On the Regulation of Repetitive Firing of Lumbar Motoneurones During Fictive Locomotion in the Cat

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ON THE REGULATION OF REPETITIVE FIRING OF LUMBAR MOTONEURONES DURING FICTIVE LOCOMOTION IN THE CAT

BY

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"Everything should be made as simple as possible, but not simpler"

- A. Einstein (1879-1955)

ABSTRACT

Repetitive firing of mammalian motoneurones has been studied since the late 1920s (eg. Adrian & Bronk, 1929). In anæsthetised animals, the afterhyperpolarizations (a.h.p.s) following the action potentials are a major determinant of the repetitive firing behaviour of motoneurones when injected with depolarizing current (eg. Granit, Kernell, & Shortess, 1963a). The experiments presented here were designed to elucidate the role of the a.h.p. in the regulation of the repetitive firing of motoneurones during movement.

Using intracellular recordings from motoneurones during fictive locomotion, it was shown that the a.h.p.s were decreased in amplitude compared to the a.h.p.s following action potentials produced by sustained depolarizing current injections. It was further demonstrated that the a.h.p.s following spikes evoked by short duration pulses of depolarizing current were reduced in amplitude and time course during fictive locomotion.

The frequency-current (f-I) relations of motoneurones were also examined. During fictive locomotion, the classical relation between the amount of current injected and the frequency of repetitive firing was usually not seen; in one cell there was a large increase in the slope of the f-I relation. It is postulated that there is therefore a

reduction in the a.h.p. conductance, and that the a.h.p. is not the sole determining factor in the regulation of the repetitive firing of motoneurones during fictive locomotion.

Although adaptation of firing rate of motoneurones and changes in firing level associated with frequency of firing have been well established in current injection studies, it was shown that these motoneurone characteristics are not present during fictive locomotion. It is also suggested that accommodation of both somatic and initial segment sodium channels can account for the depolarization of the firing level, the reduced action potential overshoot, and the increased rise time of the action potentials during fictive locomotor episodes.

It is proposed that the reduction of the a.h.p. and the resulting elimination of adaptation of firing rate permits motoneurones to fire at sustained high rates during fictive locomotion. Furthermore, it is hypothesised that the reduction of the a.h.p. conductance during fictive locomotion may contribute to an increase in the sensitivity of motoneurones to their various inputs.

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TABLE OF CONTENTS

Acknowledgements.....ii

Abstract.....iv

Introduction.....1

Repetitive firing.....1

Modification of the a.h.p. conductance.....15

Fictive locomotion.....18

Methods.....21

Preparation.....21

Data collection and analysis.....23

Sustained current injection.....24

Short pulse current-evoked action potentials.....25

Frequency-current relation.....27

Adaptation and firing level.....28

Accommodation.....29

Results.....30

Fictive locomotion.....30

Changes in inter-spike membrane potential trajectories during fictive locomotion.....33

A.h.p. changes in short pulse current-evoked action potentials.....37

Effects of membrane potential on a.h.p. amplitude modulation.....38

A.h.p. amplitude modulation within the step cycle.....39

Effects of locomotor spiking on a.h.p. amplitude modulation.....39

Changes in a.h.p. duration during fictive locomotion.....42

Delayed depolarizations during fictive locomotion.....44

Effect of locomotor activity on a.h.p. amplitude....45

Frequency-current relation.....47

Adaptation and firing level.....52

Accommodation.....56

Discussion.....60

Fictive locomotion.....60

A.h.p. trajectory changes.....64

Potassium accumulation.....69

Frequency-current relations.....70

Adaptation and firing level.....75

Accommodation.....81

Delayed depolarization.....84

Evidence that the decrease in a.h.p. amplitude is due to a decrease in a.h.p. conductance....86

Possible mechanisms of a.h.p. reduction.....88

Production of action potentials.....91

Motoneurone output.....96

Figures.....100

References.....135

INTRODUCTION

Repetitive firing

As early as 1926, Adrian & Zotterman (1926) noted that with a sustained increased load on a muscle, the rate of firing of the sensory nerve fibres of that muscle would increase. They explained this as being due to the "... natural consequence of the refractory period.... " of the neurone, and the "... return of excitability in the end-organ." In 1929, Adrian & Bronk (1929) found that in the spinal flexion reflex, "... each volley of afferent impulses seems to be directly transmitted to the motor neurones...." They found this to be in contrast to the crossed extension reflex elicited by sustained stimulation of sensory nerves, where the motor fibres would fire with a rhythm dependent on the strength of the stimulus, but independent of the frequency of stimulation (Adrian & Bronk, 1929). In this latter case, they suggested that "... the cause of the discharge may well be a more lasting excitatory state produced in the synaptic regions as a result of the passage of each impulse...." (Adrian & Bronk, 1929). In the same year, Denny-Brown (1929) also showed that motoneurones discharge repetitively to sustained asynchronous synaptic bombardment. Eccles & Hoff (1932) suggested that repetitive firing is produced when motoneurones are presented with constant excitatory bombardment from interneurones, which "... activates the rhythmic centre of

the motoneurone... The more intense the bombardment, the faster is the rhythm of this discharge...." They postulated that the "central excitatory state" of the motoneurone was the parameter of significance in controlling the firing rate. Following each action potential, this central excitatory state decreased, and would slowly build up again until the motoneurone had enough excitation to fire a succeeding action potential. If an action potential was elicited antidromically prior to this naturally occurring action potential, then the central excitatory state would decrease by an amount equal to the normal decrease following an action potential, but from its already low state. This would cause the succeeding action potential to be delayed from the time when it would normally occur. As it turns out, Eccles & Hoff (1932) had in fact postulated a mechanism similar to that of linear summation of the action potentials' afterhyperpolarization (a.h.p.) conductances two decades prior to the advent of intracellular recording from mammalian motoneurones (see Ito & Oshima, 1962; Baldissera & Gustafsson, 1971b)!

Subsequent to the studies of Eccles & Hoff (1932), Barron & Matthews (1938) found that in response to electrical stimulation of peripheral nerves, there were slow electrotonic potentials which preceded the repetitive discharges recorded in ventral roots. From this they concluded that steady depolarizations are built up in motoneu-

rones by the activity of primary afferent terminals or interneurones to produce repetitive discharges. They also noted that the frequency of discharge of a ventral root was related to both the amplitude of the potential recorded from that ventral root, and the rate of rise of this potential. They thus concluded that these slow electrotonic potentials which they recorded were indices of the central excitatory states of the motoneurones. Barron & Matthews (1938) also showed that sustained stimulation of the surface of the lateral funiculus of the spinal cord with a cathode would induce motoneurones to fire repetitively. They demonstrated that the frequencies of the motoneurone discharges were related to the intensity of the spinal cord stimulation. In addition, they showed that when a motoneurone was induced to fire repetitively by stimulation of the spinal cord in this manner, the frequency of its discharge could be increased by simultaneous stimulation of afferent pathways. Conversely, direct current of the opposite sign was shown to decrease the ventral root discharge produced by afferent stimulation. As will be seen below, these latter findings qualitatively parallel situations in which excitatory and inhibitory synaptic currents respectively add to and subtract from intracellularly injected current to alter the output of motoneurones (Granit, Kernell, & Lamarre, 1966; Kernell, 1965a; Schwindt & Calvin, 1973a; Shapovalov, 1972; Shapovalov, Kurchavyi, & Strogonova, 1966).

In the next decade, Kugelberg & Skoglund (1946) studied the repetitive activity in motor units in man with the discharges evoked by either electrical stimulation of peripheral nerves or by voluntary activation. The electrical stimulation was either with constant or linearly rising current. These investigators found that the response of motor units during voluntary contraction was very similar to that found with constant current stimulation. They concluded that this similarity corroborated the theory of Barron & Matthews (1938) that natural activation of motoneurones resulted in depolarization of the neurones and repetitive firing. They thus supported the view that "... it is the inherent autorhythmic properties of the neuron which determine the frequency of the voluntary discharges" (Kugelberg & Skoglund, 1946). That is to say that they felt that inherent properties of motoneurones determined the repetitive firing behaviour seen.

The advent of microelectrode techniques for intracellular recording from mammalian neurones (Brock, Coombs, & Eccles, 1952) led to the study of membrane events responsible for action potentials and their subsequent post-spike potentials. Shortly after the introduction of these techniques, it was shown that the previously noted positive after-potential seen when recording extracellularly from motoneurones was indeed a hyperpolarizing after-potential, or afterhyperpolarization (a.h.p.), when recording intra-

cellularly (Brock, Coombs, & Eccles, 1952; Coombs, Eccles, & Fatt, 1955). Coombs et al (1955) further demonstrated that this a.h.p. resulted from the net outward movement of potassium ions across the motoneuronal membrane. It was later shown that this extrusion of potassium ions was dependent upon the intracellular calcium activity, and the concept of a calcium-dependent potassium conductance was born (frog: Barrett & Barrett, 1976; cat: Krnjević, Puil, & Werman, 1978). The increase in calcium activity was suggested to be due to the influx of calcium during the action potential (Barrett & Barrett, 1976; Krnjević et al, 1978).

