CONDUCTANCE PATTERNS IN IDENTIFIED LUMBAR SPINAL CORD MOTONEURONS DURING FICTIVE LOCOMOTION

A Thesis
Presented to the
University of Manitoba

In Partial Fulfillment of the Requirements
for the Degreee

MASTER OF SCIENCE
IN
PHYSIOLOGY

by

Susan J. Shefchyk
September, 1982

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Acknowledgements

I would like to take this time to express my gratitude to those people who made this experience both rewarding and enjoyable.

I want to especially thank my parents, Marge and Frank Shefchyk, for thier love and support during this period of work. An added thanks should go to my Mother whose dormant secretarial skills produced the final version of this thesis.

I would like to extend my many thank to the people directly associated with the reasearch: my supervisor, Dr. Larry M. Jordan for his patience and guidance; Brian Noga for his cooperation and technical assistance; my fellow graduate students for their cooperation (and patience during difficult times); and to Dr. R.B. Stein from the University of Alberta for his cooperation during some of the data analysis.

This research was supported by a Medical Research Council (MRC) grant to Dr. Larry M. Jordan and by the MRC Studentship program.

Abstract

A variety of models for the central pattern generator for locomotion have been proffered, but the details of the synaptic drives which influence the motoneurons and their activity during locomotion have not been defined. In this study the synaptic input to flexor and extensor motoneurons in the lumbar spinal cord was evaluated by monitoring the conductance patterns of the motoneurons during the step cycle.

Fictive locomotion was produced by stimulation of the mesencephalic locomotor region in decerebrate cats paralyzed by Flaxedil. Intracellular recordings were obtained using 3 M potassium chloride or potassium acetate filled microelectrodes. The motoneurons were identified on the basis of their monosynaptic and antidromic responses to stimulation of the nerves to semimembranosus-anterior biceps, posterior biceps-semitendinosus, vastus (all branches except rectus femoris), sartorius, gracilis, tibialis anterior, lateral and medical gastrocnemius, and soleus muscles. Conductance measurements were obtained using an active bridge circuit for injection of the hyperpolarizing pulses through the recording electrode. Two pulse measurements were used to monitor the membrane resistance: the voltage deflection produced by the injected hyperpolarizing current and the slope of the decay of the injected pulse.

The results indicated that none of the flexor or extensor motoneuron populations examined displayed a change

in conductance during the depolarized and hyperpolarized phases of the step cycle. However, individual examples of cells from both flexor and extensor groups were observed to have an increase in conductance during the depolarized phase of the step cycle.

In addition to the conductance measures, monosynaptic EPSP modulation during locomotion was examined. It was found that 47% of the motoneurons examined had EPSPs that were modulated during the step cycle, with the largest amplitude occurring during the depolarized phase. In another 41% the EPSPs were not modulated during locomotion and in 12% the EPSP amplitudes were largest during the hyperpolarized phase of the step cycle.

The influence of IaIn IPSPs on motoneuron membrane oscillations during locomotion also was addressed. It was observed that reversal of the IPSP with Cl^- injections produced a decrease in the Em oscillations during locomotion, hence, the IPSP appears to contribute to the membrane oscillations. It does not determine the firing pattern, however.

An EPSP time-locked to the MLR stimulation was observed in both flexor and extensor motoneurons. This EPSP had a latency of 4-6 ms and an amplitude up to 6 mv.

The conclusion drawn from the results was that both flexor and extensor motoneurons receive an excitatory output from the central pattern generator and that any model of such a generator must consider this requirement.

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Abbreviations

MLR	Mesencephalic Locomotor Region
PB	Posterior Biceps
ST	Semitendinosus
SMAB	Semimembranosus- Anterior Biceps
TA	Tibialis Anterior
LG	Lateral Gastrocnemius
MG	Medial Gastrocnemius
Vastus	All branches of quadriceps except for rectus femoris.
Sart.	Sartorius
Grac.	Gracilis
EPSP	Excitatory postsynaptic potential
IPSP	Inhibitory postsynaptic potential
L6	Lumbar 6
L7	Lumbar 7

Introduction

Generally it is accepted that the mechanisms responsible for the generation of rhythmic alternating movements, such as swimming and walking, are centrally located in the nervous system (Friesen and Stent, 1978). In vertebrates the central pattern generator (CPG) for locomotor activities appears to be limited to the spinal cord itself (Grillner, 1975). Such a CPG can generate the rhythmic activity in the absence of descending input from higher centers or peripheral sensory feedback as has been demonstrated using spinal and/or deafferented preparations (Sherrington, 1910; Brown, 1911, 1914). It should be noted that the CPG and its output can be, and is, influenced by these outside sources (Rossignol et al., 1980; Grillner, 1975). However, these inputs are not essential to the generation of the basic pattern of activity.

Although the problem has been subject to investigation for over seventy years, progress has been largely restricted to defining the role of sensory feedback and the identification of what is <u>not</u> essential for the generation of the basic activity. The details of the elusive locomotor central pattern generator remain undefined, or unconfirmed, in the mammalian systems examined. The cat preparation has served as the vehicle for investigation through the years with the result being the generation of a variety of hypotheses each with some degree of experimental support.

When addressing the issue of the generation of rhythmic activity generated by cells one can consider two general possibilities. The first is the existence of endogenous "pacemaker" cells which under the appropriate circumstances will display a phasic behavior (Kristan et al., 1977). The other possibility is that the rhythmic behavior or output is the product of the interaction of a circuit, or network of cells, which because of their interaction produce the rhythm (Kristan et al., 1977; Pearson, 1976). Since no evidence for the "pacemaker" cell has been found in the in vivo mammalian spinal cord, the circuit concept is the one generally pursued.

In a series of experiments using deafferented cat preparations and the phenomenon of narcosis progression, T. Graham Brown (1911, 1914) obtained results which compelled him to propose that the efferent neurons (motoneurons) alone, without the other components of the reflex arc, were capable of generating rhythmic alternating stepping activity. Specifically, Brown (1911) proposed that the rhythmicity was not a function of the efferent neurons themselves, but rather was a "property of the interconnexions of the anatagonist nerve cells and (the rhythmic alternating activity) is set up during equal and opposite activation of them". Brown proffered the concept of reciprocal inhibitory connections between these antagonistic pairs, or "half-centers" as he termed them. In situations of unequal activation of the pair of half centers, the reciprocal organization allowed for one

half center to dominate while the other was being inhibited. In order for the alteration between the two halves to occur some switching mechanism was required. The mechanism Brown (1914) proposed to function as the limiting factor was what he coined "depreciation of inhibition" or an unspecified fatigue process. The details of such a phenomenon have yet to be elucidated.

Support for the half center organization came with evidence for the existence of reciprocally organized cells in the lumbosacral spinal cord. This information was obtained during a series of experiments examining the effects of L-DOPA on the acutely spinalized cat (Anden et al., 1966 a,b,c; Jankowska et al.,1966; Jankowska et al.,1967 a,b). Anden and co-workers (1966a) demonstrated that the effects of flexion reflex afferent (FRA) stimulation in the acute spinal cat changed dramatically after the intravenous administration of L-DOPA. The short latency dorsal root potential (DRP's) evoked by FRA stimulation in the absence of L-DOPA were depressed and long latency, long duration discharges were observed. The change in response pattern affected both the pattern of excitatory and inhibitory inputs to motoneurons from the FRA's. The evidence indicated that the L-DOPA effect was due to the liberation of a catecholamine, most likely noradrenalin (NA), from a descending system (Anden et al.,1966b). The NA functioned to inhibit the short latency FRA response thereby facilitating the late, long duration response (Anden et al.,1966 a,b).

The effects after L-DOPA revealed not simply an ipsilateral (ipsi) effect, but also the comparable contralateral (co) changes (Anden et al., 1966c; Jankowska et al., 1966). This indicated that the short latency FRA pathway not only inhibited the ipsilateral late, long duration responses observed after L-DOPA, but also the contralateral long latency, long duration responses observed after L-DOPA.

Jankowska and co-workers (1967 a) examined motoneuron discharge patterns, as well as intracellular records after L-DOPA administration and noted a long latency, long duration dishcarge which parallelled that observed in earlier experiments using the DRP recordings. Specifically, with stimulation of the ipsi FRAs one observed flexor motoneuron activation and extensor motoneuron inhibition on that side. With stimulation of the co FRAs the reverse was observed with extensor activation and flexor inhibition occurring. Using the conditioning-test paradigm Jankowska et al.,1967a) were able to demonstrate that activation of the ipsi FRA could effectively inhibit the actions of the co FRA and visa versa. With bilateral FRA stimulation either flexor or extensor motoneurons were activated but never both. This indicated the presence of some sort of reciprocal inhibition between the pathways mediating the two effects. The intracellular data indicated that the inhibition was not mediated postsynaptically at the motoneurons, nor did it appear to be presynaptic inhibition of the primary afferent terminals.

The mechanism the authors proposed was a reciprocal organization at an interneuronal level.

Jankowska and co-workers (1967 b) proceeded to examine the lumbosacral spinal cord in an effort to identify interneurons which could mediate the late, long duration effects from the FRA after L-DOPA. Several groups of interneurons found in the lateral region of REXED's lamina 7 were excellent candidates. One group of cells were excited from the ipsi FRAs and inhibited from the co FRAs suggesting an excitatory route to ipsilateral flexor motoneurons. A second group of cells were excited by co FRAs and inhibited by ipsi FRAs indicating an excitatory output to ipsilateral extensor motoneurons. These two groups are of particular interest since they have the firing pattern compatable with the late, long duration FRA evoked discharge observed in motoneurons after the administration of L-DOPA. The indications are that these two groups of interneurons are the ones that are reciprocally organized and mediate the effects observed after L-DOPA.

