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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April 1979

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

p.p.s. This thesis was skillfully typed by Janet Greer, who never cried once.

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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