Coombs, Eccles, & Fatt (1955) showed that two distinct post-spike hyperpolarizing potentials could be seen depending on the presence or absence of somatodendritic action potentials. In the absence of a somatodendritic spike there was a brief (about 5 ms) post-spike hyperpolarization, while in its presence, they found a true long-lasting hyperpolarization (about 100 ms) which they termed the hyperpolarizing after-potential. They implied that the repolarization of action potentials and the subsequent hyperpolarizing after-potential resulted from two distinct processes, both involving increases in potassium conductance. Kolmodin & Skoglund (1958) also differentiated between two phases of spike repolarization: an initial rapid repolarization which remained very constant regardless of the way in which the action potential was ini-

tiated; and a second, later and longer lasting phase which was more variable. Barrett & Barrett (1976) later demonstrated in the frog that the post-spike hyperpolarization can indeed be separated into two components, the early repolarization component followed by the later a.h.p. component. Each of these components was attributed to a distinct potassium conductance. It was shown by Nelson & Burke (1967) that the early component was remarkably constant, independent of the pre-spike membrane potential. This was taken to indicate that following each action potential was a high state of membrane conductance which then rapidly shut off (Nelson & Burke, 1967). This early component has been further shown to be distinct from the a.h.p. in that it is independent of intracellular calcium concentration and likely reflects the opening of the voltage-dependent delayed rectifier potassium channels (Krnjević et al, 1978). However it should be noted that a recent study has indicated that there may indeed be a fast acting calcium-dependent potassium current which contributes to the repolarization of action potentials in hippocampal neurones (Storm, 1987).

The ability to record intracellularly from mammalian neurones also enabled further study of the repetitive discharge of motoneurones. Some early studies consisted of recording from motoneurones during asynchronous synaptic bombardment. The detailed analysis of Kolmodin & Skoglund

(1958) demonstrated that changes in sensory input (limb position in their case) resulted in changes in the firing frequencies of motoneurones. They further found that, in response to this asynchronous synaptic excitation, the membrane potential at which action potentials are initiated, or the firing level, remained very constant for any given motoneurone firing at any given rate. However, when the firing rate was increased by increasing the intensity of the synaptic bombardment, the firing level became more depolarized, approximating a linear relation between the firing level and the impulse interval. In addition, Kolmodin & Skoglund (1958) showed that although the immediate post-spike period remained very constant throughout repetitive firing, the second, slower phase showed greater variation, including a "... pronounced reduction with increasing frequency of the rhythmical activity...." This second component was, of course, the afterhyperpolarization.

The introduction of intracellular recording techniques also afforded the opportunity to stimulate neurones by the injection of current through the microelectrodes. The use of intracellular stimulation rapidly became an alternative approach to the study of repetitive firing in motoneurones. This approach has been used by many investigators to determine the intrinsic factors responsible for regulating the autonomous repetitive discharge of alpha-motoneurones (eg. Baldissera & Gustafsson, 1971b; Calvin & Schwindt, 1972;

Granit, Kernell, & Shortess, 1963a; Granit, Kernell, & Smith, 1963). It has not been determined to what extent the mechanisms elucidated by these studies are used by repetitively firing motoneurones during the behaviour of an animal. Hence little is known about how the nervous system regulates the firing of these cells, be it through their intrinsic properties or rather through the extrinsic organisation of the nervous system, such that motor output is appropriate for the organism to best perform the necessary motor tasks.

In the extensive studies that have been done to assess the repetitive firing behaviour of mammalian alpha-motoneurones during controlled intracellular current injection (Baldissera & Gustafsson, 1971b; Calvin & Schwindt, 1972; Granit, Kernell, & Shortess, 1963a; Granit, Kernell & Smith, 1963), characteristic properties have been observed. Motoneurones will begin to fire at high rates, and then rapidly adapt to slower, steady-state rates (Baldissera & Gustafsson, 1974; Granit, Kernell, & Shortess, 1963a; Kernell, 1965b, 1972). This decrease in firing rate in response to a sustained stimulus is called "adaptation." The interspike interval is thought to be governed by the time course of the conductance of the afterhyperpolarization following each action potential (Baldissera & Gustafsson, 1971b). As this conductance decays, the cell will again depolarize and another action potential will be ini-

tiated (Granit, Kernell, & Shortess, 1963a). With striking similarity to the proposal by Eccles & Hoff (1932), it has been proposed that the a.h.p. conductance of the second action potential summates with any a.h.p. conductance remaining from the first interval thus producing a longer duration second interval (Baldissera & Gustafsson, 1971b; Ito & Oshima, 1962). The summation of these a.h.p. conductances from consecutive spikes is thought to lead to the subsequent decrease in the firing rate; the saturation of this conductance would lead to the leveling off of the firing rate to a steady state (Baldissera & Gustafsson, 1974).

This pattern of firing of motoneurones was found to be similar to the optimal pattern of firing needed to activate skeletal muscle fibres. Stein & Parmiggiani (1979) found that a short first interval (to activate the contractile mechanism of the muscle) followed by a longer second interval (to prevent the tension from transiently exceeding its optimal level) and then intermediate subsequent intervals (to maintain an optimal steady state tension) provided the most effective stimulation pattern to produce a rapid rise of tension to a steady state in the soleus muscle. Combining these results with the found importance of the a.h.p. in regulating the repetitive firing of motoneurones in response to current injection, Stein & Parmiggiani (1979) suggested that motoneurones were "... well designed by evolution and developmental factors to generate optimal pat-

terns for activating the muscles they innervate." The extent to which the nervous system relies on the intrinsic properties of the motoneurones to activate skeletal muscle remains to be demonstrated.

Another property of spinal motoneurones seen in current injection studies is the relationship between the amount of current (I) injected and the frequency (f) at which a motoneurone fires (the f-I relation: see Granit, Kernell, & Shortess, 1963a). Generally, in the pentobarbitone-anæsthetized cats in which most of this work has been carried out, this frequency-current relationship consists usually of two phases. The first phase is a low slope relation from the lowest attainable rate of repetitive firing up to a certain frequency (mean 52 impulses sec^{-1} (30 - 74); Kernell, 1965c). This phase is known as the "primary range" of firing (average slope 1.9 impulses sec^{-1} nA^{-1} (0.4 - 4.5); Kernell, 1965b). The primary range is usually followed by a sudden change to a higher slope relation, called the "secondary range" (average slope 4.6 impulses sec^{-1} nA^{-1} (4.0 - 7.5); Kernell, 1965c). Sometimes, there is a third linear region known as the "tertiary range" which may be of either higher or lower slope than the primary and secondary ranges (Schwindt, 1973). When the instantaneous firing frequency for the first inter-spike interval produced in response to current injection is plotted versus the amount of current injection, the

same relations are found as in steady state firing, yet the slopes of the primary and secondary range are steeper than those seen in steady state firing (Kernell, 1965c). The transition frequency from primary to secondary range firing, however, remains constant amongst the different intervals studied in any given cell (Kernell, 1965c).

There are reportedly characteristic changes in the a.h.p. trajectories seen with changes in firing frequency. Increases in firing rate within the primary range are said to be accompanied by earlier and smaller peak amplitudes of the a.h.p.s, with the trajectory of the a.h.p. decay phase remaining fairly constant (Schwindt & Calvin, 1972). Secondary range trajectories differ from those in the primary range in that the early portion of the a.h.p. is said to become convex-upward (Schwindt, 1973). Here too, the last portion of the trajectory of the decay remains stereotyped and can be superimposed on those seen in the primary range (Schwindt, 1973).

It is interesting to note that secondary range firing is not always seen. Granit, Kernell, & Lamarre (1966) did not find secondary range firing in motoneurones in unanæsthetized decerebrate cats. Also, a difference has been reported in the f-I relations between anæmically decorticated versus spinalized cats (both anæsthetized with pentobarbitone; Baldissera & Gustafsson, 1971a). These investigators found that in steady state firing, the

motoneurones of spinalized cats did not have secondary ranges of firing, whereas those of anæmically decorticated cats did. It was suggested that in spinalized cats the time constant of decay of the a.h.p. conductance is decreased, or the magnitude of this conductance is increased. Baldissera & Gustafsson (1971a) then raised the possibility that there is a descending action in intact or anæmically decorticated cats which acts to decrease the a.h.p. conductance.

Few studies have been done to determine if synaptic excitation regulates motoneuronal repetitive firing in the same way as does depolarizing current injection (Granit, Kernell, & Lamarre, 1966; Kernell, 1965a; Schwindt & Calvin, 1973a; Shapovalov, 1972; Shapovalov, Kurchavyi, & Strogonova, 1966). In one report, Schwindt & Calvin (1973a) determined that tonic excitation from stimulation of either segmental afferents or the red nucleus, as quantified by motoneurone firing rate, seemed to add algebraically with the injected current. That is, the f-I relation was shifted to the left with excitatory synaptic input, and to the right with inhibitory synaptic input, with no change in the slopes of either the primary or secondary range relations (Schwindt & Calvin, 1973a). From the amount of this shift they directly determined the amount of current provided by the synaptic activity. Further, these investigators reported that the a.h.p. trajectories at any given

firing rate produced by any combination of synaptic and injected current remained identical (Schwindt & Calvin, 1973a). They interpreted this as meaning that the mechanisms of action of the two forms of excitation are similar, and that "... only the net driving current.... " is important to produce repetitive firing in motoneurones. This was in partial agreement with the results of Shapovalov et al (1966), who showed that depolarizing synaptic current produced in lumbar motoneurones by stimulation of the vestibular nerve is additive to injected current, while hyperpolarizing synaptic current is not. On the other hand, Shapovalov (1972) demonstrated that synaptic current in motoneurones receiving monosynaptic excitation from stimulation of reticulospinal cells was not additive to the current injected through microelectrodes. Also, in one study, Granit, Kernell, & Lamarre (1966) found that segmental excitation and inhibition were additive to injected current; however in an earlier study, Kernell (1965a) found that with synaptic excitation from tonic stimulation of either the ipsilateral brain stem in the region of the red nucleus or a peripheral hindlimb nerve, effects were not necessarily additive to the injected current. This indicates that in some cases there is involvement of a mechanism(s) for the regulation of repetitive firing by synaptic activation which is distinct from that revealed by current injection. Clearly there are situations in which the excitatory synaptic current may act upon the

motoneurones in the same way as does injected current. However, there are situations in which there must be a difference between the mechanisms of action of the two sources in the production of repetitive firing in motoneurones. The nature of this mechanism(s) is yet to be determined, but one might suspect that an alteration in the a.h.p. conductance could be involved.