Once given L-DOPA, the normally non-stepping acute spinal cat can be made to walk, supposedly via the actions of NA. This last point rests on the fact that clonidine, an agonist of NA, can produce the effect as well (Forssberg and Grillner, (1973). Stepping can also be evoked in the decerebrate cat by electrical stimulation of the MLR (Orlovsky et al., 1966). Grillner and Shik (1973) demonstrated that such stimulation can not only produce locomotion, but it

also produces the same pattern of FRA response changes as those observed in the acute spinal L-DOPA treated animals described earlier. This suggests a relationship between the two phenomena and has stimulated investigation into the concept of a reciprocal organization for the spinal cord CPG for locomotion (Lundberg, 1980).

Miller and Scott (1977) proposed a model for the locomotor CPG using an electronic analogue neuron arrangement based on identified spinal neurons. The model included two sets of three coupled neurons, one set for extensor and another for flexor motoneuron activity. The neurons included were alpha motoneurons, Ia inhibitory interneurons (IaIn) and Renshaw cells (RC). Miller and Scott proposed "half-centers" composed of groups of IaIn interneurons which were reciprocally inhibiting as well as having an inhibitory action on antagonistic motoneurons. An integral part of the model was the presence of a tonic excitatory input to both IaIns and the alpha motoneurons. This bistable oscillatory activity between flexor and extensor activity was a function of the phasic IaIn inhibition of the alpha motoneurons and the paired IaIn half center on a background of tonic excitation to both the IaIn and motoneurons. The patterning of the IaIn output was in turn influenced by the inhibitory action of the RCs on the IaIns coupled to them. The RC activation was via the motoneuron axon collaterals, hence the RC functioned as a negative feedback element. The oscillatory behavior of the system was believed to be a

function of the delays associated with the RC recurrent pathway and its influence on the IaIn interneurons.

Pearson and co-workers working on the cockroach proposed a central pattern generator for locomotion they called the flexor burst generator (FBG) (Pearson, 1976). The FBG phasically activates flexor motoneurons and phasically inhibits extensor motoneurons. Interneurons intercalated between the generator and the motoneurons were identified and when stimulated could produce bursting in flexor motoneurons. The inhibition to extensors was not elaborated although a tonic excitatory drive to this group was suggested. Hence, an asymmetry in the pattern of input to the flexor and extensor motoneurons is evident from the model. A mutual inhibitory arrangement between FBG's was proposed to prevent adjacent legs from stepping at the same time. Based on similar patterns of sensory feedback modulation of the step cycle, including stance and swing variations in both the cockroach and cat, the FBG has been extended to include the cat CPG for locomotion.

The fact that each of the previously described models predicts a different pattern of synaptic activity from the CPG onto motoneurons in the lumbar spinal cord during locomotion provides a tool with which the models can be tested. By monitoring the conductance changes in identified flexor and extensor coupled motoneurons during locomotion, one can evaluate the pattern of synaptic activity acting on the motoneurons. To illustrate this, the following section will

outline the pattern of synaptic activity to flexors and extensors which each model would suggest.

The half-center organization (Brown, 1914; Lundberg, 1980) requires a phasic excitatory output from the CPG to both flexor and extensor motoneurons. The issue of the source of the phasic inhibition, either from the CPG directly or via an independent activation of IaIns was not addressed. Based on this, one would expect the membrane resistance to vary little during the step cycle if both excitatory and inhibitory synaptic input alternated. This would be the same for both flexor and extensor motoneurons. If the inhibition was ommitted or played only a very minor role, one would expect an increase in conductance during the active depolarized phase of the step cycle when an increase in active synaptic excitation was occurring.

The Miller and Scott (1977) model requires tonic excitation to both flexor and extensor motoneurons with phasic inhibition to both groups during locomotion. Hence, all motoneurons would display an increase in membrane conductance during the hyperpolarized phase of membrane oscillations during the step cycle.

The FBG proposed by Pearson (1976) would have flexor and extensor motoneurons displaying very different conductance patterns during the step cycle. Flexor motoneurons should have increased conductance only during their active burst phase while the extensor motoneurons are hypothesized to receive tonic excitation interrupted with a phasic

inhibitory output the FBG. Thus the extensors would display the greatest membrane conductance during the active inhibition of the interburst hyperpolarization. Clearly a distinct difference should be seen between the conductance patterns for the two groups of motoneurons.

The purpose of this project was to examine the synaptic input from the locomotor central pattern generator to identified alpha motoneurons in the cat lumbar spinal cord. Flexor and extensor coupled motoneurons acting across the hindlimb hip, knee and ankle joints were sampled in an effort to characterize similarities or differences in the CPG output to different functional types of motoneurons during fictive locomotion. From this information a general statement about the CPG can be made and provide further avenues to pursue in establishing the spinal cord neurons involved in the generation of stepping movements.

Methods

1. Surgical Preparation and Anesthesia

The anesthetic of choice was halothane carried in a mixture of oxygen and nitrous oxide administered using a Dragger halothane vaporizer. Initial induction was achieved using a direct flow of 4% halothane, with maintenance using 0.8% to 3% halothane delivered via a face mask and later through a tracheal catheter. The animal's body temperature was maintained between 36-38°C throughout the surgery using a heating pad placed beneath the animal.

The right common carotid artery was cannulated using silastic tubing filled with a lactated Ringer's and heparin solution (4:1). The catheter was attached to a Strathen pressure transducer to allow for the continuous monitoring of blood pressure with a Grass polygraph. The right external jugular vein was cannulated using polyethelene tubing filled with lactated Ringer's. This route was used for the intravenous (i.v.) administration of fluids and drugs as required during the experiment.

The left common carotid artery was dissected free from surrounding tissue and a loose tie was placed around it in a fashion that would allow the temporary occlusion of the vessel by pulling the tie. This procedure was done to control excessive bleeding at the time of the decerebration.

The next phase of the surgery involved the dissection and cuffing of the following peripheral muscle nerves; PB,

ST, LG, MG,Vastus, Sart., Grac., and TA. In the majority of animals unilateral nerve dissections were performed with the remainder of the preparations having the procedure done bilaterally. The nerves were dissected free from the surrounding connective tissue using glass probes. A minimum of stretching and manipulation was the rule during the procedure, and exposed nerves were bathed regularly in warm saline to prevent damage or deterioration of the tissue.

The nerve cuffs used to stimulate the peripheral nerves were made of 4.0 to 6.0 mm long silastic tubing slit along one side to facilitate placement of the intact nerve within the lumen of the cuff. Copper wire (#40 Single Beldsol) was threaded through the cuffs and bared, providing the current flow when attached to the output of the stimulator. Once the nerve was placed within the cuff the entire cuff was carefully covered with soft elastomere (Silastic, 382 Medical Grade) to secure the nerve and cuff in place.

A laminectomy extending from L4 to L7 was performed. The spinal canal was widened to provide good access for the dorsal and ventral root identification and dissection. The exposed spinal cord, with dura intact, was covered with gauze soaked in warm saline to maintain a moist environment until a heated mineral oil pool could be arranged.

II. Mechanical Support

After the initial phases of surgery were completed, the animal was transferred to a shielded room and placed into a modified Transvertex frame. Two metal clamps, one placed

at the thoracic level and a second at the L2-L3 region, were used to suspend the animal. The animal's head was placed in a stereotaxic headholder in preparation for the decerebration and placement of the brainstem stimulating electrode. Metal pins placed tightly against the iliac creats of the pelvic secured the lower spinal cord and hindlimb region, but allowing the hindlimbs freedom to move. The vertebral column in the area of the lumbar enlargement was fixed firmly in position using L-shaped metal clamps placed on either side of the column. This procedure functioned to decrease movement artifacts which would confound the intracellular recording procedures. The skin of the back was tied in such a manner as to create a mineral oil pool over the exposed spinal cord. The pool was maintained at approximately 37°C using a heating lamp.

III. Craniotomy and Decerebration

Once the animal's head was securely placed in the stereotaxic headholder, the bone over the parietal and temporal regions was removed. A blunt spatula was used to remove the two cerebral hemispheres and to transect the brainstem. The transection extended from the rostral edge of the superior colliculi on the dorsal surface to the caudal edge of the mammillary bodies ventrally, thereby creating a precollicular, postmammillary mesencephalic preparation (for figure refer to Schwindt, 1981). All brain tissue rostral to the transection was removed and the floor of the cranium

packed with absorbable hemostat (Surgical). Once the transection was completed the anesthetic was removed and a slow i.v. infusion of 6% Gentran (Dextran 75) was carried out. The amount of Dextran given was dictated by the blood pressure recovery characteristics. The exposed brainstem was covered with a 4% Agar solution to prevent drying and deterioration of the tissue during the two hour recovery period that followed the decerebration.

Later locomotion was evoked in the decerebrate preparation using electrical stimulation of the mesencephalic locomotor region (MLR) (Shik et al., 1966; Grillner and Shik, 1973). An insulated monopolar stimulating electrode (exposed tip 0.25 mm, diameter 0.1 mm) was positioned on the dorsal surface of the exposed midbrain 4.0 mm lateral to the midline of the two superior colliculi and approximately 1.0 mm caudal to the division between the inferior and superior colliculi. The electrode was lowered 4.0 mm to place it above the dorsal border of the MLR. Square wave pulses (duration 0.5 msec, rate 30 Hz) were delivered through a constant current generator. The electrode was lowered in increments of 0.5 mm in order to locate the region (usually 5-6 mm deep) which displayed the lowest threshold (25 to 150 microamperes) for evoking locomotion.

IV. Spinal Cord Dissection

The L6 and L7 dorsal and ventral roots were identified and separated. A small filament from the L7 ventral

root was isolated, cut, and the end proximal to the cord was placed on a bipolar recording electrode. The filament was classified as extensor or flexor coupled based on its participation in reflex firing patterns and/or peripheral nerve stimulation. In addition, the filament's activity was characterized relative to a Vastus or PB electromyogram or neurogram activity pattern during MLR evoked locomotion in the air. A filament which was phasically active during walking in the air was selected to serve as the monitor for locomotion during the fictive locomotion trials after the animal had been paralyzed.

