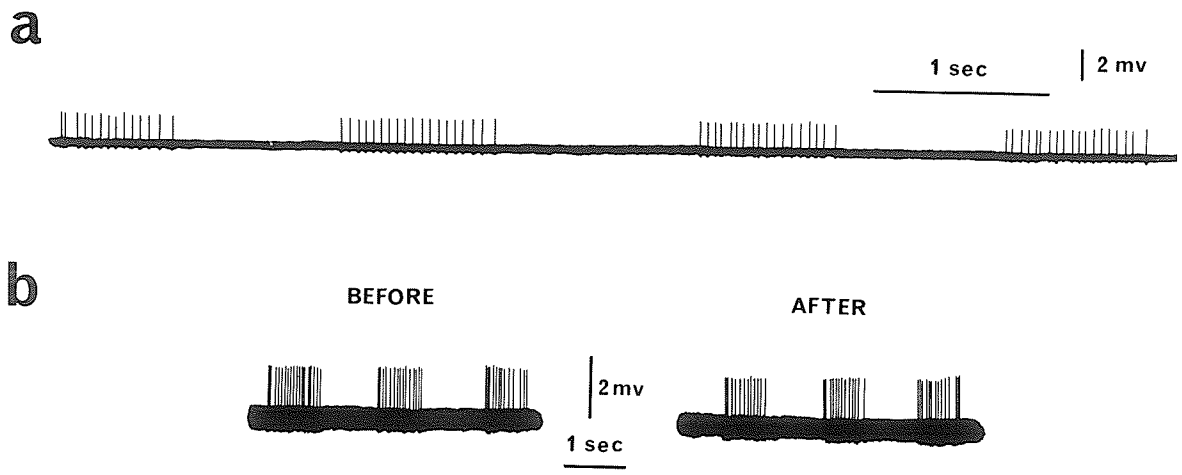

a
1 sec
2 mv
b
BEFORE
AFTER
2mv
1 sec

Figure 6: Spontaneous rhythmic activity of a Ia inhibitory interneuron during fictive locomotion. The horizontal bars above the abscissa are periods of ventral root filament discharge (fictive "steps"). The insert is a sample of the moving film records used to measure the interspike interval; the interneuron spikes being on the upper trace and the ventral root filament activity on the lower.

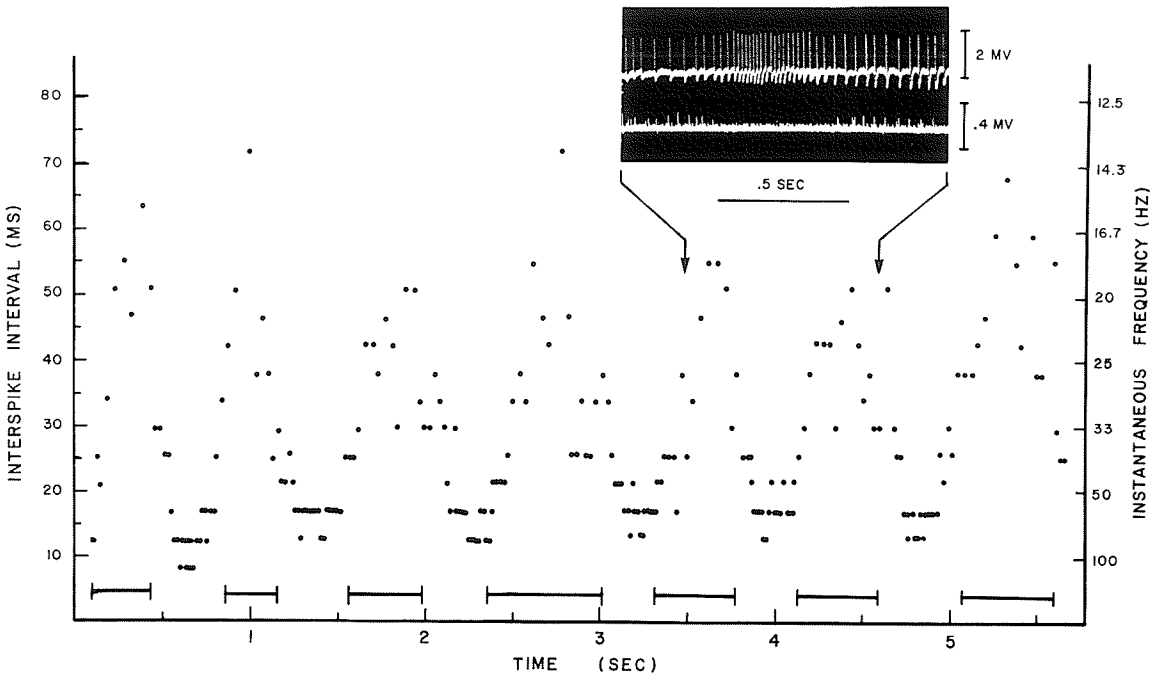
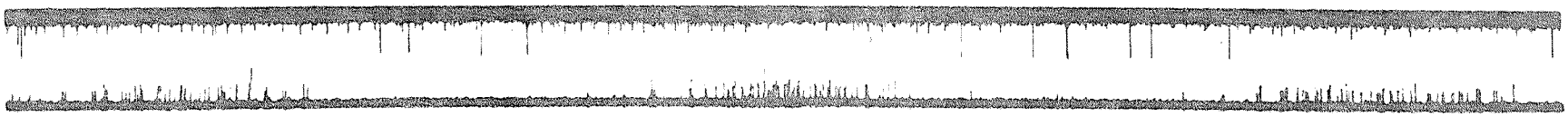