These studies demonstrating similarities between synaptic and injected current, along with the study of the effects of small membrane potential fluctuations (synaptic noise) on repetitive firing led Schwindt (1973) to postulate that the functional use of the primary range is to maintain "... a regular, rhythmic discharge in the face of potentially perturbing influences such as synaptic noise and accommodation." He also postulated that the secondary range "... occurs only transiently under sustained synaptic input," and perhaps has "... little functional significance" (Schwindt, 1973). However, it should be emphasised that the study of repetitive firing and the role of the a.h.p. in regulating this firing has not been determined in motoneurones with more natural forms of activation such as that present during a behaviour. Therefore, the predictive value of results obtained in current injection studies for determining the activity of neurones during behaviour remains to be seen.

Before continuing with this discussion of repetitive

firing, however, it is first necessary to digress and discuss the extent to which the a.h.p. conductance can be modified by neuroactive substances.

Modification of the a.h.p. conductance

It has been shown that the a.h.p. in many types of neurones can be modified by such substances as histamine (Haas & Konnerth, 1983), noradrenaline (Haas & Konnerth, 1983; Haas & Rose, 1987; Madison & Nicoll, 1982; Yoshimura, Polosa, & Nishi, 1986), dopamine (Benardo & Prince, 1982), corticotropin releasing factor (Aldenhoff, Gruol, Rivier, Vale, & Siggins, 1983), bradykinin (Weinreich, 1986), serotonin (Holz, Shefner, & Anderson, 1986; van Dongen, Grillner, & Hökfelt, 1986), neuroleptics (Dinan, Crunelli, & Kelly, 1987), and muscarinic agonists (North & Tokimasa, 1983). Also, Grafe, Mayer, & Wood (1980) have demonstrated that the a.h.p. conductance in guinea pig myenteric neurones can be decreased by synaptic activation on stimulation of interganglionic connectives. The mechanisms of these modifications to the a.h.p. may differ with the different cell types and different substances involved. In rat hippocampal CA1 pyramidal cells, for instance, application of noradrenaline directly affects the calcium-dependent potassium channels and greatly reduces the a.h.p. (Madison & Nicoll, 1986a). On the other hand, it has been shown that in dorsal root ganglion cells of the bullfrog, serotonin decreases calcium influx during the action

potential, thus effecting a reduced activation of the calcium-dependent potassium conductance (Holz et al, 1986). A third situation is thought to occur in guinea pig myenteric neurones, where activation of muscarinic acetylcholine receptors is thought to decrease the availability of calcium ions from the intracellular membrane binding site regulating the calcium-dependent potassium channel (North & Tokimasa, 1983).

When comparing effects of neuroactive substances on the a.h.p.s in different cell types, it must be kept in mind that even though certain neurochemicals may effect a similar biochemical pathway in the different cell types, the final result may be different. An example of this is seen by the effects produced by intracellular injections of either phorbol esters or protein kinase C into either hippocampal cells or motoneurones. In the former, the a.h.p. is reduced in amplitude (Baraban, Snyder, & Alger, 1985), while in the latter it is increased (Zhang & Krnjević, 1987a).

Perhaps the best studied of these situations is that of the action of noradrenaline on hippocampal pyramidal cells (Madison & Nicoll, 1982, 1986a,b). These investigators have determined that noradrenaline blocks the adaptation seen in pyramidal cell discharge (Madison & Nicoll, 1982). In these cells, noradrenaline reduces neither the size of the calcium action potential in the presence of

tetrodotoxin and tetraethylammonium, nor the amplitude of calcium current recorded under voltage clamp (Madison & Nicoll, 1986a). Furthermore, Madison & Nicoll (1986b) showed that increased activity of intracellular cyclic adenosine 3',5'-monophosphate (cyclic AMP) mimics the action of extracellularly applied noradrenaline, and concluded that noradrenaline acts at beta-receptors to increase the intracellular concentration of cyclic AMP. They further concluded, in accordance with others (eg. Woodward, Moises, Waterhouse, Hoffer, & Freedman, 1979) that the actions of noradrenaline are to increase the signal-to-noise ratio of the cell, making it more responsive to excitatory stimuli by the blockade of the a.h.p. conductance, and less responsive to small stimuli by the slight tendency to hyperpolarize the cells. Similar postulates have been forwarded regarding the functional significance of other substances affecting the a.h.p.

As there are many different substances intrinsic to the central nervous system which can alter the a.h.p.s in many different cell populations, there is no reason to believe that such substances cannot physiologically act on the motoneurone membrane. Kernell (1966) suggested that under different states of synaptic activation, the a.h.p. in motoneurones could have different durations and thus respond differently to sustained synaptic inputs. In fact, as mentioned above, Baldissera & Gustafsson (1971a) have

found differences in the repetitive firing behaviour of alpha-motoneurones in acute spinalized cats versus anæmically decorticated cats. They suggested that descending pathways may act to decrease the magnitude of the spike-evoked a.h.p. conductance.

Fictive locomotion

One means by which one may examine the firing behaviour of motoneurones during more natural activation is in the precollicular-postmammillary decerebrate cat induced to walk by stimulation of the mesencephalic locomotor region (see Shik, Severin, & Orlovsky, 1966). When the neuromuscular junctions of these cats are blocked, their peripheral nerves exhibit rhythmic activity similar to that observed during treadmill locomotion (Jordan, Pratt, & Menzies, 1979). This pattern of neural activity which occurs in the absence of muscular contractions and thus also without phasic afferent input is termed fictive locomotion (see Perret, 1983). Using this controlled locomotion preparation, one can record intracellularly from lumbar motoneurones, which reveal rhythmic alternating periods of relative depolarization and hyperpolarization of the membrane potential known as locomotor drive potentials (Jordan, 1983). These locomotor drive potentials have been shown to be caused by alternating excitatory and inhibitory synaptic inputs (see Jordan, 1983). This pattern is thought to derive from input from interneurones of the

spinal cord circuitry responsible for the rhythmic alternating output of flexion and extension. When a motoneurone repetitively fires during fictive locomotion, this firing occurs during the depolarized phases of the locomotor drive potentials. If the intrinsic mechanisms of the cell determine the pattern of firing during fictive locomotion, then one would expect the synaptic current producing this depolarized phase to supply the motoneurone with a "net driving current" (see Schwindt & Calvin, 1973a) that acts on the cell in an identical manner to current injected through the microelectrode. Under these circumstances, the repetitive firing behaviour should be the same as that observed during controlled current injection. This is the view presented by Zajac & Young (1980) from their study of firing patterns of ventral root filaments during locomotion induced by stimulation of the mesencephalic locomotor region. They found that the discharge patterns in these filaments were similar to those of motoneurones subjected to intracellularly injected depolarizing current. Thus the firing pattern, they reasoned, is "... controlled by the mechanism that produces afterhyperpolarization.... " (Zajac & Young, 1980). However, in order to determine the role of the a.h.p. in governing the pattern of firing observed in motoneurones during locomotion, it is necessary to record intracellularly from these cells to examine the events responsible for the production of repetitive firing.

The following experiments were performed to investigate this role of the a.h.p. and to elucidate other factors which may contribute to the regulation of the repetitive firing of alpha-motoneurones during fictive locomotion induced by stimulation of the mesencephalic locomotor region. Preliminary data have been presented (Brownstone, Jordan, Kriellaars, & Noga, 1987; Brownstone, Jordan, Kriellaars, Noga, & Shefchyk, 1986; Jordan & Shefchyk, 1984), and have shown that the a.h.p. is reduced during fictive locomotion.

METHODS

Preparation

The data were obtained from motoneurones of cats of either sex weighing between 2.0 and 4.0 kg. Animals were anæsthetised with an oxygenated mixture of nitrous oxide and halothane, their blood pressures monitored through common carotid cannulae, at least one vein cannulated in each animal for delivery of fluids and drugs intravenously, and the tracheas intubated. Most animals were given intravenous injections of dexamethasone (4 mg, Hexadrol phosphate, Organon) to decrease brain stem swelling otherwise often seen following decerebration. Muscle branches of the sciatic nerves were dissected free bilaterally, so that they could be mounted on bipolar electrodes used for either stimulating or recording. These nerve branches included anterior biceps, posterior biceps, semimembranosus, semitendinosus, medial gastrocnemius, lateral gastrocnemius - soleus, flexor digitorum longus, and tibialis anterior. In addition, branches of the femoral nerves were unilaterally dissected free, cut, and bipolar nerve cuffs placed around them for stimulating or recording. These nerves included branches to the three vasti and all branches to sartorius. The contralateral femoral nerve was cut to assure symmetric tonic afferent input to the spinal cord; this was found to enhance the efficacy of stimulation of the mesencephalic locomotor region in producing fictive

locomotion. Following removal of the fourth to seventh lumbar vertebral laminae, the cats were suspended in a stereotaxic headframe with their limbs pendant. Back pools were formed, filled with mineral oil, and maintained near 38 degrees Celsius by a heating lamp controlled with a feedback circuit. The sciatic nerves were extended horizontally and placed in specially designed plastic trays filled with mineral oil. The animals were then decerebrated at a precollicular-postmammillary level and the anæsthesia discontinued. Following a one hour recovery period, animals were paralysed with intravenous injections of gallamine triethiodide (10 - 15 mg Flaxedil; Rhone-Poulenc), and artificially ventilated, maintaining the end tidal CO_2 between 2.5 and 5.0%. Periodic injections of Flaxedil were used throughout the experiments to keep the animals in states of flaccid paralysis. Dextran was administered intravenously to maintain plasma volume. If the animals became hypotensive during the experiments, noradrenaline was infused intravenously at a rate titrated to maintain a reasonable mean arterial pressure.