The identification of the exposed lumbar and sacral spinal cord segments allowed for the selective placement of the intracellular recording microelectrode depending on which of the motoneuron pools were to be sampled. A small hole was made in the pia mater so as to facilitate the entrance of the glass microelectrode into the spinal cord tissue.

V. Fictive Locomotion

The animal was paralyzed using gallamine triethiodide (Flaxedil 100, 20mg/ml) and artificially ventrilated. The end tidal CO_2 was monitored and maintained between 4% and 6% using volume and/or rate adjustments on the ventilator.

Locomotion was induced in the same way as it was evoked prior to paralysis. During fictive locomotion the rhythmic activity of the ventral root filament served as the monitor for locomotion.

VI. Intracellular Recording Procedures

The intracellular recordings were made using glass microelectrodes filled with 3.0 M potassium acetate or 3.0 M potassium chloride solutions. The microelectrodes were characterized by tip diameters of 2 mm or less with tip resistances ranging from 5 to 25 megohms. A WPI-M4A active bridge circuit was used to allow recording and current injection through a single barreled microelectrode. The current injections were necessary for monitoring membrane conductance during locomotion and short (5 to 10 msec) hyperpolarizing pulses (10pps) were used.

In one series of experiments, potassium chloride filled microelectrodes were used to reverse inhibitory postsynaptic potentials (IPSPs) by injecting Cl- ions into the motoneuron. Several technical and theoretical problems arose and are discussed in later sections.

VII. Data Collection and Analysis

During the experiments, records of the ventral root filament activity, intracellular high gain AC and low gain DC traces, the current monitor (associated with the hyperpolarizing pulse injections) and, when possible electroneurograms and electromyograms were stored on FM tape (8 Channel Vetter). The tapes were later played back for photographic and analytical purposes.

Two parameters of the injected hyperpolarizing pulses were used to provide information about the membrane resistance, or conductance, changes in the motoneurons during

fictive locomotion. The first parameter was the amplitude of the hyperpolarizing pulse during the step cycle. With an increase in membrane conductance, shunting of the injected current pulse occurs and subsequently a decrease on the pulse amplitude is observed. With an increase in the pulse amplitude the conductance of the cell is decreased thus less shunting of the injected current occurring (Engberg and Marshall, 1979; Krnjevic et al., 1977; Mauritz et al., 1974). Knowing the amount of current (I) injected and measuring the amplitude of the pulse (E) allows one to determine the membrane resistance (R) (R=I/E) throughout the depolarize phase as well as the interburst interval of the motoneuron membrane activity during the step cycle.

The second parameter examined was the time constant (T_m) of the decay of the injected hyperpolarizing pulse. The time constant is a function of the resistance multiplied by the capacitance (C) (T_m=RC). Since the membrane capacitance does not change significantly under these conditions, changes in the T_m are reflections of resistance changes (Burke and ten Bruggencate, 1971; Nelson and Lux, 1970). A comparison of the slope of the lines from the semilog plot of change in voltage over change in time (dv/dt) versus time for the hyperpolarizing pulses during the depolarized and hyperpolarized phases of the step cycle was performed.

The amplitude and pulse decay data was taken from either discrete samples or from averages obtained from several step cycles (30 to 200 samples per average).

The discrete samples were sorted according to membrane potential values while the averages were triggered using ventral root filament activity and/or membrane potential levels.

Results

General Motoneuron Information

The motoneurons used in this analysis had an average membrane potential (Em) of -60 mv (range: -45 to -75 mv). Cells which displayed damage by the microelectrode impalement, as reflected in a decreasing Em or poor spike production, were not used. The Em level was obtained from the DC levels recorded on a pen recorder during the experiments. The mean membrane resistance (R) was 1.30 megohms (range: 0.7 to 3.1 megohms) and was calculated using the value for the injected current (I) associated with the hyperpolarizing pulse and the amplitude (V) of that pulse during resting conditions (R=V/I). The conduction velocity of the cell was calculated using the distance between the spinal cord motoneuron recording site and the peripheral nerve stimulating region (cuffed portion of the peripheral nerve) in addition to the latency of the antidromic spike. All values so obtained indicated that the cells were within the range which corresponds to alpha motoneurons (Gasser and Grundfest, 1939; Hursh, 1939). For those motoneurons associated with bifunctional muscles (i.e. posterior biceps, semitendinosus), classification as flexor or extensor was based on their activity pattern during fictive locomotion as related to the identified extensor or flexor coupled ventral root filament activity.

Conductance Results

The membrane conductance of the motoneuron was monitored during the step cycle using an injected current pulse, in this case a short hyperpolarizing pulse as described in the Methods (Fig. 1). In some circumstances the microelectrode tip resistance changed during a trial, creating a situation where the WPI-M4 active bridge circuit was not compensating for the electrode resistance. Hence, the voltage deflection produced by the current injection was a reflection of both the motoneuron resistance and the microelectrode resistance. In these cells only the T_m measures were made (Fig. 2) because the T_m measures are independent of the pulse amplitude values. A number of cells (10) were analysed using both pulse amplitude and T_m techniques with excellent reliability between the two methods. Based on the good correlation between the two measures and their equal ability to address the conductance change issue at hand, it was decided that both measures were not necessary for every cell and that either method alone was sufficient.

Insurance that the technique of the pulse injection was sensitive enough to detect changes in conductance was obtained using records of the pulse during events known to have increased conductance (i.e. inhibitory postsynaptic potentials, after hyperpolarization of a spike). Clear decreases in the injected pulse amplitude were observed,

Figure 1 : A typical example of the raw data collected on a motoneuron. The AC intracellular microelectrode record from a soleus motoneuron (A) consists of a calibration pulse (2mv, 1msec) followed by a monosynaptic EPSP and an injected hyperpolarizing pulse. The upper trace in B shows the AC record for the same cell (calibration same as in A) during a period of suprathreshold stimulation of the soleus peripheral nerve to evoke an antidromic spike in the motoneuron. In B the injected hyperpolarizing pulse is occurring during the afterhyperpolarization of the spike and although the same amount of current is being injected in A and B, the voltage deflection in B is smaller indicating a period of increased conductance. The lower trace in B is that of the low gain DC record. In C the DC microelectrode record (upper trace) and the ventral root filament record (lower trace) of three step cycles is shown with the start of the steps marked with an arrow. The extensor coupled ventral root filament fires in phase with the depolarized phase of the extensor motoneuron Em oscillations during the step cycles.