2 MV
.4 MV
.5 SEC
INTERSPIKE INTERVAL (MS)
80
70
60
50
40
30
20
10
INSTANTANEOUS FREQUENCY (HZ)
12.5
14.3
16.7
20
25
33
50
100
1
2
3
4
5
TIME (SEC)

Figure 7: Spontaneous activity of a Renshaw cell (upper trace, large spikes during fictive locomotion. Lower trace is the activity of a ventral root filament illustrating three fictive steps.



1 SEC

Figure 8: Rhythmic activity of a flexor-coupled Renshaw cell during locomotion. Motoneuron discharges recorded from a ventral root filament (lower trace in a) were compared with discharges recorded from a cuff electrode around the quadraceps nerve (Q) to establish that the filament discharges occurred during the swing phase of locomotion (prior to paralyzation) when the flexor motoneurons are active. A Renshaw cell identified on the basis of its burst discharge in response to ventral root (VR) stimulation (b) was shown to be predominantly excited from posterior biceps (PB), a flexor motor nerve (righthand trace in b). In (c) it is clear that the Renshaw cell (upper trace) discharges in phase with the flexor filament (lower trace).

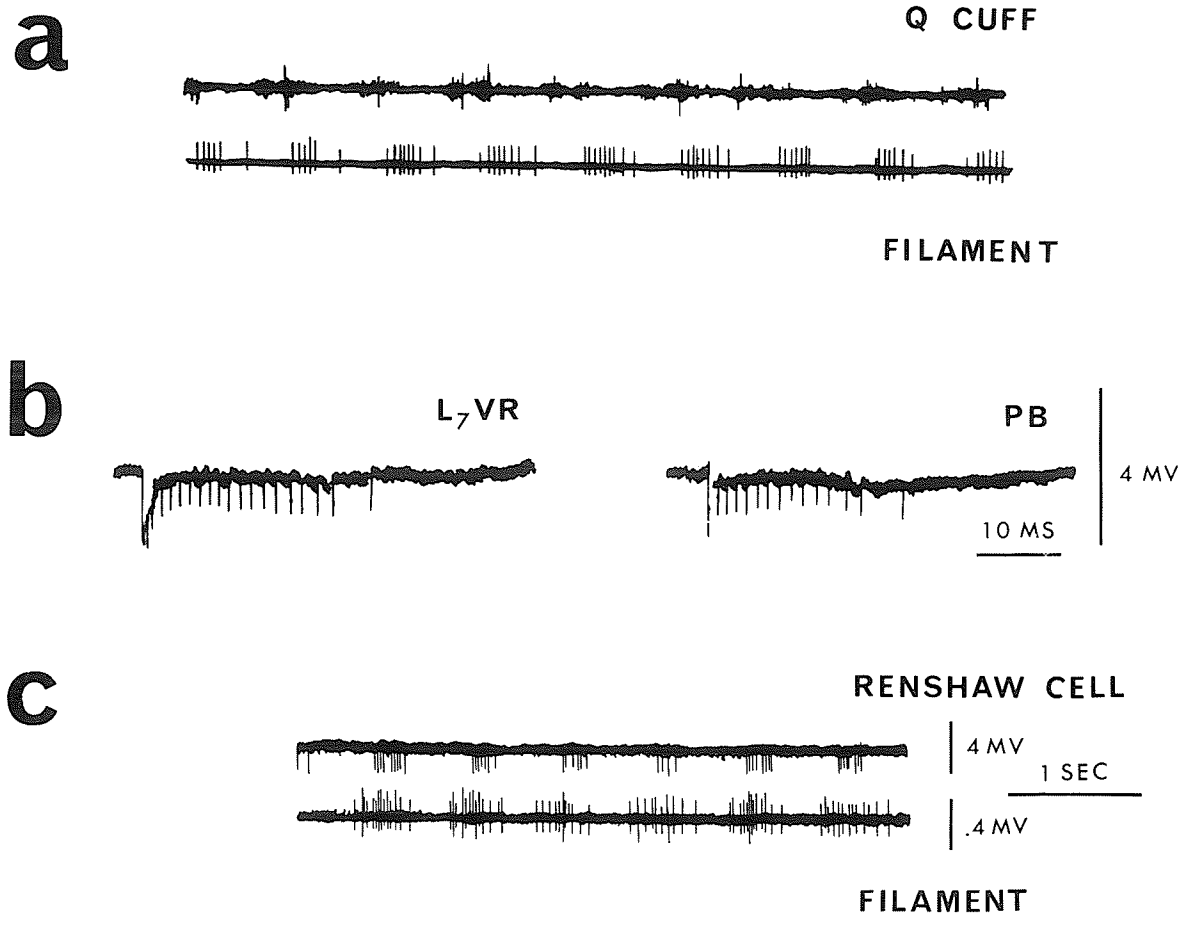

a
Q CUFF
FILAMENT
b
L_7VR
PB
4 MV
10 MS
c
RENSHAW CELL
4 MV
1 SEC
.4 MV
FILAMENT

Figure 9: Rhythmic activity of an extensor-coupled Renshaw cell during locomotion. The cell's identification as a Renshaw cell was determined on the basis of its response to stimulation of the L_7 ventral root (A), and its excitation from the nerve to the lateral gastrocnemius (LG) muscle establishes it as an extensor coupled Renshaw cell (B). The Renshaw cell fired rhythmically during locomotion (D) induced by stimulation of the mesencephalic locomotor region (artifacts in D). Its firing occurred out of phase with that of a filament previously shown to be active during the swing phase of locomotion in the manner described from Figure 8.