Locomotion was initiated by stimulation (50 - 200 uA, 0.5 - 1.0 ms rectangular pulses, 10 - 20 Hz) of the mesencephalic locomotor region (Shik et al, 1966) with an insulated monopolar stimulating electrode as previously described (Jordan, Pratt, & Menzies, 1979). The rhythmic activities of two or more peripheral nerves plus in some

cases a seventh lumbar segment ventral root filament were used as monitors of fictive locomotion.

Data collection and analysis

Intracellular recordings from single-barrelled glass micropipette microelectrodes filled with 2 M potassium citrate (resistance less than 10 Mohms, tip diameter less than 2 um) were obtained from lumbar alpha-motoneurones. In most cases, the motoneurones were identified by antidromic action potentials elicited by stimulation of peripheral nerves. When this was not possible, attempts were made to identify the motoneurones by their synaptology (Eccles, Eccles, & Lundberg, 1957), and/or their period of activity within the step cycle. High gain a.c.-coupled and lower gain d.c.-coupled intracellular signals were recorded along with the cord dorsum potential, ventral root filament activity, electroneurogram (e.n.g.) activity, and a timing pulse, on an eight-track frequency-modulated magnetic tape recorder (bandwidth 0 to 2250 or 4500 Hz) for later analysis, and/or collected directly through a 1 MHz sixteen channel analogue-to-digital converter (Masscomp AD12F) and stored on computer (Masscomp MC563) disk. In order to capture the action potentials with adequate resolution, the d.c. trace was digitized through the analogue-to-digital converter at 10 kHz. All analyses of membrane potential trajectories were performed on d.c.-coupled waveforms. E.n.g.s and ventral root filament recordings were passed

through first an analogue rectifier and then a low pass filter; these signals were then digitized with a sampling rate of 200 Hz.

Sustained current injection

Sustained depolarizing currents (2 - 50 nA) were injected through the microelectrodes into motoneurones to induce repetitive firing to compare with the repetitive firing seen during fictive locomotion. In motoneurones which fire during fictive locomotion, the spiking occurs during the depolarized phase of the locomotor drive potential. Some action potentials were elicited directly from the short-latency excitatory post-synaptic potentials produced by the stimulation of the mesencephalic locomotor region (see Shefchyk & Jordan, 1985a), while others were not directly related in time to the stimulus. These latter spikes which are not directly evoked by the brainstem stimuli will be referred to as "locomotor" spikes. Only cells in which these locomotor action potentials predominated were considered to be repetitively firing. Digitized action potentials were averaged to facilitate comparing the inter-spike trajectories seen during sustained current injection with those seen during fictive locomotion. Due to the fact that there are no identifiable baselines during repetitive firing in either fictive locomotion or current injection, accurate quantification of a.h.p. amplitude and duration differences was impossible. The

a.h.p.s were visually compared using the firing level of the action potential as a guideline (see below).

Short pulse current-evoked action potentials

Short pulses (0.5 ms) of depolarizing current were injected into 10 motoneurones to elicit action potentials every 100 to 200 milliseconds throughout the step cycle. The intensity of the injected current was just above the threshold for eliciting action potentials, and varied from cell to cell. By eliciting spikes throughout the step cycle, it became possible to measure changes in the a.h.p.s that may be related to the phase of the step cycle in which the spike occurred. Only action potentials elicited more than five milliseconds after a locomotor spike and preceding the next locomotor spike by a time sufficient to allow the visualisation of the peak amplitude of the a.h.p. were included in subsequent analyses. The action potentials could be sorted and averaged by two different schemes in order to determine patterns in the observed changes in a.h.p.s. The first scheme involved the determination of the membrane potential prior to the onset of each action potential. The action potentials were then sorted into equally divided bins depending on this value (see Shefchyk & Jordan, 1985a). The action potentials within each bin were averaged, and comparisons made between the a.h.p.s in the depolarized and hyperpolarized phases of the locomotor drive potentials. The second scheme involved dividing each

step cycle into an equal number of time intervals. The action potentials elicited during each interval of every step cycle were then averaged. By using both these schemes, it was possible to determine whether changes in the a.h.p. were related to changes in the membrane potential, or whether changes were associated more with the timing of the spiking within the step cycle. Note that for the first type of analysis, the phases of the locomotor drive potentials will be referred to as depolarized and hyperpolarized phases, while for the the latter type, they will be called active and inactive phases.

Several measurements were made in order to characterise the a.h.p.s following action potentials elicited by short pulse current injection (Figure 1). All a.h.p. measurements were made on d.c. recorded waveforms. For the a.h.p.s following the short pulse current evoked spikes, the amplitude was determined in the standard method by measuring the amount of deflection below the prespike membrane potential (Figure 1, point A to point B; see Coombs, Eccles, & Fatt, 1955). Determination of the a.h.p. duration did not follow the standard method because the end of an a.h.p. trajectory was often impossible to determine (Figure 1, point D). Instead, the time when the membrane potential returned half way to the pre-spike potential was determined (Figure 1, point C) and measured relative to the onset of the spike. This measurement was termed the time

to half decay of the a.h.p. Also, the half-width of the a.h.p. was defined as the duration the membrane potential remained more hyperpolarized than the voltage at the time of half decay. Changes in a.h.p. half-width in most cases paralleled changes in the time to half decay of the a.h.p., as would be expected if there were no significant changes in either the action potential duration or its delayed depolarization. As occasionally a hump-type delayed depolarization (see Kernell, 1964) would decrease the half-width measurement substantially, the time to half decay was used as a more reliable indicator of a.h.p. duration. Measurements of a.h.p. time courses were only possible for the short pulse current-evoked spikes (see above).

Frequency-current relation

By injecting various amounts of sustained depolarizing current, the frequency-current (f-I) relation of motoneurones could be determined in the absence of locomotor activity. This was done by plotting the mean steady-state frequency of firing against the amount of current injected into the cell (Granit, Kernell, & Shortess, 1963a). In order to compare the effects of the synaptic current present during locomotion with current injected through the microelectrodes, this input-output relation of the cell was then tested during fictive locomotion. That is, various currents (up to 24 nA) were injected into the motoneurone, sustained throughout several step cycles, and the firing

rates measured. The maximum current injected was determined by the appearance of a large degree of accommodation of the motoneurone, as judged by the action potential amplitude. This f-I relation during fictive locomotion could then be compared with that obtained at rest.

Adaptation and firing level

In order to assess the presence of adaptation during fictive locomotion, the frequency of repetitive firing was plotted versus the time in the step cycle, with the start of either the depolarized phase or the repetitive firing signaling the start of the step cycle. To assess the firing level of action potentials in a motoneurone, the intracellular signal was digitally differentiated. This differentiated signal was used as a trigger in order to discriminate the action potentials based on their rate of rise. The firing level was defined as the membrane potential at which this differential reached ten volts per second. (A differential of ten volts per second is equivalent to a depolarization of one millivolt in one 100 microsecond sample point.) All triggered events were examined in order to assure that only action potentials were discriminated. In some cells slightly higher trigger levels were used in order to exclude triggering from events such as calibration pulses and membrane potential fluctuations with high frequency components. This level did not exceed twenty-five volts per second. If the trigger

occurred too high on the rising phases of the action potentials in any given cell, the trigger level was maintained but the firing level was chosen to be the potential of a digitized point 100 to 300 microseconds prior to the triggered point; this ensured a firing level threshold of close to ten volts per second. The firing level was then analysed in relation to both the instantaneous firing frequency and the time of action potential occurrence in the step cycle.

Accommodation

Several changes in intracellular behaviour generally accompany accommodation of a neurone, defined as the phenomenon whereby an increase in the threshold current intensity to initiate an action potential accompanies a lowering in the rate of rise of the depolarizing stimulus. These include decreased action potential overshoot, decreased rate of rise of an action potential, and a depolarization of the firing level (see Bradley & Somjen, 1961; Richter & Heyde, 1975). Motoneurones in which the action potential overshoot decreased during the locomotor episode were examined to determine if these other signs of accommodation were also present.

RESULTS

Fictive locomotion

The data were obtained from 29 motoneurones in 16 cats in which fictive locomotion was elicited by stimulation of the mesencephalic locomotor region. Locomotor activity was apparent when the activity of the peripheral nerves innervating various hindlimb muscles consisted of bilateral alternating periods of flexion and extension in a rhythmic pattern similar to that seen during overground locomotion (Figure 2).

The results presented here are from pooled data from different species of motoneurones across the different hindlimb joints and likely with different biophysical profiles (and hence of different types). Whether the results are consistent across these different motoneurone populations was not determined from these studies.