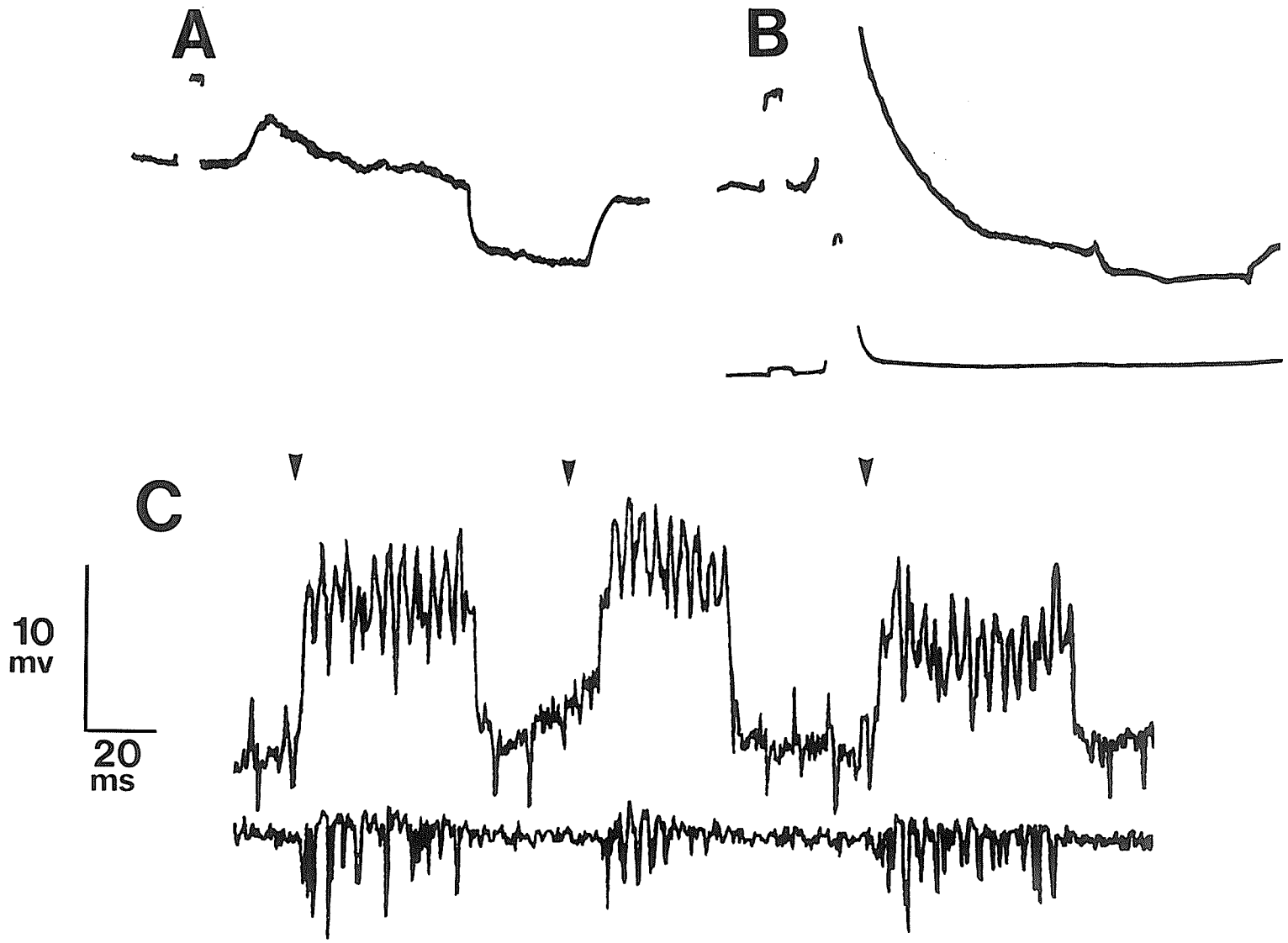
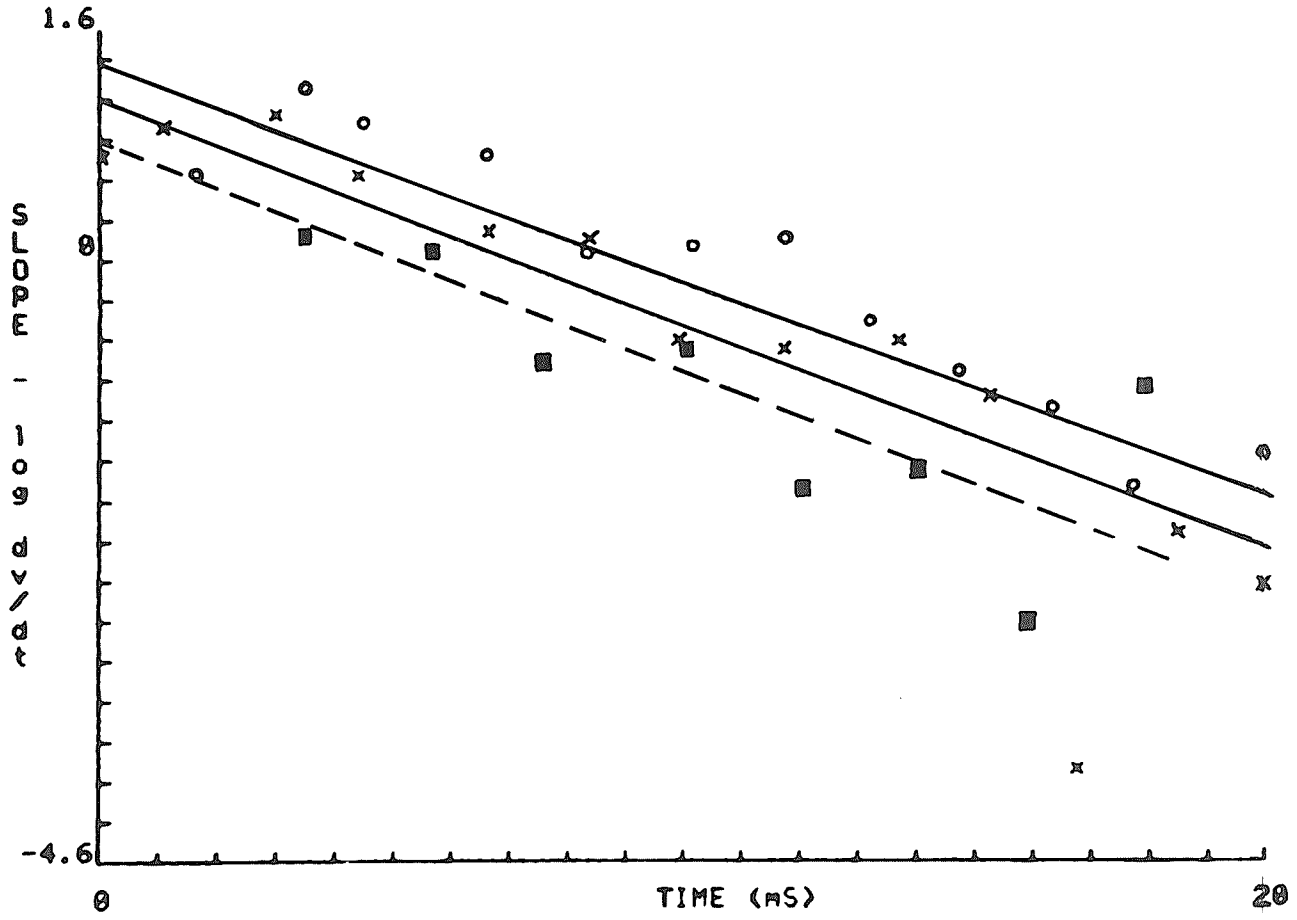

A
B
C
10
mv
20
ms

Figure 2: Semilog plot of the slope of the decay of the injected hyperpolarizing pulse (or T_m measures). The dots (●) represent the depolarized phase and the crosses (×) the hyperpolarized phases of the step cycle. The slopes of the best fit lines (solid lines) are 0.17 and 0.16 respectfully. The squares (■) and the dashed line represents the data from the control, non-walking period (slope=0.17). In this example, none of the samples are significantly different, indicating no conductance changes occurred.



o Hyperpolarized phase of step cycle ——

× Depolarized phase of step cycle ——

■ Non-walking Control – – – – –

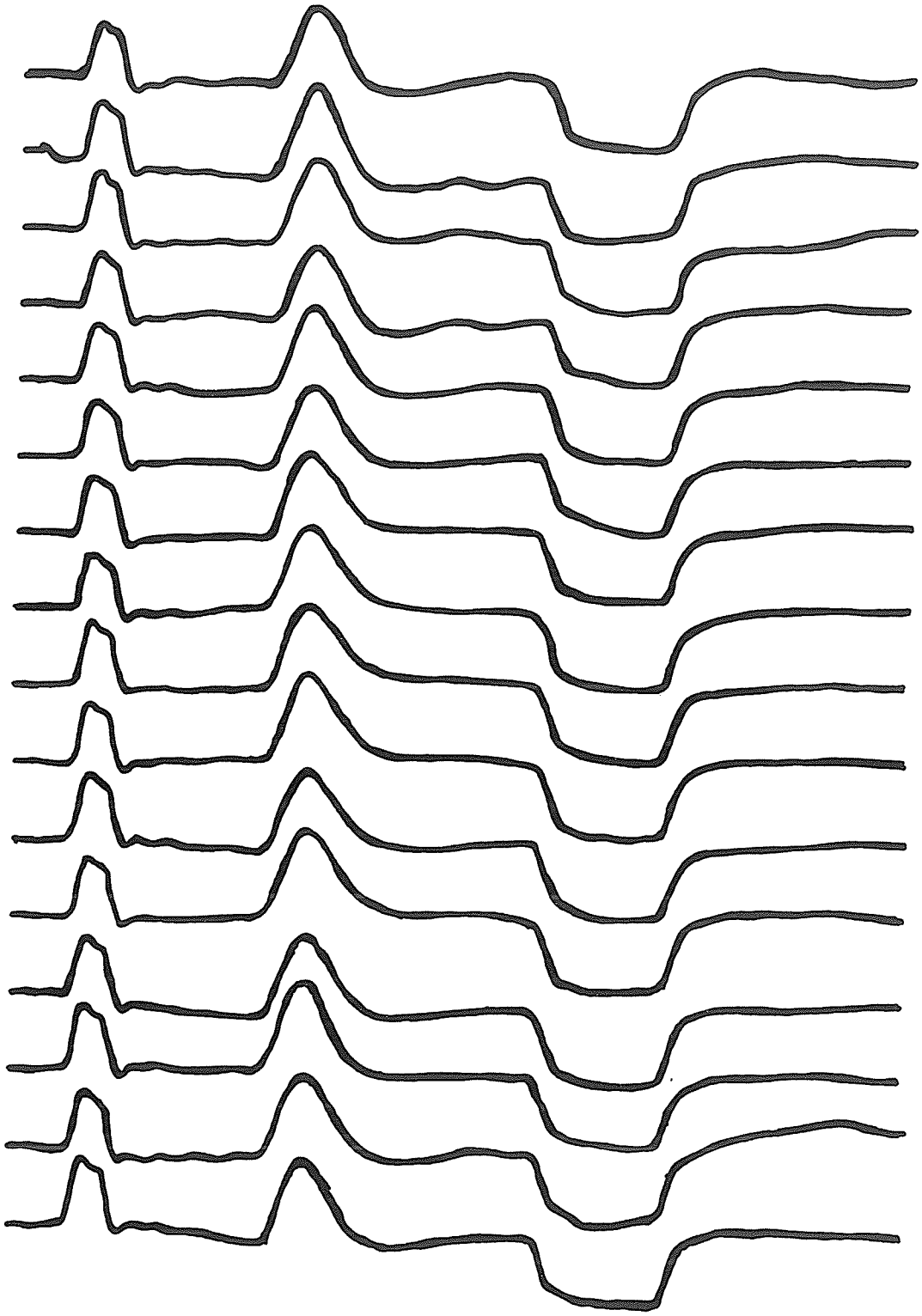
indicating that the method was capable of detecting changes on conductance should they occur (Fig. 1A,B).

The averages of the hyperpolarizing pulses during several step cycles obtained using the DIDAC averager were cued to the Em levels of the cell, thus dividing the step cycle into a depolarized and a hyperpolarized phase. Because of the fluctuations in the number of samples taken for the two phases, the averages obtained were normalized prior to further analysis using the calibration pulse (2 mv, 1 ms) associated with each average.

The averages of the hyperpolarizing pulses using a PDP-11 system were triggered using the Em levels or the rhythmic bursting of the ventral root filament during fictive locomotion. The step cycles were normalized by dividing each into 16 bins. The AC traces were averaged during each of the 16 bins across several step cycles, thereby providing 16 averages for the step cycle (Fig. 3). By noting the VR filament characterization and the identity of the recorded motoneuron, the depolarized and hyperpolarized phases of the step cycle could be identified. In those trials in which the VR filament firing varied in amplitude within a burst, erroneous triggering was a confounding variable. In these circumstances the triggering was done using the Em level.