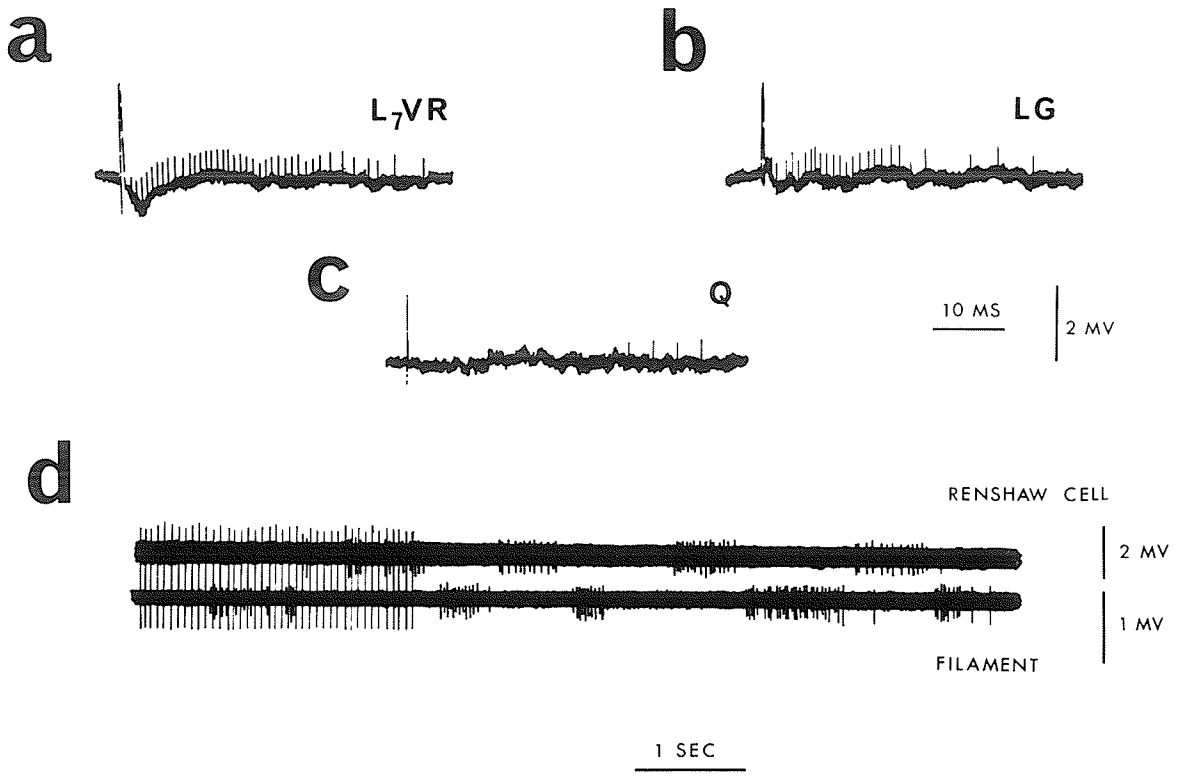

a
L7VR
b
LG
c
Q
10 MS
2 MV
d
RENSHAW CELL
2 MV
1 MV
FILAMENT
1 SEC

Figure 10: Renshaw Cell excitability testing. Oscilloscope records; the upper trace is the Renshaw activity from ventral root stimulation (fast sweep speed). The lower trace is the ventral root filament activity before (a) and during (b,c) fictive locomotion (slow sweep speed). The dot above the slow trace indicates the period in which the fast trace occurred. The graph illustrates the average numbers of action potentials elicited in this Renshaw cell in the various conditions. Camera frames were examined and placed into categories of no locomotion (as in trace a), discharge occurring in phase (trace b), or out of phase (trace c) with ventral root discharge fictive locomotion.
N refers to the number of frames counted and the numbers above the points on the graph are the mean number of Renshaw spikes elicited.

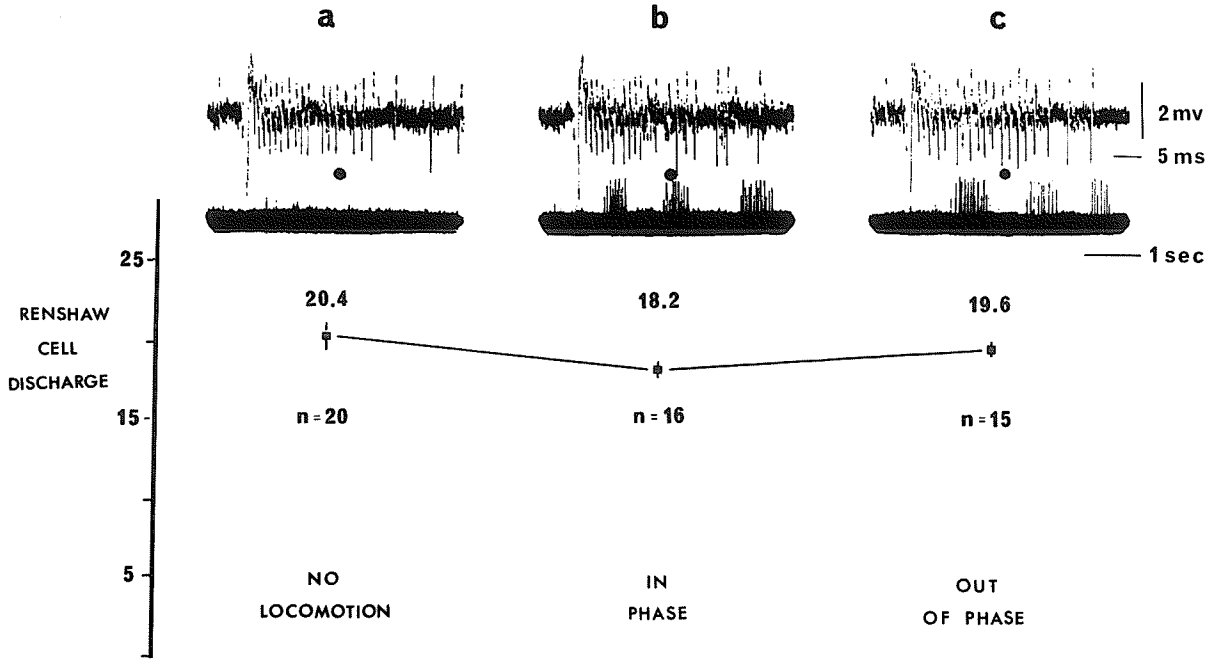

a
b
c
2 mv
5 ms
1 sec
25
15
5
RENSHAW
CELL
DISCHARGE
20.4
18.2
19.6
n = 20
n = 16
n = 15
NO
LOCOMOTION
IN
PHASE
OUT
OF PHASE

Figure 11: Excitability testing in a Renshaw cell (cell 2, Table I). The upper trace (fast sweep speed) is the microelectrode record of the Renshaw cell. The lower trace is the ventral root filament (slow sweep speed). The stimulus artifacts (panel 2) are from dorsal root stimulation which evoked fictive stepping (panels 3,4,5). The arrow represents the time on the lower trace at which the upper trace was triggered.