The duration of the step cycle during fictive locomotion varied considerably, ranging from 442 to 2730 msec (average: 1037 msec) per step cycle (Figure 3). Step cycle duration is given as the time from the onset of one depolarized phase to the onset of the next, averaged over the locomotor episode. In 18 of the cells, step cycle duration was less than one second. In two cells, the length of the step cycle was very long (greater than 2 seconds). If these two cells are eliminated from the average because of

the unusually slow stepping rate, the mean step cycle duration becomes 931 msec. Duration of the active phase of the step cycles was measured in two ways: either the duration of spiking or the duration of the depolarized phase (or both) was measured. Samples of the motoneurones presented here were chosen for detailed measurements. In 22 cells, the motoneurone fired repetitively for a mean of 33.1% of the step cycle (range: 8.7% to 67.8%). In 11 cells, the duration of the depolarization lasted from 32.0 to 77.2 percent of the step cycle (mean: 55.5%). In 5 cells in which both measurements were made, the depolarization lasted longer than the repetitive firing by a mean of 20.2% (range: 9.1 to 39.8%) of the step cycle.

If one examines the rates at which motoneurones discharge during fictive locomotion, it is clearly seen that motoneurones fire very rapidly, usually with a mean rate in the order of 40 to 50 impulses per second or faster. For example, in the seven motoneurones used for the frequency-current analysis in this study (see Figures 12, 13, 14), the average rate of firing during fictive locomotion was 45 impulses sec^{-1}. Six of these 7 motoneurones discharged with average instantaneous frequencies between 41 and 57 impulses sec^{-1}; the seventh discharged at 22 impulses sec^{-1}. Conversely, in response to large amounts of sustained depolarizing current injection (28 to 50 nA), these motoneurones discharged at average maximum rates of

21 to 40 impulses sec^{-1} (mean: 30 impulses sec^{-1}). Even with these high amplitude current injections, the 6 of 7 motoneurones would not fire at the rates seen in fictive locomotion; i.e. the f-I curve would either fall off or reach a plateau at firing rates lower than those seen during fictive locomotion. Considering the high frequencies at which motoneurones fire during fictive locomotion, one might conclude that either: (a) if the synaptic current were to create repetitive firing in a manner similar to current injected through a microelectrode, its magnitude would have to be very large; or (b) the factors controlling repetitive firing during fictive locomotion would be different than those controlling repetitive firing during sustained current injection. In 2 of these 7 cells, sustained hyperpolarizing current was injected during fictive locomotion. In both of these, as well as many other motoneurones (unpublished observations; Figure 4), very small amounts of hyperpolarizing current (less than 6 nA) would abolish the repetitive firing in motoneurones during fictive locomotion (Figures 12, 13). It thus seems unlikely that the synaptic current is of large magnitude; the second possibility above is thus favoured.

Even the time course of the synaptic currents underlying the locomotor drive potentials in fictive locomotion is difficult to estimate because of the combination of inhibitory and excitatory currents (Shefchyk & Jordan, 1985a),

and the likely overlap of these currents. Because of the unknown and not quantified nature of the synaptic current, no attempt was made to emulate this current with current injected through the microelectrode. Rather, square wave injection of sustained depolarizing current was used to initiate repetitive firing in the absence of fictive locomotion.

Changes in inter-spike membrane potential trajectories during fictive locomotion

The inter-spike membrane potential trajectories during fictive locomotion were compared to those seen in sustained depolarizing current injection in 17 cells. During fictive locomotion, there was no apparent relationship between any given interspike trajectory and the associated interspike interval. The intracellular record from one of these cells is shown in Figure 5. Figure 5A illustrates the repetitive firing of this motoneurone during fictive locomotion. During 35 nA of repeated sustained depolarizing current injections, the same cell fired as shown in Figure 5B. A segment of repetitive firing during fictive locomotion and one during sustained current injection are expanded in time and amplitude in the lower part of the figure in order to better visualise the interspike trajectories. It can be seen that these trajectories are similar throughout the period of current injection, yet quite variable during fictive locomotion. It is clear that some of the interspike

trajectories observed during fictive locomotion are very different from those observed during sustained current injection. It is also clear that during fictive locomotion similar interspike intervals often display completely different interspike trajectories (arrows). Although the inter-spike trajectories are different, it remains to be seen whether the a.h.p. current has changed, or if the a.h.p. trajectory is simply masked by excitatory synaptic current. However, it appears unlikely that the a.h.p. alone governs the interspike interval.

As in the cell shown in Figure 5, the amplitudes of action potentials during fictive locomotion were usually lower than the amplitudes of those spikes either evoked by short pulse current injection or occurring during the early period of sustained current injection. This is not secondary to a deterioration of the penetration of the cell, as these decreases during fictive locomotion were completely reversible following the locomotor episode. A possible explanation for this phenomenon would be that during fictive locomotion there is a degree of accommodation of the fast sodium channels. This issue will be discussed further below.

Differences in the a.h.p.s during fictive locomotion compared to those during sustained depolarizing current injection were observed in all of the 17 motoneurones examined. Although a.h.p. trajectories were present during the

current injections in all 17 cells (for example, Figure 6A), during fictive locomotion there was only a very small, if any, downward convexity in the post-spike period in 12 of these cells (for example, Figure 6B). In 9 of these 12 cells, there remained a post-spike hyperpolarization of very brief duration and small amplitude such as that seen in Figure 6B. In the remaining 5 of the 17 motoneurones, although a.h.p.s persisted during fictive locomotion, they were clearly smaller in amplitude than during sustained current injection.

The absolute magnitude of the reduction of the a.h.p. amplitude was impossible to assess due to the lack of an identifiable baseline from which to measure these amplitudes. Furthermore, to facilitate comparisons between post-spike trajectories during fictive locomotion and those during sustained current injection, all locomotor spikes with subsequent short inter-spike intervals were excluded from averaging. That is, only inter-spike intervals of longer duration (and therefore larger amplitude a.h.p.s - see Figure 16C) were used in these averages. Hence, the observed decreases in a.h.p. amplitudes may have been even greater than the averages indicated.

Two methods were used to aid in the comparison of the a.h.p.s during sustained current injection and fictive locomotion. In the first method, the firing level was assumed to be at a relatively constant level of membrane

potential. When the mean action potential produced during sustained current injection was overlaid with the mean action potential during fictive locomotion using this assumption, it became quite evident that the a.h.p. amplitude is greatly reduced during fictive locomotion (Figure 6C).

In the second method, the post-spike repolarization potential was assumed to be a relatively constant voltage. Potentials similar to these small amplitude, brief duration hyperpolarizations which are sometimes seen following the locomotor action potentials (Figure 6B) have been attributed to the delayed rectifier potassium conductance responsible for the falling phase of the action potential. (Barrett & Barrett, 1976; Krnjević et al, 1978). It is interesting to note that the absolute membrane potential at the end of the falling phase of the locomotor spikes remained very constant throughout the penetration of a cell (unpublished observations; also see Figure 10). This is a similar effect to that noted by Nelson & Burke (1967), who showed that this rapid repolarization voltage was very stable regardless of both the method of spike initiation and the concurrent synaptic input. They indicated that this stability reflects a high conductance state of the motoneurone soma, effectively shunting any current flow from the dendrites during the immediate post-spike period. They stated further that this conductance shuts off rapidly

(Nelson & Burke, 1967). It was because of the noted constancy of this voltage that it was thought that this potential might be similar during sustained current injection and fictive locomotion. When this post-spike repolarization potential was used to compare the a.h.p.s during sustained current injection with those during fictive locomotion, large differences in a.h.p.s were still evident (Figure 6D). Results similar to those shown in Figure 6 were seen in all 17 cells.

A.h.p. changes in short pulse current-evoked action potentials

The observed reduction in the a.h.p. amplitude could result from factors having three possible time courses: (a) it may be reduced uniformly throughout the locomotor episode; (b) it may be reduced only during the depolarized phases of the locomotor drive potentials; or (c) the reduction may be associated with the production of repetitive firing in the motoneurone, and thus only evident while the cell is spiking. In order to distinguish between these alternatives, brief (0.5 ms) depolarizing current pulses were injected into 10 cells to elicit action potentials every 100 to 200 milliseconds throughout the locomotor cycles, regardless of whether or not repetitive firing was present.

In 7 of the 10 cells, the membrane potential variations during fictive locomotion covered a range such that

some action potentials were elicited at membrane potential levels similar to resting potential. These spikes were averaged and the a.h.p.s compared to those seen following action potentials evoked at rest. In 5 of these 7 cells, the average a.h.p.s following spikes evoked near resting potential during fictive locomotion were smaller in amplitude than those following spikes evoked at rest (average decrease 37%; range 18% - 59%). In the two other cells, the mean a.h.p.s were increased in amplitude at membrane voltages similar to resting potential during fictive locomotion (31% and 119%).

Effects of membrane potential on a.h.p. amplitude modulation

In order to examine the modulation of the a.h.p.s during fictive locomotion, the evoked action potentials were sorted into six bins depending on the pre-spike membrane potential. Although the a.h.p.s were usually decreased in amplitude during fictive locomotion, they did show a progressive increase in amplitude as the membrane potential became more depolarized. This modulation of a.h.p. amplitude would be expected because the membrane potential is further from the a.h.p. reversal potential during the depolarized phase than during the hyperpolarized phase (Coombs, Eccles, & Fatt, 1955). Three of the 10 cells showed minor variations in this, with the a.h.p. amplitude decreasing in one bin as the cell depolarized, then increasing again with further depolarization.