The analysis of the 16 averages consisted of plotting the DC offset as well as the peak amplitude of any postsynaptic event that was recorded and the peak amplitude of

Figure 3: Averages of the AC microelectrode record representing the 16 divisions of the step cycle. Included in each average is the calibration pulse (2 mv, 1 msec) followed by the LG monosynaptic EPSP and the injected hyperpolarizing pulse.



the hyperpolarizing pulse. The DC offset was the offset the computer required to adjust the Em level to an arbitrary zero value. Thus the DC offset reflected the oscillations of the Em or the locomotor drive potentials observed during the step cycle. Since waves were fitted to each of the plots to illustrate changes in the variable during the step cycle as well as any possible relationship such a change may have had to the Em and the step cycle (Fig. 4A,B,C).

In a small number of motoneurons discrete hyperpolarizing pulses were examined rather than the averages from several step cycles. The samples were correlated with the Em level throughout the step cycle. Hyperpolarizing pulse amplitudes and T_m measures were made for these samples.

The results from each of the described analytical techniques indicated that, overall, no single population of flexor or extensor motoneurons examined displayed any significant change in membrane conductance during the step cyle (Table 1). However, examples of individual motoneurons from both flexor (TA) and extensor (LG) populations were observed to display an increase in conductance during the depolarized phase of the step cycle (Fig. 5). In addition to the conductance during the step cycle, a comparison of the prestimulation non-walking periods and the MLR-evoked walking periods was done. This revealed that some cells displayed no change in the conductance between the two periods while other cells showed an increase in membrane conductance with the onset of MLR stimulation to evoke locomotion.

Table 1: The table summarizes the cells which showed no change in conductance during the step cycle. The number in brackets after each name represents the number of cells examined in the group. For full names refer to the list of abbreviations in the Methods section.

	EXTENSORS	FLEXORS
HIP	Grac. (1) Sart. (1)	SMAB (5)
KNEE	Vastus**	PB (1) ST (1)
ANKLE	LG (7) MG (1) Soleus (3)	TA (14)

** All branches except rectus femoris

Figure 4: Graphs obtained from the 16 averaged bins depicted in Figure 3. The horizontal axis represents the 16 bins averaged during the step cycle--in this example a time span of 800 msec. The DC OFFSET shows a clear locomotor drive potential with the peak of the depolarized phase of the cycle at about 450 msec. The graph of the peak EPSP amplitude shows modulation of the EPSP in phase with the depolarized phase of the step cycle. The hyperpolarizing pulse amplitude plot shows no significant change in the amplitude of the pulse--hense no conductance change during the step cycle.

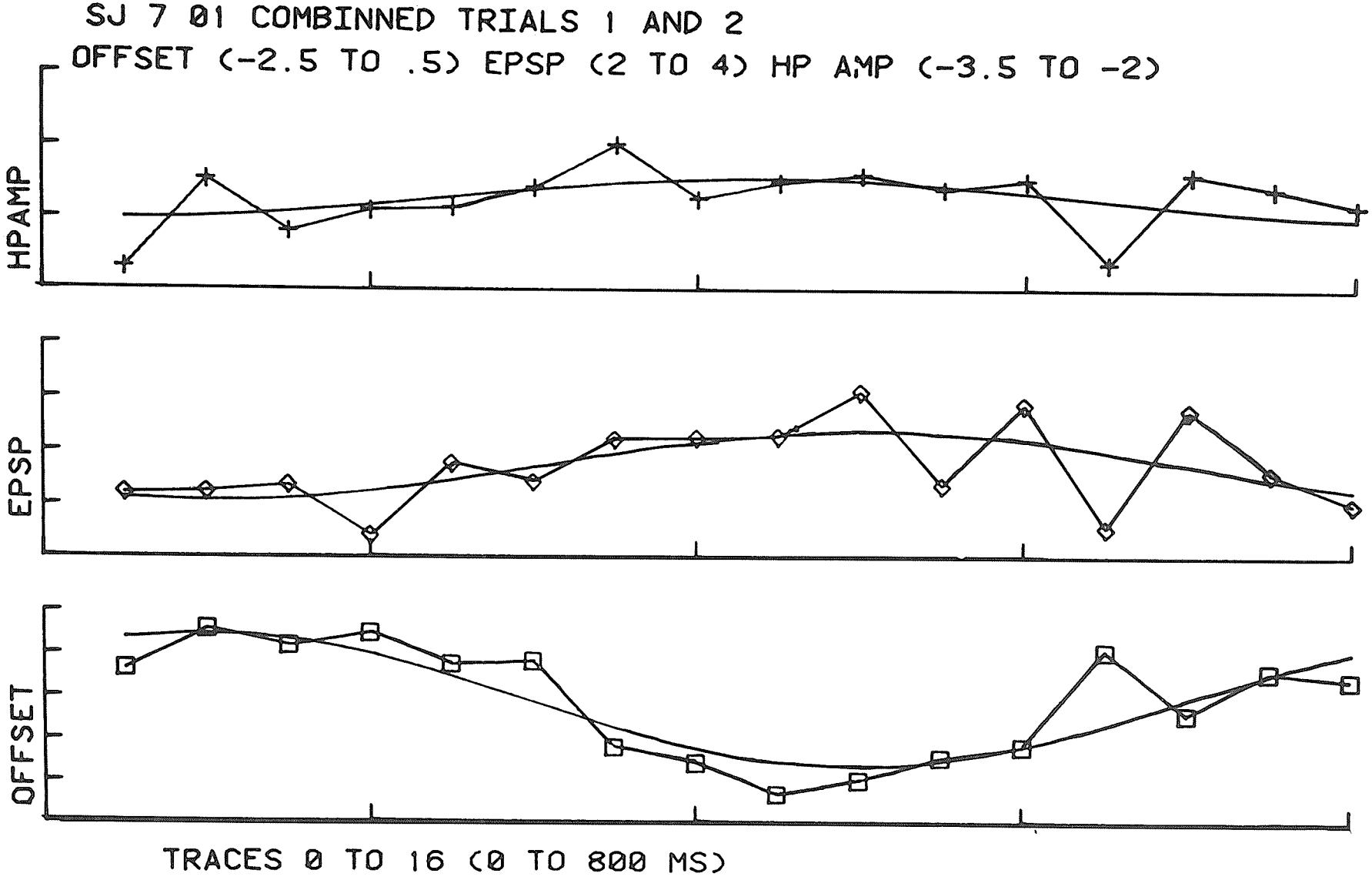

SJ 7 01 COMBINNED TRIALS 1 AND 2
OFFSET (-2.5 TO .5) EPSP (2 TO 4) HP AMP (-3.5 TO -2)
HPAMP
EPSP
OFFSET
TRACES 0 TO 16 (0 TO 800 MS)

Figure 5: Averages from an extensor (LG) motoneuron (A,B) and a flexor (TA) motoneuron (C,D). Panel A and C show the averages from the depolarized phase of the step cycle in each cell respectfully, while traces B and D show the averages from the hyperpolarized phase. Each trace consists of a calibration pulse 2 mv, 1 msec), a monosynaptic EPSP and the injected hyperpolarizing pulse. It is clear that in both cells the hyperpolarizing pulse is decreased in amplitude during the depolarized phase of the step cycle (A,C) as compared to the hyperpolarized phase (B,D).

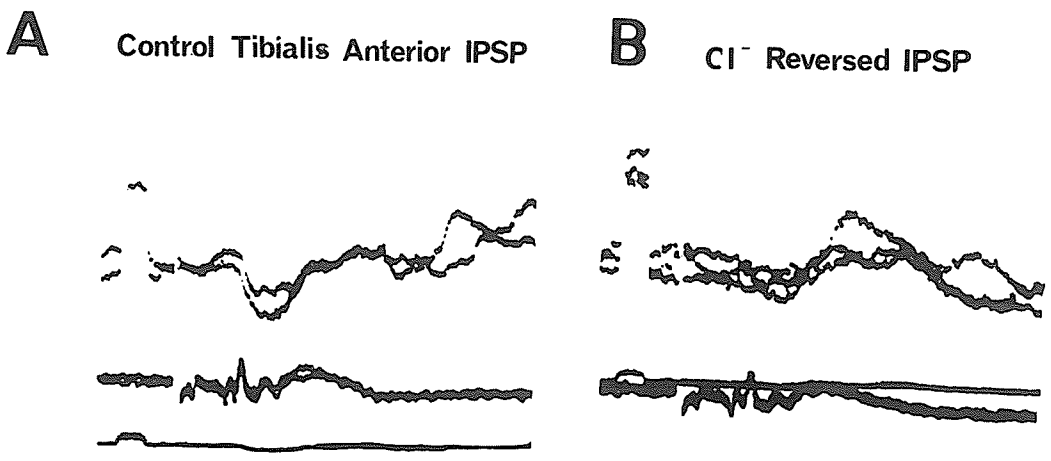
IPSP Reversal Results

The contribution of the Ia inhibitory interneuron (IaIn) disynaptic inhibitory postsynaptic potential (IPSP) to the activity of the motoneurons during locomotion was examined during several experiments. Using 3 M KCl filled microelelctrodes, attempts were made to inject Cl^- into the cell and reverse the IPSP. The IPSPs were classified according to which peripheral nerve, when stimulated, produced the IPSP. The IPSPs were classified as disynaptic if they had a latency of between 1.0 and 1.5 msec.