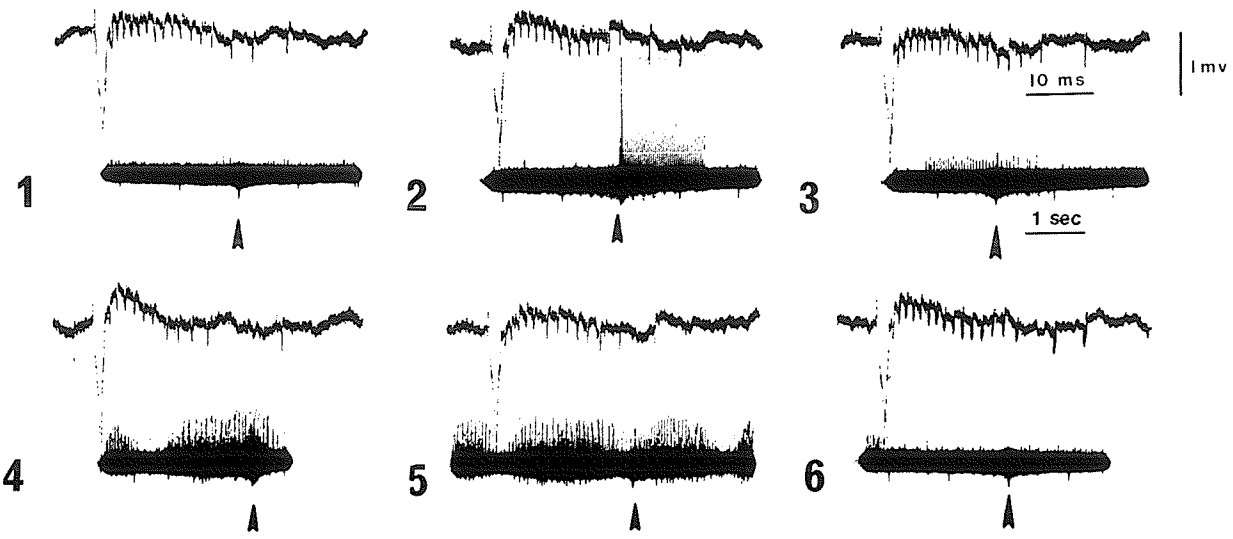

1
2
3
10 ms
1 mv
1 sec
4
5
6

Figure 12: Intracellular records from a motoneuron during fictive locomotion. Upper trace is the DC record from this cell. The lower trace is the ventral root filament discharge at the same slow speed as the upper trace. The middle trace is the fast record of the recurrent IPSP produced by ventral root stimulation. The dot indicates the time of occurrence of the fast trace. The tops of the action potentials on the upper trace are off the scale of the oscilloscope screen. The 5 frames (a-e) were taken sequentially at 6 second intervals.