A.h.p. amplitude modulation within the step cycle

When the current evoked spikes from these same cells were divided into bins dependent on their timing within the step cycle, rather than the prespike membrane potential, 7 out of the 8 cells in which there were an adequate number of spikes per bin to obtain an average with a low level of noise displayed a decrease in the average a.h.p. amplitude and time to half decay during at least a portion of the active phase of the step cycle. This decrease had no consistent timing within the active phase of the fictive step cycle from cell to cell. This demonstrates that the reduction in a.h.p. is modulated within each single step cycle and not uniformly reduced throughout the locomotor episode.

Effects of locomotor spiking on a.h.p. amplitude modulation

A difference was noted in the modulation of the amplitudes of the a.h.p.s following short pulse current evoked spikes between the situation where there was fictive locomotion with locomotor drive potentials but no repetitive firing and the situation where there was repetitive firing in the active phase of the fictive step cycle. This is illustrated by the example seen in Figure 7. It can be seen that when this cell was not exhibiting locomotor spiking (Figure 7A), the average a.h.p. amplitude varied with the membrane potential (Figure 7A-1). This modulation could also be seen when the action potentials were averaged

dependent on their timing in the fictive step cycle (Figure 7A-2), being largest during the active phase of the cycle, at which time the membrane was most depolarized. However, when the same cell was repetitively firing during fictive locomotion (Figure 7B), there was no obvious relation between a.h.p. amplitude and membrane polarization (Figure 7B-1). In this case, the averaged a.h.p. following short pulse current-evoked spikes in the active phase of the step cycle had the smallest amplitude (Figure 7B-2, trace #2).

To judge the significance of this modulation of amplitude, the a.h.p. amplitude and motoneurone membrane potential were both plotted versus time in the step cycle (Figure 7C, D). When the motoneurone was not repetitively firing (Figure 7C), the changes in a.h.p. amplitude paralleled the changes in membrane potential as would be expected if the a.h.p. was either not affected or uniformly depressed throughout the step cycle; that is, changes in the a.h.p. amplitude reflect a passive dependence on the membrane potential. However, when the motoneurone was repetitively firing (Figure 7D), the a.h.p. amplitudes did not follow the changes in membrane potential, but rather were very often reduced during a portion of the depolarized phase (see also Figure 9B). This variation in a.h.p. amplitude therefore can not be explained by a passive dependence on the membrane potential; it can be explained by a decrease in the a.h.p. amplitude during a portion of the active

phase of the step cycle. The fact that no obvious relationship between the a.h.p. amplitude and the membrane potential was seen when the action potentials were averaged into bins dependent on the pre-spike membrane potential (Figure 7B-1) can be explained because this type of averaging does not distinguish between events occurring at similar membrane potentials but at different times within the step cycle. (Note that some a.h.p. amplitudes measured at the very end of the active phase of the step cycle may be artefactually large because of the repolarization of the membrane following the depolarized phase of the step cycle. Also note that although the amplitude of the locomotor drive potential appears larger in Figure 7D than in Figure 7C, the presence of repetitive spiking (Figure 7D) precludes accurate determination of the amplitude of this potential. Therefore the average depolarization of the membrane potential may be exaggerated.) These data are consistent with a selective depression of the a.h.p. conductance during only a portion of the active phase of the step cycle; alternative explanations will be presented below (see Discussion).

Of the two cells that did not show a decrease in a.h.p. amplitude and duration following the current-evoked spikes, one did not repetitively fire during fictive locomotion, and the other fired only a few locomotor spikes at the beginning of each depolarized phase. In this latter

cell, when spikes were evoked in the midst of the locomotor spiking activity, the a.h.p. amplitudes and durations following these current evoked action potentials were markedly reduced compared to those following spikes evoked during non-spiking periods. This can be seen in Figure 8, where one step cycle is shown in a semimembranosus motoneurone (Figure 8A). Five locomotor spikes are evident in this segment of the locomotor episode, as are three current evoked spikes: one prior to the locomotor spikes, one in the midst of these spikes, and one following the train (arrows). These latter two spikes are shown on a larger scale in Figure 8B, where it is evident that the a.h.p. in the active phase of this cell is about one-third the amplitude and has less than one-half the time to half decay of the a.h.p. during the non-spiking phase. In this cell, the a.h.p.s following most locomotor spikes are also very small. It is interesting to note that the first locomotor spike in each step cycle in this cell exhibits a large a.h.p., while subsequent ones, other than the final spike of each cycle, do not (Figure 8C). This lends further support to the above evidence that the a.h.p. amplitude is reduced for only a portion of the active phase of the step cycle.

Changes in a.h.p. duration during fictive locomotion

The times to half decay of the a.h.p.s following these short pulse current-evoked action potentials were studied

in 8 motoneurones. In 5 of the 7 cells in which comparisons could be made at membrane potentials similar to resting potential, the time to half decay of the a.h.p. was decreased during fictive locomotion by an average of 27% (range 15 - 41%). In one cell there was no change in this parameter. In the final cell this duration was greater during locomotion than at rest (37%); in this cell, the a.h.p. amplitude was also increased. There was no consistent relation detectable between the variations in membrane potential and the time to half decay of the a.h.p. However, in 7 of the 8 cells in which these measurements could be made accurately throughout the locomotor cycle, the time to half decay decreased in at least one bin between the most hyperpolarized potential and the most depolarized potential. (In the eighth motoneurone, the time to half decay increased with depolarization; this change paralleled an increase in a.h.p. amplitude.) One example is depicted in Figure 9, which shows the current evoked spikes averaged into six bins dependent on their time of occurrence in the step cycle. The average with the smallest amplitude a.h.p. occurs during the active portion of the step cycle; the duration of the averaged a.h.p. is also shortest at this time (Figure 9A). Figure 9B illustrates the average membrane potential, the a.h.p. amplitudes, and the average a.h.p. times to half-decay. It can be seen that the a.h.p.s with the smallest amplitudes and shortest durations occur during the active phase of the

step cycle. In summary, both the amplitudes and durations of the a.h.p.s following short pulse current evoked action potentials appear to be modulated within the step cycle.

Delayed depolarizations during fictive locomotion

Of the 10 cells in which action potentials were elicited by the injection of short pulses of current throughout the step cycle, only two consistently showed "hump-type" delayed depolarizations following the evoked action potentials (see Kernell, 1964). These two cells both appeared to have larger hump amplitudes at depolarized than at hyperpolarized membrane potentials during the step cycle (Figure 10A), as would be expected with a passive dependency associated with changes in the membrane potential (Nelson & Burke, 1967). At all membrane potentials and at all times in the step cycle in these two cells the absolute membrane potential following each evoked spike and preceding its hump type delayed depolarization was remarkably constant (Figure 10A). As mentioned above with respect to the locomotor spikes, this constancy likely reflects the high conductance state of motoneurones following repolarization of their action potentials (Nelson & Burke, 1967). The remaining cells had smoothly declining delayed depolarizations. In these 8 cells, the declining portion of the spike began to slow at more depolarized levels during the depolarized phases of the step cycle, and in three of these motoneurones a hump-type delayed

depolarization is seen at the most depolarized potentials (Figure 10B).

Effect of locomotor activity on a.h.p. amplitude

Figures 5 through 9 demonstrate a reduction in the a.h.p. amplitude in most cells during fictive locomotion, either by comparing to the a.h.p.s in the absence of locomotor activity or by comparing the a.h.p.s in the different phases of the step cycle. Is it possible that this reduction is due largely to accumulation of potassium in the extracellular space and is otherwise unrelated to the production of fictive locomotion? It has been shown that potassium can accumulate in the extracellular space due to the activity of motoneurone pools and can then reduce the potassium equilibrium potential, thus causing a reduction in the a.h.p. amplitude (Richter, Camerer, & Sonnhof, 1978; Syková, 1981; Wallén, Grafe, & Grillner, 1984; Webber & Pleschka, 1981). Figure 11 illustrates that the reduction in the a.h.p. is dependent on locomotor activity in the motoneurone. A lateral gastrocnemius motoneurone is shown during fictive locomotion with simultaneous intracellular depolarizing current injection. The rhythmic oscillations of the membrane potential stopped after the first locomotor drive potential shown (Figure 11A), yet the cell continued to fire due to the sustained current injection. During this last locomotor drive potential, there was, on the average, no a.h.p. following the locomotor spikes, but only

a brief duration, small amplitude post-spike hyperpolarization (Figure 11B). However, the a.h.p. returned once the locomotor drive potentials were no longer present (Figure 11C), even though fictive locomotion persisted with no change in the rhythmic activity of the other seventh lumbar segment motoneurones represented in Figure 11A. Note that both the motoneurone and the ventral root filament are extensors from the seventh lumbar segment. Since fictive locomotion continued, it would be expected that any potassium accumulation would still be present, making it unlikely that the reduction of the a.h.p. amplitude can be accounted for by an accumulation of potassium. Further support for this is that the a.h.p. sometimes returns at the end of the depolarized phase, despite a reduction earlier in the same phase (see Figure 8C). Also, a change in the potassium equilibrium potential would be reflected in a depolarization of the post-spike repolarization potential, since this potential is likely due to the delayed rectifier potassium conductance (Barrett & Barrett, 1976; Krnjević et al, 1978). Since this membrane potential remains very constant throughout fictive locomotion (see above and Figure 10A), it is unlikely that the potassium equilibrium potential is affected. Therefore, it is very doubtful that potassium accumulation can explain the reduction in the a.h.p.

It should also be noted that although the specific

synaptic events occurring at the motoneurone membrane during fictive locomotion are not known, the synaptic events giving rise to a particular level of depolarization do not appear to account for the changes seen in the a.h.p. This has been demonstrated above (Figures 7, 9), and can also be observed in Figure 11A where the a.h.p. returns following the cessation of locomotor activity in the cell, despite the membrane potential remaining approximately the same. It therefore appears that the reductions in the a.h.p. amplitude and duration are specifically related to the presence of locomotor activity in the motoneurones.