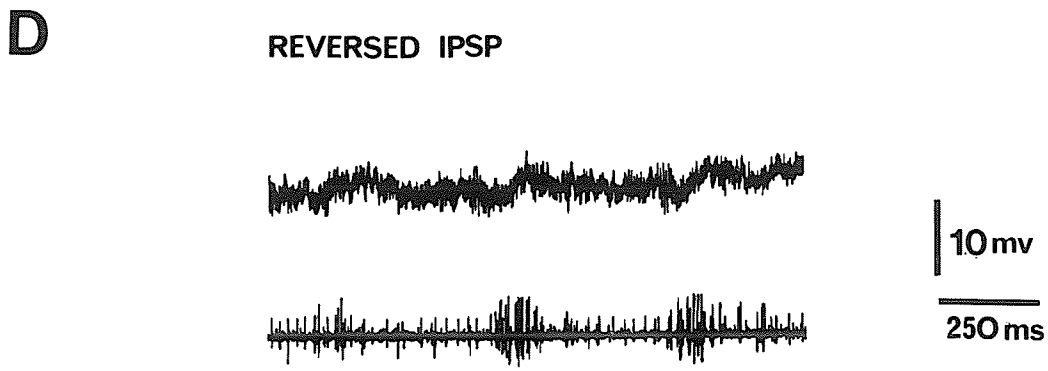
The method of Cl^- injection could successfully reverse the disynaptic IPSP, however, certain problems did arise with these reversals. At the start of the locomotion segment, the IPSP was reversed, but during the segment the dynamic state of the cell created an efflux of Cl^- sufficient to allow the return of the IPSP. The Cl^- injection was not sufficient to maintain a reliable steady reversal of the IPSP.

It did become apparent during the IPSP reversal trials that the removal or reversal of this inhibitory event produced a decrease in the Em oscillations or LDPs during locomotion in both extensor and flexor motoneurons. In the examples where the IPSP returned during the segment, a corresponding increase in the LDP also was observed. In one segment of locomotion one could see the IPSP changes and their corresponding effects on the motoneuron's activity during locomotion (Fig. 6).

Figure 6: The effects of the reversal of a IaIn disynaptic IPSP on the motoneuron membrane oscillations during locomotion. The IPSP prior to reversal is shown in A (upper trace: AC record; middle trace: cord dorsum record; lower trace: DC record). The reversed IPSP is shown in B and a later EPSP now can be observed (upper trace: AC record; middle trace: DC record; lower trace: cord dorsum). The calibration pulse in both A and B is 2 mv and 1 msec. The shift in the DC trace after the IPSP reversal (B) reflects a depolaration of the cell due to the removal of the inhibitory influence. In C and D the effects of the IPSP reversal can be seen in the decrease of the Em oscillations during the step cycle (upper traces in C and D). The ventral root filament activity (C and D lower traces) is largely unchanged after the reversal of the IPSP.


A
Control Tibialis Anterior IPSP
B
Cl⁻ Reversed IPSP


C
CONTROL
10 mv
250 ms


D
REVERSED IPSP
10 mv
250 ms

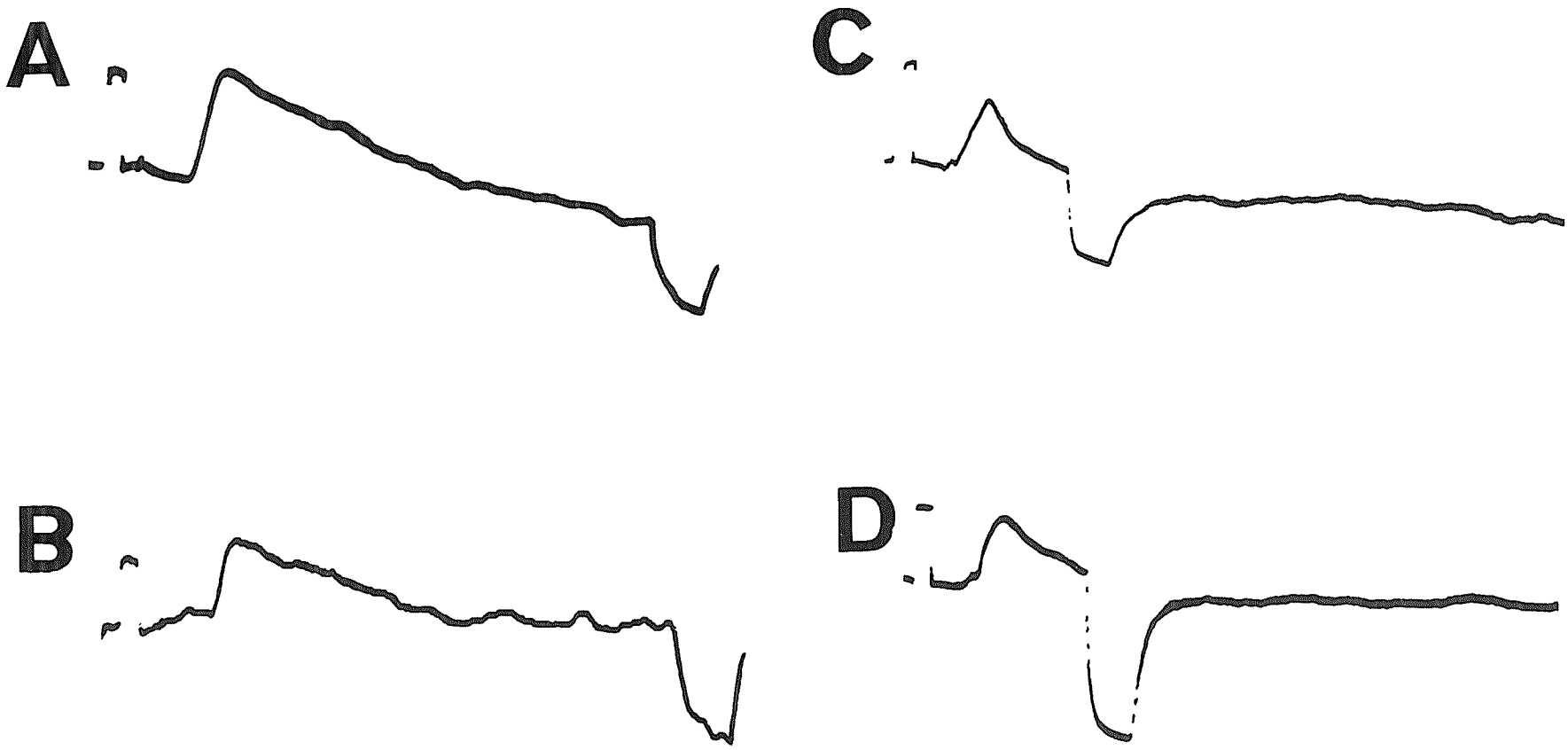
Monosynaptic EPSP Modulation

In a series of experiments analyzed using the PDP-11 system described earlier, not only conductance but also the modulation of the monosynaptic EPSP was investigated. The source of the monosynaptic EPSP was identified using electrical stimulation of the cuffed peripheral nerves (latency of the EPSP less than 8 msec) and a constant train of stimuli was delivered to evoke the monosynaptic EPSP repeatedly throughout the step cycle. The peak amplitude of the EPSP was calculated for the 16 bins of the step cycle. The triggering methods were the same as described earlier. The results indicated that 41% of the identified motoneurons displayed no modulation of the monosynaptic EPSP during the step cycle while 47% showed an increase in the EPSP peak amplitude during the depolarized phase. The remaining 12% showed the largest EPSP amplitude during the hyperpolarized phase of the step cycle. There was no apparent correlation between the monosynaptic EPSP modulation and conductance changes in the cells and the conduction velocity or after-hyperpolarization amplitude of the cell. The conduction velocity and the size of the after-hyperpolarization are both indicators of cell size. It became evident that those motoneurons which had the largest membrane potential oscillations or locomotor drive potentials during the step cycle displayed the greatest modulation of the monosynaptic EPSP during locomotion (Shefchyk, Stein and Jordon, 1982).

MLR-Evoked EPSPs in Motoneurons

During the course of these experiments, it was observed that many motoneurons displayed a general depolarizing shift in the Em level with the onset of MLR stimulation. Superimposed on this shift were the LDPs associated with the cells' rhythmic activity during locomotion (Fig. 7). In addition to this phenomena, EPSPs that were time locked to the MLR stimulus were recorded in both flexor (TA, St) and extensor (LG, Soleus) motoneurons (Fig. 8). These EPSPs appeared after the delivery of each MLR stimulus with a latency of 4-6 msec. The amplitude of these EPSPs ranged between 4 to 6 mv.

Figure 7: The low gain DC trace of an LG motoneuron. The cell became depolarized with the onset of MLR stimulation (marked with an arrow) with the LDP's superimposed on this depolarization later. This all displays spiking during the depolarized phase of the step cycle.