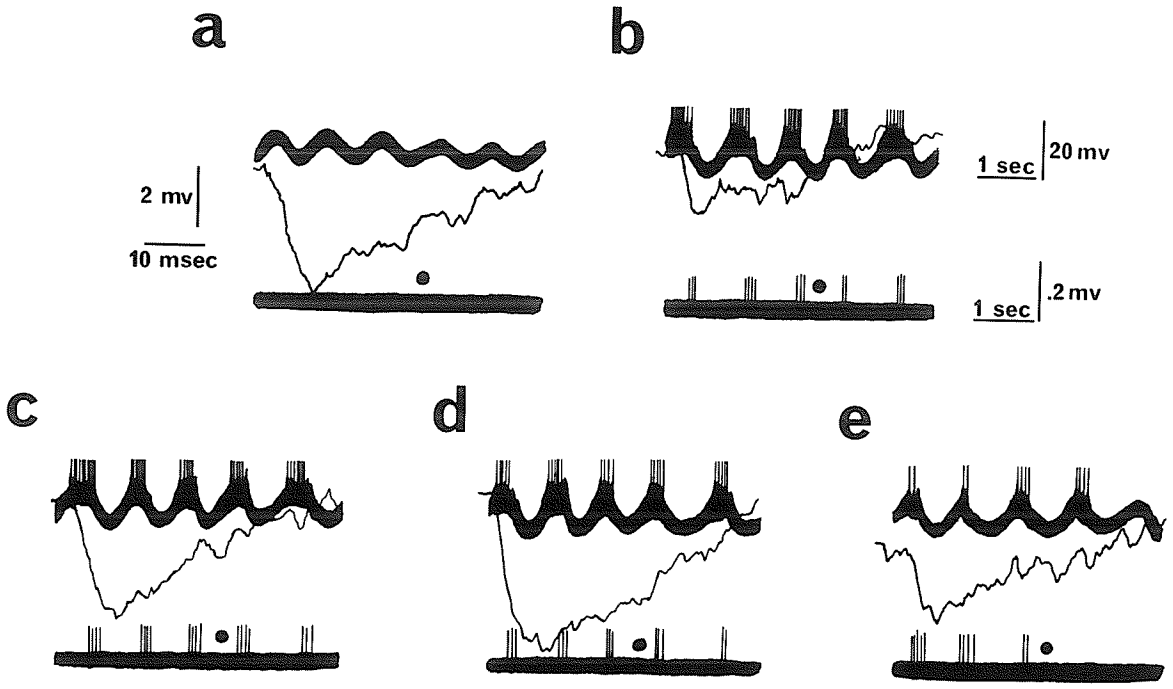

a
b
2 mv
10 msec
1 sec
20 mv
1 sec
.2 mv
c
d
e

Figure 13: Relationship of motoneuron membrane potential and R-IPSP amplitude. A and B are intracellular microelectrode recordings obtained from two motoneurons during fictive locomotion. The arrows below the traces indicate the time of occurrence of single stimuli to the cut ventral root. The peak magnitudes of the R-IPSPs produced were plotted against the DC potential of the motoneuron (Em). C is the plot obtained from the motoneuron in trace A and shows a correlation coefficient of $r = .95$ ($p < .005$). D is from the cell in trace B and gives $r = .75$ ($p < .005$).