Frequency-current relation

Although the a.h.p. trajectory is clearly depressed during fictive locomotion, the contribution of the a.h.p. conductance to the production of repetitive firing can be further established by testing the frequency-current (f-I) relationship of motoneurones. If the a.h.p. conductance is not affected during locomotion and the repetitive firing is produced by a step depolarization of the membrane voltage, then one would expect that the synaptic current would add algebraically with the injected current, and the f-I line would simply be shifted to the left during locomotion with no change in slope (Granit et al, 1966; Schwindt & Calvin, 1973a; Shapovalov et al, 1966). In order to establish if this is indeed the case, the f-I relation was tested in 7 motoneurones; a typical result is illustrated in Figure 12.

In the absence of fictive locomotion, when the steady-state mean frequency was plotted versus the current injected (Figure 12A), these cells displayed only primary ranges of firing (see Baldissera & Gustafsson, 1971a). The f-I relations had slopes averaging 0.74 impulses sec^{-1} nA^{-1} (range 0.44 - 1.3). These slopes are comparable to the lower end of the range found by Kernell (1965b) in pentobarbitone-anæsthetized cats. There was always a high correlation between the frequency of firing and the injected current (mean Pearson correlation coefficient $r = 0.78$, range 0.61 - 0.94). The minimum current needed to induce repetitive firing in a motoneurone averaged 21.7 nA, ranging from 10 to 32 nA. The f-I relationship was then tested during fictive locomotion. The amount of current injected during fictive locomotion was always less than or equal to that injected in the control condition as the synaptic currents already present and the associated depolarization limited the amount of current that could be injected without blocking the action potentials. Figure 12B (circles) illustrates that during the repetitive firing produced during fictive locomotion in the same motoneurone as that illustrated in Figure 12A, there no obvious relationship between the quantity of current injected and the frequency of firing of the motoneurone. With each step of current injected, large standard deviations of firing frequency were seen, and the Pearson correlation coefficient of this relationship was, in all cells excepting the one discussed

below (Figure 14), near zero (mean r = -0.11, range -0.35 - 0.37).

One possible explanation for this lack of correlation between frequency and current during fictive locomotion must be ruled out: that is, perhaps the f-I relation reaches a plateau at high frequencies. If this were the case, then the injection of hyperpolarizing current into the cell should reveal a f-I relation similar to that produced in the absence of fictive locomotion. However in many motoneurones, as has been shown above, very small amounts of hyperpolarizing current (less than 6 nA) will completely eliminate the repetitive firing seen in fictive locomotion (Figure 4). It can be seen that in the motoneurone shown in Figure 12, during a subsequent period of fictive locomotion, 2 nA of hyperpolarizing current completely abolished the repetitive firing (Figure 12B, diamonds). This result further demonstrates that the depolarizing synaptic currents in motoneurones during fictive locomotion are not sufficiently large to initiate repetitive firing at the rates seen by simply utilising the motoneurone repetitive firing mechanisms which have been elucidated in current injection studies.

A second example of the loss of a frequency-current relation during fictive locomotion is shown in Figure 13. In this cell, injection of 5.8 nA of sustained hyperpolarizing current eliminated repetitive firing, except for

occasional spikes in some of the step cycles. It can also be seen in Figure 13 that there is no correlation between injected current and the frequency of firing during fictive locomotion, even within a range of firing rates where such a correlation has been demonstrated with current injection alone. These results preclude the possibility that the f-I relations during fictive locomotion are simply on plateaux to the right of the f-I relations at rest.

The loss of a significant relationship between firing frequency and injected current is good evidence in support of the conclusion that the control of the interspike interval during fictive locomotion is different from the control during intracellular current injection. Taken together with the well established importance of the a.h.p. in maintaining a relationship between frequency of firing and injected current (Granit, Kernell, & Shortess, 1963a), this suggests that there is little, if any, contribution of the a.h.p. conductance to the regulation of repetitive firing in fictive locomotion.

It is interesting but not surprising that the classical relation between injected current and firing frequency cannot be demonstrated during fictive locomotion. Although the use of sustained injections of depolarizing current has elucidated several intrinsic properties of motoneurones as have been discussed, there is no reason to believe that the central nervous system will rely on these properties in the

production of repetitive firing during movements. This will be discussed further below (see Discussion).

In the one motoneurone referred to above, the Pearson correlation coefficient was not near zero during locomotion (Figure 14A). At first, although the motoneurone was receiving rhythmic input as was evident from the locomotor drive potentials with rhythmic bursts of repetitive firing, the e.n.g.s were quite silent (Figure 14B; left tibialis anterior e.n.g.). During this time, the slope of the f-I relation (0.70 impulses sec^{-1} nA^{-1}; r = 0.653; Figure 14A, line a) was similar to that observed in the absence of locomotor activity (0.78 impulses sec^{-1} nA^{-1}; r = 0.892; Figure 14A, line b). Subsequently, the quality of locomotion improved (Figure 14C; left tibialis anterior e.n.g.), the a.h.p. was reduced but not eliminated, and the slope of the f-I line increased dramatically (to 2.9 impulses sec^{-1} nA^{-1}; r = 0.738; Figure 14A, line c). Following this episode of fictive locomotion, the f-I slope was not appreciably different than the initial control (0.86 impulses sec^{-1} nA^{-1}; r = 0.848; Figure 14A, line d). This suggests that in this case as opposed to the others, the a.h.p. conductance played a role in the production of repetitive firing during fictive locomotion. However, it was clearly reduced to a fraction of its control conductance. It is stressed that the a.h.p. conductance could not simply be shunted by the increase in membrane input conductance dur-

ing fictive locomotion (Shefchyk & Jordan, 1985b), as one would have then expected no change in the f-I slope (Kernell, 1969; Schwindt & Calvin, 1973a).

Adaptation and firing level

One other property of repetitive firing in motoneurones which is demonstrated by current injection studies is that of early adaptation of firing rate (Granit, Kernell, & Shortess, 1963a). An example of this is demonstrated in the motoneurone shown in Figure 15. When repetitive firing is produced by sustained injection of rectangular depolarizing current, the firing rate is initially higher than once it reaches steady state (Figure 15A). This decrease in firing rate after a brief period of constant depolarization is thought to be due to summation of the a.h.p. conductances (Baldissera & Gustafsson, 1974; Ito & Oshima, 1962). During fictive locomotion, however, repetitive firing rarely follows this pattern. Figure 15B shows the same motoneurone as in Figure 15A but during fictive locomotion. It can clearly be seen that there is neither a consistent pattern to the firing (Figure 15B, top), nor an initial high rate of firing (Figure 15B, bottom) during fictive locomotion (see also Figure 5). The analysis of the various motoneurone firing patterns during fictive locomotion is beyond the scope of the experiments presented here. However, it is important to note that although during fictive locomotion, the pattern of firing within a step cycle

in some motoneurones is consistent with an adaptive process occurring, many other cells show firing patterns strikingly different than this (see also Figure 16C). In many cells, the first action potential in each fictive step is followed by a larger a.h.p. and longer interspike interval than subsequent spikes (see Figures 8C, 14C). In other cells there is an increase in firing rate toward the end of the depolarized phase of the locomotor drive potential. Figure 16A depicts four step cycles from an anterior biceps motoneurone which had quite variable inter-spike intervals during fictive locomotion. When the average instantaneous firing frequency for the first nine inter-spike intervals of fifteen consecutive fictive step cycles is examined, the classical pattern of adaptation (seen with injection of depolarizing current) is not seen (Figure 16B). Note that the first inter-spike interval has the widest variation of firing rates, while the subsequent intervals have much less variability.

If this seeming lack of adaptation during fictive locomotion were still consistent with the a.h.p.s being responsible for the control of repetitive firing, then one might expect to see changes in the post-spike trajectory associated with changes in the instantaneous firing frequencies. Since, as mentioned above, no secondary range firing was seen in the motoneurones reported here, characteristic changes in membrane potential trajectories seen at

different firing rates within the primary range might be expected (Schwindt & Calvin, 1972). These authors reported that as the inter-spike interval becomes shorter in duration (within the range of primary firing), the rise of the trajectory preceding the subsequent action potential maintains a constant slope, but the "scoop," or the early, most hyperpolarized portion of the a.h.p., becomes shallower and of shorter duration.

In order to examine the inter-spike trajectories during the various intervals, the action potentials were averaged into bins dependent on the succeeding inter-spike interval. Averages of intervals from five to thirty-five milliseconds are shown in Figure 16C-1 and with increased gain in Figure 16C-2. In this cell, following the post-spike small amplitude short duration hyperpolarizing potential, there is what appears to be a small a.h.p. at the longer intervals. At the shorter intervals (for example, the second and third shown), this trajectory is quite flat. Thus it can be seen in this example that the changes in a.h.p. amplitude characteristic of spikes produced by intracellular current injection are not seen during fictive locomotion. Similar results were seen in all eight motoneurones examined in this manner.