A
C
B
D

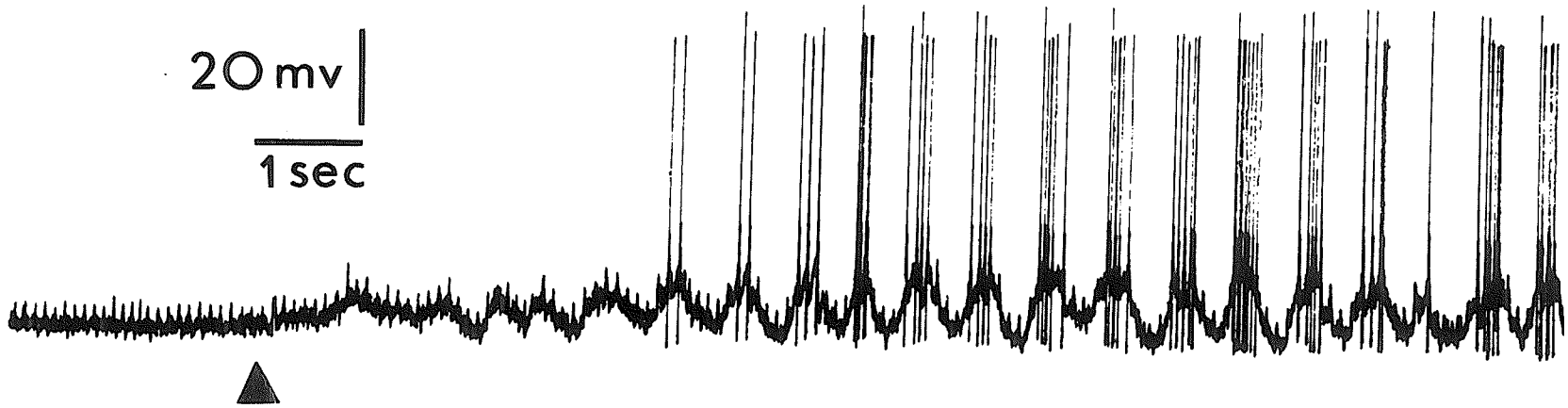
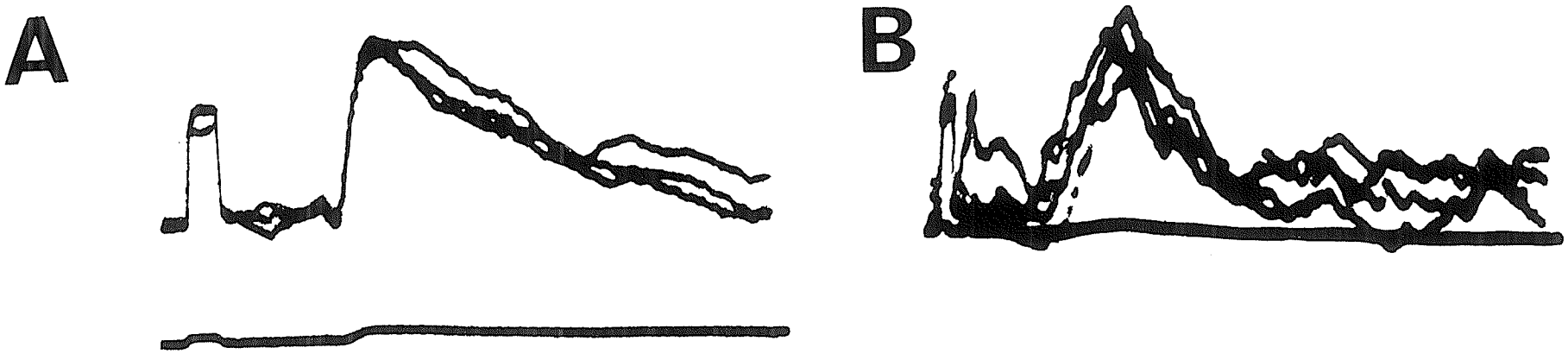

20 mv
1 sec

Figure 8: The AC intracellular record from an LG motoneuron (A). Depicted is the calibration pulse (2 mv, 1 msec) and the monosynaptic EPSP evoked from stimulation of the LG peripheral nerve. In B the AC record, at a slower sweep speed, is triggered from the MLR stimuli and shows the EPSP which is time locked to those stimuli. Calibration pulse is the same as in A.


A
B

Discussion

Monitoring the conductance patterns of identified extensor and flexor alpha motoneurons during locomotion provides the first step in defining the drives necessary to produce the motoneuron rhythmicity. As stated in the Results section, none of the flexor or extensor populations examined displayed a significant change in membrane conductance or resistance during the step cycle. If one turns to the existing theories for the central pattern generator model, it becomes obvious that some models do not provide an adequate account of the motoneuron events observed in this work.

For instance, the assymmetry between extensor and flexor motoneurons stressed in Pearson's flexor burst generator (PBG) was not observed. The flexor and extensor populations examined received a similar pattern of synaptic input during locomotion as reflected in both groups' unchanging conductance during the step cycle.

If one addressed the issue of increased conductance during the interburst hyperpolarization proposed for extensor motoneurons (Pearson, 1976) or both flexor and extensor motoneurons (Miller and Scott, 1977), one finds no support for this in the results. Such an expected increase would be reflected in a decrease in the hyperpolarizing pulse amplitude during the hyperpolarized phase of the step cycle. No group of extensors or flexors displayed this. Any individual

examples of increases in conductance during the step cycle for either flexor or extensor motoneurons were observed during the depolarized phase, not the hyperpolarized phase of the cycle. In addition, an increase during the depolarized phase suggests the presence of a phasic excitatory synaptic input to the motoneurons during locomotion. Such an input is not a part of either the FBG (Pearson, 1976) or the Miller and Scott (1977) model, since both models proposed a tonic excitatory input to extensor or both extensor and flexor motoneurons, respectfully.

The Miller and Scott (1977) model is further challenged by the fact that Cl^- injections could reverse the IaIn mediated IPSPs and produce a decrease in the Em oscillations of the cell, but did not prevent rhythmic activity. Further evidence against the IaIn IPSPs significance in determining the motoneurons' rhythmicity has been obtained using the intravenous administration of strychnine (Pratt and Jordan, unpublished observations). Strychnine antagonizes the IaIn glycine mediated inhibition and can successfully remove the interburst hyperpolarization of the motoneuron. However, the cells continued to display rhythmic depolarization and spiking after the strychnine administration. It would appear that the importance placed on the IaIn in determining the rhythmicity of the motoneurons by the Miller and Scott model is not consistent with the experimental evidence.

The half-center hypothesis (Brown, 1911, 1914;

Jankowska et al., 1967; Lundberg, 1980) provides for an equal activation of flexor and extensor motoneurons. The reciprocal inhibition between the half-centers functions to prevent co-activation of the two groups. If one fuses the pathways described in the L-DOPA work, the MLR-evoked locomotion and descending pathways, and the identified spinal cord neurons active during walking, a scheme begins to evolve (Fig. 9). Figure 9 illustrates the FRA early short (A) and late long pathways (B). A descending NA pathway is shown, and it functions to inhibit the A pathway thereby allowing the extression of the B pathway effects. The switching between these two pathways has been observed to occur after treatment with L-DOPA, the NA precursor in acute spinal cats (Anden et al., 1966 a,b,c) and during MLR-evoked locomotion (Grillner and Shik, 1973). In the latter situation there is evidence of proximity between the MLR region and catecholamine containing cells which descend to the spinal cord (Steeves et al., 1975). However, the descending NA path is not necessary for MLR-evoked locomotion since depletion of NA and serotonin did not prevent MLR-evoked walking (Steeves, et al., 1980). This should not diminish the role of NA completely since recent work in acute spinal cats indicates that intrathecal administration of NA can produce weight support and stepping (Omeniuk and Jordan, 1982).

The MLR-evoked locomotion does require a descending pathway which runs in the ventral lateral funiculi of the

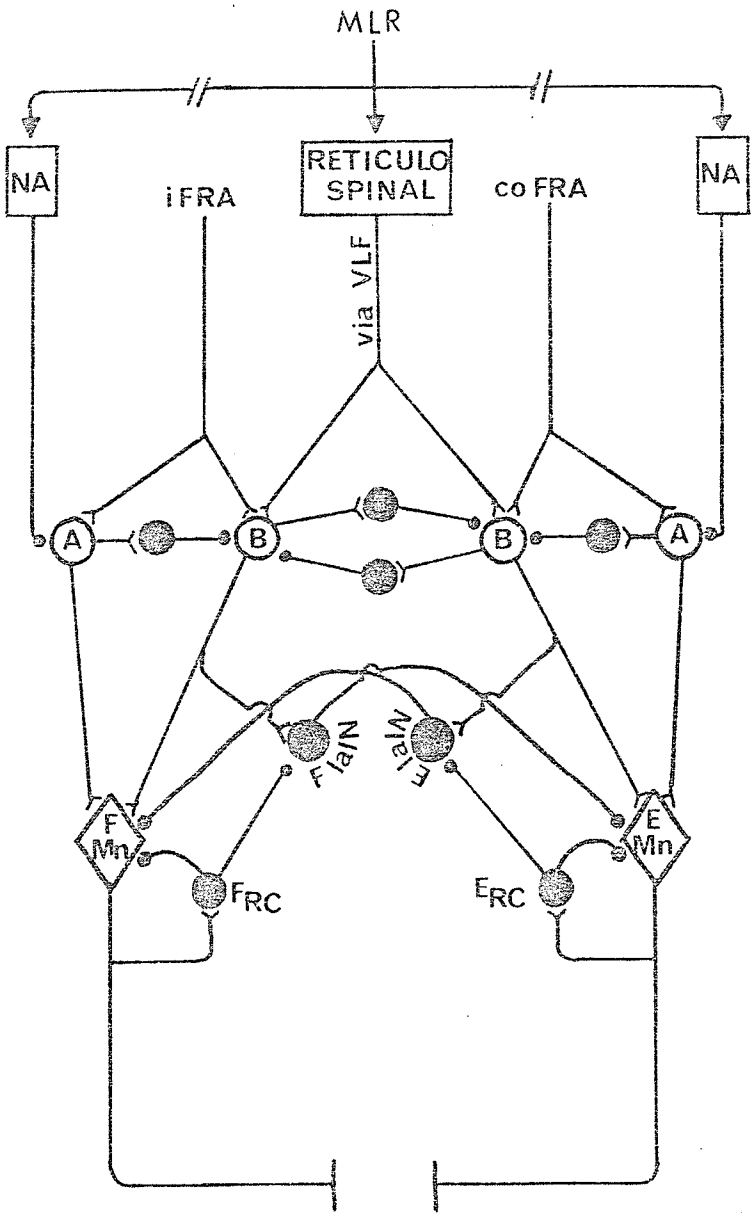
spinal cord (Steeves and Jordan, 1980; Eidelberg et al., 1981). The evidence indicates that the necessary pathway is a reteculospinal tract (Jell, Jordan and Ireland, 1981; Jankowska et al., 1967); Kuypers and Maisky, 1977).