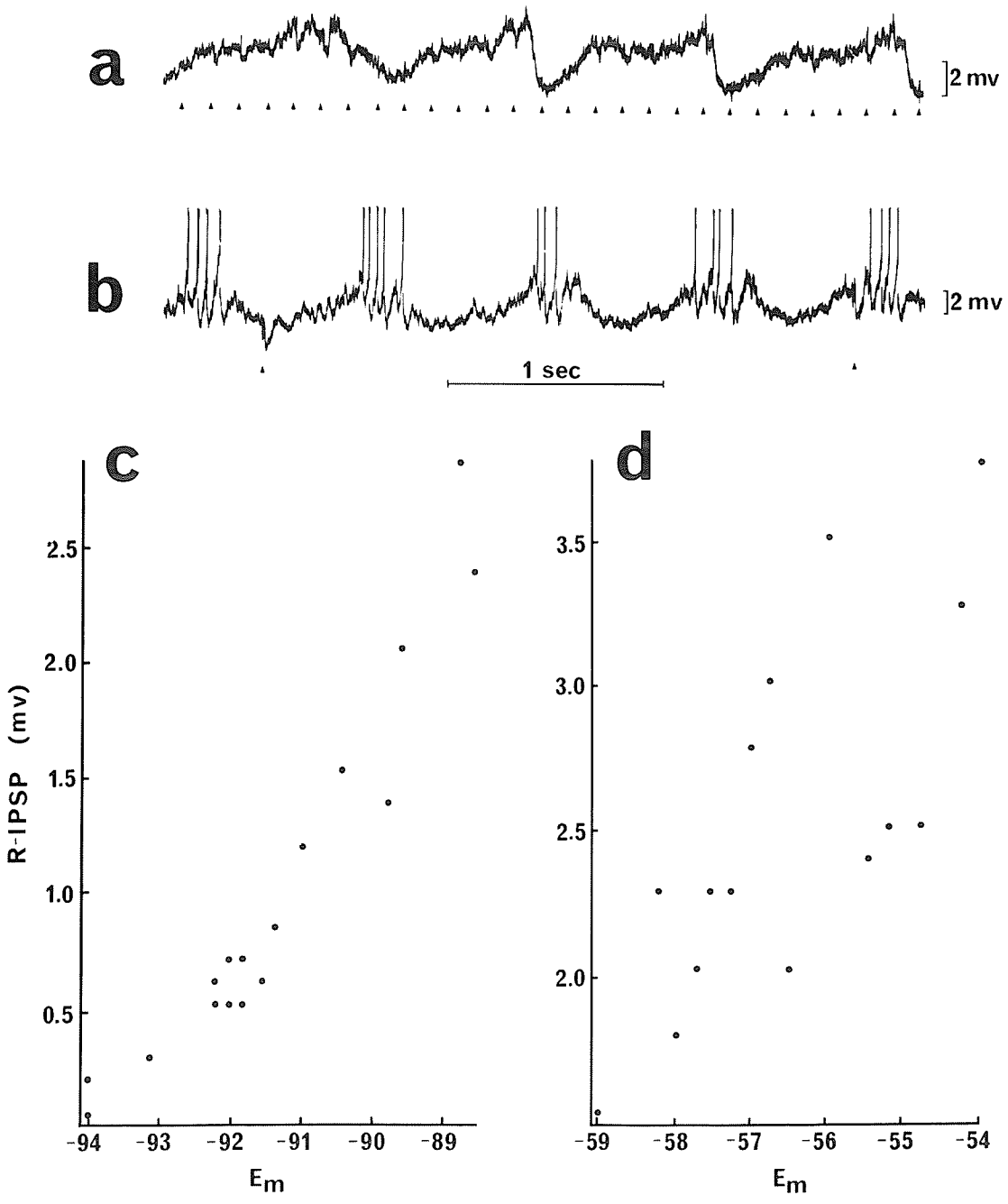

a
]2 mv
b
]2 mv
1 sec
c
d
R-IPSP (mv)
2.5
2.0
1.5
1.0
0.5
-94
-93
-92
-91
-90
-89
Em
3.5
3.0
2.5
2.0
-59
-58
-57
-56
-55
-54
Em

Figure 14: Recurrent facilitatory potentials during fictive locomotion. Stimulation of the ventral root produces in this motoneuron a recurrent inhibitory and facilitatory potential (upper traces: A,B,C,D at fast sweep speed). The lower traces at slow sweep speed show fictive locomotor activity (B,C) in an isolated ventral root filament. The arrowhead beneath the slow trace shows the time of occurrence of the fast trace.

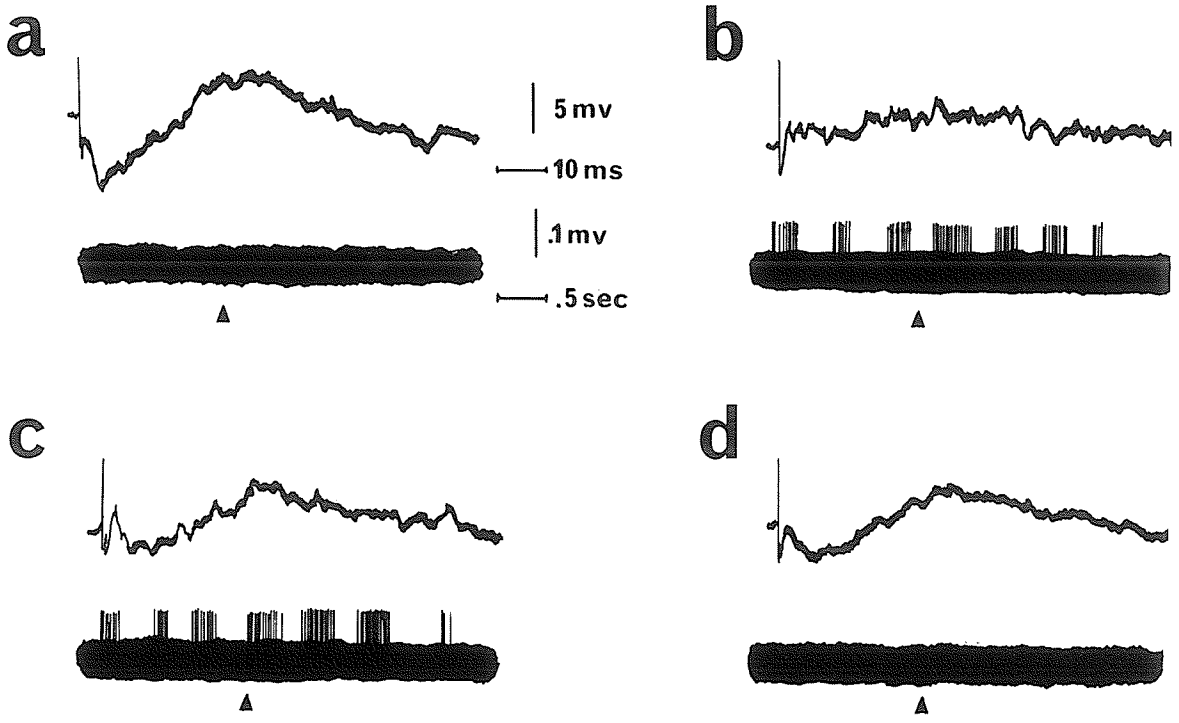

a
5 mv
10 ms
.1 mv
.5 sec
b
c
d

Figure 15: The effect of atropine and mecamylamine on locomotion. EMG records were obtained from the left hindlimb during MLR evoked locomotion on a treadmill. The leftmost traces are control just before atropine was injected I.V. (1.5 mg/kg). Locomotion was induced 35 minutes later (middle traces, fast chart speed). Mecamylamine was injected and locomotion again evoked (rightmost traces).