Also included in the scheme are identified spinal cord interneurons, the IaIns and the Renshaw Cells (RCs), which are known to be rhythmically active during locomotion (McCrea et al., 1980; Pratt and Jordan, 1980; Pratt and Jordan, unpublished observations). These interneurons contribute to the Em oscillations during locomotion but when these events were removed pharmacologically with strychnine for IaIns (Menzies and Jordan, unpublished observations) or mecamylamine, for RCs (Jamal et al.,1982), the rhythmicity of the motoneurons can still be observed.

Much of the data presented provides indirect evidence for the necessity of an excitatory drive from the CPG to motoneurons. It was not until the observance of the MLR-evoked EPSPs in both flexor and extensor motoneurons during locomotion that more direct proof was available. These EPSPs appeared with a latency of 4 - 6 msec and their amplitude increased with repetitive stimulation, indicative of a polysynaptic pathway from the MLR to the motoneurons. Relays at the brainstem reticular formation and the lumbar cord interneurons such as the B cells described by Jankowska et al., (1976) are serious possibilities (Fig. 9).

The evaluation of monosynaptic EPSPs during locomotion was done in an effort to elucidate the mechanisms of

Figure 9: Proposed scheme of brainstem and spinal cord mechanisms for locomotion. Included are the flexion reflex afferent pathways (FRA), the descending NA pathway and the flexor coupled Renshaw cells (F_{RC}) and Ia inhibitory interneurons (FIaIN) and the Extensor coupled cells (EIaIN and ERC).


MLR
NA
iFRA
RETICULO SPINAL
coFRA
NA
via VLF
A
B
B
A
FIa/N
MIa/N
F Mn
E Mn
FRC
ERC

monosynaptic reflex (MSR) modulation during locomotion observed by Aldridge et al., (1981). Specifically the issue addressed was whether the recruitment of motoneurons into the MSR during locomotion was a function of interneuronal gating and/or cyclic motoneuron membrane potential changes. The results revealed that those cells with very large Em oscillations during locomotion tended to have the greatest EPSP modulation thereby implying that the recruitment of motoneurons into the MSR during walking may be largely a reflection of the motoneuron Em variations (Shefchyk et al., 1982).

The usefulness of such reflex modulation has been dealt with by Lundberg (1968) who proposed that the propriospinal stretch reflex could facilitate the stance (extension) phase of locomotion. For example, the MSR activation of extensors and accompanying IaIn inhibition of flexors could facilitate the desired muscle activation patterns necessary for the stance. Hence, the half-center CPG output supported by Lundberg (1968, 1980) and the reflex activity could work in a supportive fashion to create the necessary reciprocal muscle activity.

At this point it is useful to examine not only the facts learnt from the results, but also to evaluate new avenues to pursue. It has become clear that to understand the spinal cord organization and mechanisms responsible for the generation of rhythmic alternating motoneuron activity, an interneuronal system delivering an excitatory input to

both flexor and extensor motoneurons must be considered. The most exciting observation made during these experiments was that of the MLR-evoked EPSPs observed in both flexor and extensor motoneurons. These EPSPs may provide a tool for the identification of both the brainstem relays and spinal interneurons functional during walking. In the event that these EPSPs are an epiphenomenon and not directly related to locomotion, the search for excitatory elements in the spinal cord which could mediate the necessary phasic excitation to motoneurons can still proceed. With such leads as the identification of the B cells (Jankowska et al., 1967b) and a better understanding of reflex changes and interactions during locomotion the spinal cord interneuron work can advance. To define the elements of the CPG for locomotion and their mode of action will allow for the eventual understanding and pharmacological manipulation of this CPG in both normal and pathological conditions.

References

Aldridge, J.W., R.B.Stein, K. Akazawa and J.D. Steeves (1981) Modulation of stretch reflexes during locomotion in the decerebrate cat. Soc. Neuroscience Abstract, Los Angeles, 7: 560.

Anden, N.E., M.G.M. Jukes, A. Lundberg, and L. Vyklicky (1966a) The Effect of DOPA on the Spinal Cord. 1. Influences on transmission from primary afferents. Acta physiol. scand., 67: 376-386.

Anden, N.E., M.G.M. Jukes, and A. Lundberg (1966b) The Effect of DOPA on the Spinal Cord. 2. A pharmacological analysis. Acta physiol. scand., 67: 387-397.

Anden, N.E., M.G.M. Jukes, A. Lundberg, and L. Vyklicky (1966c) The Effect of DOPA on the Spinal Cord. 3. Depolarization evoked in the central terminals of ipsilateral Ia afferents by volleys in the flexor reflex afferents. Acta physiol. scand., 68: 322-336.

Brown, T.G. (1911) The intrinsic factors in the act of progression in the mammal. Proc. R. Soc. Lond. (Biol.), 84: 308-319.

Brown, T.G. (1914) On the nature of the fundamental activity of the nervous centers. J. Physiol. (Lond.), 48: 18-46.

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

Engberg, I. and K.C. Marshall (1979) Reversal potential for Ia excitatory postsynaptic potentials in spindle motoneurons of cats. Neuroscience, 4: 1583-1591.

Forssberg, H. and S. Grillner (1973) The locomotion of the acute spinal cat injected with clonidine i.v. Brain Res., 50: 184-186.

Friesen, W.D. and G.S. Stent (1978) Neural circuits for Generating rhythmic movements. Ann. Rev. Biophys. Bioeng., 7: 37-61.

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

Grillner, S. (1977) "On the neural control of movement- A comparison of different basic rhythmic behaviors." In Function and Formation of Neural Systems, Ed. G.S. Stent, Dahlem Konferenzen 1977: Berlin, pp. 197-224.

Jamal, J., B. Noga, S.J. Shefchyk, and L.M. Jordan (1982) Testing the role of Renshaw cell rhythmicity during fictive locomotion. Soc. Neuroscience Abstract, Minneapolis.

Jankowska, E., S. Lund, and A. Lundberg (1966a) The Effect of DOPA on the Spinal Cord. 4. Depolarization evoked in the central terminals of contralateral Ia afferent terminals by volleys in the flexor reflex afferents. Acta physiol. scand., 68: 337-341.

Jankowska, E., M.G.M. Jukes, S. Lund, A. Lundberg (1967a) The Effect of DOPA on the Spinal Cord. 5. Reciprocal organization of pathways transmitting excitatory action to alpha motoneurons of flexors and extensors. Acta physiol. scand., 70: 369-388.

Jankowska, E., M.G.M. Jukes, S. Lund, and A. Lundberg (1967b) The Effect of DOPA on the Spinal Cord. 6. Half-centre organization of interneurons transmitting effects from the flexor reflex afferents. Acta physiol. scand., 70: 389-402.

Jordan, L.M. (1981) Comment: Gating effects and constraints on the central pattern generator for rhythmic movements. Can. J. Physiol. Pharm., 59(7): 727-732.

Kristan, W.B., M. Burrows, N. Elsner, S. Grillner, F. Huber, E. Jankowska, K.G. Pearson, T.A. Sears and G.S. Stent (1977) "Neural Control of Movement--A Group Report" In Function and Formation of Neural Systems, Ed. G.S. Stent, Dahlem Konferenzen 1977: Berlin, pp. 329-354.

Krnjevic, K., E. Puil, and R. Werman (1977) Gaba and glycine actions on spinal motoneurons. Can. J. Physiol. Pharm., 55(3): 658-669.

Kuypers, H.G.J.M. and Maisky, V.A. (1977) Funicular trajectories of descending brain stem pathways in cat. Brain Res., 136: 159-165.

Lundberg, A. (1980) "Half-centres revisited" In Adv. Physiol. Sci. Vol. 1. Motion and Organization Principles, Eds. J. Szentagothai, M. Palkovits, H. Hamori. Pergmon Press, Budapest, pp.155-167.

McCreas, D.A., C. Pratt, and L.M. Jordan (1980) Renshaw cell activity and recurrent effects on motoneurons during fictive locomotion. J. Neurophys., 44(3), 475-488.

Miller, S. and P.D. Scott (1977) The spinal locomotor generator. Exp. Brain Res., 30: 387-403.

Nelson, I.G. and H.D. Lux (1970) Some electrical measurements on motoneuron parameters. Biophys. Journal, 10: 55-73.

Omeniuk, D. and L.M. Jordan (1982) Locomotion induced by intrathecal drug administration in spinal cats. Soc. Neuroscience Abstract, Minneapolis.

Pearson, K. (1976) The control of walking. Scientific American, 235(6): 72-86.

Pratt, C. and L.M. Jordan (1980) Recurrent inhibition of motoneurons in decerebrate cats during controlled treadmill locomotion. J. Neurophys., 44(3): 489-500.

Rossignol, S., C. Julien and L. Gauthier (1981) Stimulus-response relationships during locomotion. Can. J. Physiol. Pharm., 59(7): 667-674.

Schwindt, P.C. (1981) "Control of Motoneuron Output by Pathways Descending from the Brain Stem" Chap. 4 in Handbook of Behavioral Neurobiology, Vol. 5, Motor Coordination, Eds., A.L. Towe and E.S. Luschei, Plenum Press; New York, pp. 139-230.

Shefchyk, S.J., R.B. Stein, and L.M. Jordan (1982) How is the monosynaptic reflex modulated during the step cycle? Soc. Neuroscience Abstracts, Minneapolis.

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

Shik, M.L., F.V. Severin, and G.N. Orlovsky (1966) Control of walking and running by means of electrical stimulation of the midbrain. Biophysics, 2: 756-765.

Steeves, J.D., L.M. Jordan and N. Lake (1975) The close proximity of catecholamine containing cells to the 'mesencephalic locomotor region' (MLR). Brain Res., 100: 663-670.

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

Steeves, J.D. B.J. Schmidt, B.J. Skovgaard, and L.M. Jordan (1980) Effect of noradrenalin and 5- hydroxydopamine depletion on locomotion in the cat. Brain Res., 186: 349-362.