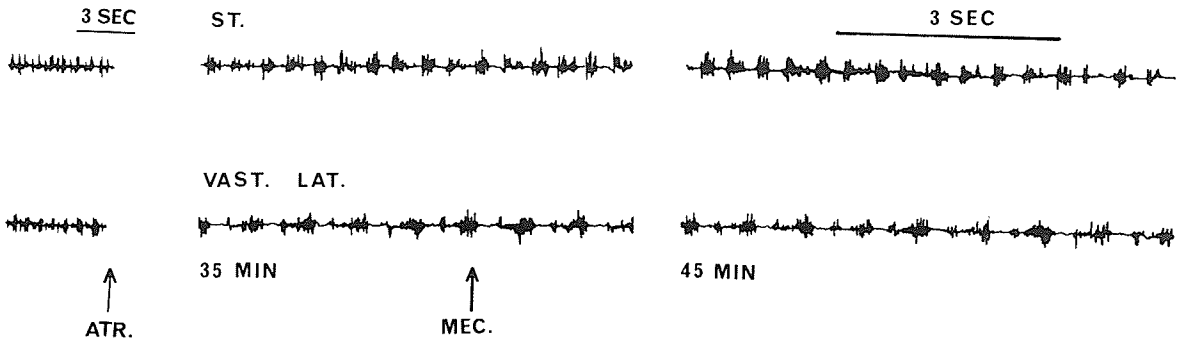
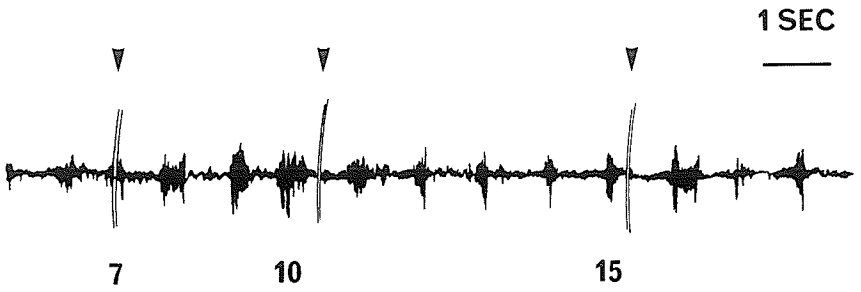

3 SEC
ST.
3 SEC
VAST. LAT.
35 MIN
45 MIN
ATR.
MEC.

Figure 16: The effect of muscle nerve stimulation of the timing of the EMG bursts during treadmill locomotion. EMG records were obtained from the adductor femoris muscle in the left hindlimb which had been deafferented 10 days prior to the experiment. Stimulus trains (200 Hz .1 msec pulse, 100 msec duration) were applied to the nerve cuff around the adductor femoris nerve (arrows). The table shows the measurements taken of the time from onset of EMG burst to the time of onset of the next EMG burst. The numbers below the trace correspond to the steps indicated in the table. The mean ($\overline{X}$) and standard deviation (s.d.) of the time between onset of EMG bursts were obtained from 29 steps of which 17 are shown in the table.

STEP	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
INTERVAL (SEC)	.88	.63	.81	1.0	.88	.72	.81	.97	.69	1.0	.94	.90	1.0	.88	.94	.91	.87

$\overline{X}$ = .90 s.d. = .106



ABBREVIATIONS

Ach	acetyl choline
A.W.G.	American Wire Gauge Standard
DBE	dihydro- β -erythroidine
DOPA	dihydroxyphenylalanine
EMG	electromyogram
EPSP	excitatory post synaptic potential
FRA	flexion reflex afferents
IPSP	inhibitory post synaptic potential
KAC	potassium acetate
MLR	midbrain locomotor region
RFP	recurrent facilitatory potential
R-IPSP	recurrent inhibitory potential
VSCT	ventral spino-cerebellar tract
Ia	lowest threshold excited afferent
IaIN	group Ia excited inhibitory interneuron

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