Furthermore, if motoneurone repetitive firing during fictive locomotion were similar to repetitive firing during sustained current injection through a microelectrode, then

one might expect to observe changes in the action potential firing level. That is, the firing level ought to become increasingly depolarized both the later in the spike train during which the action potential occurred (Schwindt & Crill, 1982), and the shorter the preceding inter-spike interval (Barrett, Barrett, & Crill, 1980; Schwindt & Crill, 1982). That is, the "... firing level increases drastically with firing rate ...." (Schwindt & Crill, 1982). For example, from inspection of their Figures 1 and 3, it can be seen that the firing rate increased from about 15 to 40 impulses sec^{-1} as injected current increased from 5 to 15 nA. Associated with this increase in firing rate, the firing level increased from about 8 mV above resting potential to 13 mV above resting potential. Stated otherwise, there was a 0.2 mV per impulses sec^{-1} rise in firing level in that cell. This depolarization of the firing level with increasing firing rate was also observed by Kolmodin & Skoglund (1958) in decapitate cats with afferent stimulation providing the necessary synaptic current to induce the motoneurone to fire repetitively.

Eight motoneurones were studied to determine changes in their firing level during fictive locomotion. One of these motoneurones is depicted in Figure 16. Indeed, a tendency for the firing level to become more depolarized later in the train was evident (Figure 16D), indicating that there was a degree of accommodation of the motoneurone

(see below). However, there was no relation between firing level and instantaneous firing frequency (Figure 16E). (If one were to assume that the slope calculated from the Schwindt & Crill (1982) data is typical, then a 0.2 mV per impulse sec^{-1} increase would mean a 10 mV depolarization of the firing level as the rate increases from, for example, 25 to 75 impulses sec^{-1}.) Schwindt & Crill (1982) do suggest that this relation no longer holds at the faster firing rates because the initial segment becomes completely accommodated and the action potentials are initiated in the soma. However, it can be seen that this is not the case here because at all inter-spike intervals, the break between the initial segment spike and the soma-dendritic spike can be distinguished in the averaged, differentiated action potentials (Figure 16F). This lack of relationship between firing level and instantaneous firing frequency therefore further supports the hypothesis that repetitive firing mechanisms in fictive locomotion are different than those during intracellular current injection. Similar results to those shown in Figure 16 were seen in all eight motoneurones examined.

Accommodation

As mentioned previously, there are several changes in intracellular behaviour which generally accompany accommodation of a neurone, defined as the phenomenon whereby an increase in the threshold current intensity to initiate an

action potential accompanies a lowering in the rate of rise of the depolarizing stimulus. When the firing behaviour of a motoneurone is observed from the onset of a fictive locomotor episode, it can clearly be seen that the action potential overshoot decreases as the episode continues, with most of this change occurring within the first four or five steps (Figure 17A, top). At the same time, the rates of rise of the action potentials decrease, and in some cases, the rate of the spike repolarization also decreases. This can be seen in the differentiated intracellular record shown in the lower trace of Figure 17A. This slowing of the action potentials leads to the production of longer duration action potentials. These changes were observed in all five cells that were studied from the onset of fictive locomotor episodes and are associated with neuronal accommodation (Bradley & Somjen, 1961; Richter & Heyde, 1975).

An additional change that has been reported with initial segment accommodation is a depolarization of the firing level, due to the fact that there is sodium channel inactivation at the membrane of the initial segment (Schwindt & Crill, 1982). Note, however, that Gustafsson & Pinter (1984) found no significant increase in the firing level of the second spike in a train of two. A change in firing level was witnessed within the step cycles of all five cells (see Figure 16D). In addition to this, in four of the five cells the firing level became increasingly more

depolarized with successive step cycles in parallel with the other signs of accommodation (Figure 17B). (Note that this cannot be explained by a deterioration in cell health. Firstly, there is partial recovery between successive steps, with early spikes always initiated at a more negative membrane potential than the later spikes of the preceding step. Secondly, if one can use action potential amplitude as a measure of cell health as is traditionally done, this cell was still very healthy following a prolonged penetration with a spike height of 80 mV.) This suggests that the initial segments do not fully recover from their increasing sodium channel inactivation from one step to the next. In the fifth cell, although the firing level became more depolarized within each step cycle, it remained fairly constant from the start to the end of the locomotor trial.

It can be seen that, in general, the first spike of each step cycle fires at a more hyperpolarized membrane potential than subsequent spikes (Figure 17B). In addition to being consistent with the findings of Schwindt & Crill (1982), this is also consistent with the finding that accommodation of the motoneurone membrane depends upon the rate of change in membrane potential (Schlue, Richter, Mauritz, & Nacimiento, 1974a). Since the membrane potential is depolarizing fairly rapidly at the onset of each step cycle, it is expected that the first spike would be ini-

tiated at this higher membrane potential.

It should be noted that although motoneurones show signs of accommodation during fictive locomotion, this accommodation does not affect motoneurone output. This can be seen in Figure 17C, which demonstrates that the instantaneous firing frequency remains constant even as the cell accommodates.

DISCUSSION

Fictive locomotion

Data have been presented which have demonstrated that the afterhyperpolarization in motoneurones does not regulate their repetitive firing behaviour during fictive locomotion initiated by stimulation of the mesencephalic locomotor region. The a.h.p. is reduced in magnitude and time course, the frequency-current relations of the motoneurones no longer hold, and properties of adaptation are not seen.

It is important to note that these results are from pooled data from different species of motoneurones across the different hindlimb joints and likely with different biophysical profiles (and hence of different types). In order to maximise the sample size of the data, they were not sorted into species or type. Further studies would be necessary to determine whether the results are consistent across these different motoneurone populations.

The mesencephalic cat in which fictive locomotion is induced by stimulation of the mesencephalic locomotor region provides a preparation which enables the examination of motoneurone activity during near-natural synaptic activation. Tonic stimulation of this region of the brainstem initiates locomotion by activating the spinal cord circuitry responsible for the production of locomotor

output (see Jordan, 1986 for review). This circuitry rhythmically activates the motoneurone pools to produce the motor output necessary for locomotion (Figure 2). As the motoneuronal pool output (i.e. the e.n.g. activity in the present study) is similar in fictive locomotion and overground locomotion (see Perret, 1983 for review), it is assumed that the input to the motoneurones is also similar in both cases, even though in the former there is no phasic afferent input. The degree to which fictive locomotion resembles whole cat overground locomotion is not relevant to this study; the main concern is the control of repetitive firing in motoneurones. The absolute pattern of activity may be slightly different in the normal cat receiving tonic and phasic afferent input, but the principles are expected to remain the same.

Stimulation of the mesencephalic locomotor region results in short latency excitatory post-synaptic potentials (e.p.s.p.s) in motoneurones (Shefchyk & Jordan, 1985a), which on occasion elicit action potentials. The action potentials examined in the present study were not related in time to the brain stem stimulation; that is, they were not directly related to these short latency e.p.s.p.s. Rather, they were repetitive action potentials which were independent of the externally applied stimulus.

Ideally, one would like to know the magnitude and time course of the synaptic current underlying the locomotor

drive potentials in order to compare the repetitive firing of motoneurones during fictive locomotion with that seen during current injection. To determine this current, however, it would be necessary to voltage clamp the motoneurones, a task which is fraught with difficulties. (Other methods, such as that proposed by Heckman & Binder (1988) would likely fail in the case of fictive locomotion because of the complicated nature of the synaptic current which includes both excitatory and inhibitory phases.) It is known that the membrane potential variations during fictive locomotion reflect both excitatory and inhibitory synaptic events (Shefchyk & Jordan, 1985a). If one examines the rising edges of the depolarized phases of the locomotor drive potentials, it can be seen that these, although variable, are usually of quite rapid onset and steep rise. The synaptic current underlying this rise is likely comprised of a combination of rapidly increasing excitation and decreasing inhibition. Because of the complicated nature of this synaptic current, it was necessary to assume that a reasonable estimate of this current could be made in order that comparisons could be made between repetitive firing produced by injection of this current with that seen during fictive locomotion. For this estimate, square wave depolarizing current was used. This is the type of current which has been studied most thoroughly in previous examinations of repetitive firing of motoneurones. Discussion of the injections of ramp and sinusoidal currents which have

been presented in the literature will be included below. Although not presented here, experiments have shown that within any cell the repetitive firing produced by injection of sinusoidal current did not resemble the repetitive firing seen during fictive locomotion (unpublished observations).

The rate at which motoneurones discharge during fictive locomotion was usually greater than 40 impulses sec^{-1}. These same motoneurones did not discharge at comparable frequencies during large amounts of current injection (up to 50 nA). However, if the synaptic current were of large enough magnitude to initiate repetitive firing by using the intrinsic properties of the motoneurones, then small amounts of hyperpolarizing current injection would not eliminate the repetitive firing altogether. However, small magnitude hyperpolarizing current does eliminate the repetitive firing of motoneurones during fictive locomotion (Figures 4, 12, 13). It therefore seems likely that the central nervous system is using mechanisms to regulate the repetitive firing of motoneurones during fictive locomotion which differ from the intrinsic firing mechanisms of motoneurone membranes which have been previously elucidated in current injection studies.

It is interesting to consider the experiments of Barrett & Barrett (1976). In blocking the slow calcium and potassium conductances of frog motoneurones by the addition

of manganese to the perfusate, these investigators found that they could not sustain repetitive firing by the injection of long-lasting steps of depolarizing current. The motoneurones would fire 3 to 5 high frequency spikes (never slower than 60 impulses sec^{-1}) and then stop firing. They suggested that the inability to produce a low frequency response in their preparation was due to the absence of the slow calcium dependent potassium conductance. The rate would then be limited only by the decay of the rapidly decaying fast potassium conductance responsible for the falling phase of the action potential, and the passive properties of the cell. During fictive locomotion, motoneurones sustain a high rate of firing. One might therefore postulate that during this event: (a) the reduction in the a.h.p. allows for the high rate of firing; and (b) the input to the motoneurones must differ from a sustained step depolarizing current in order to maintain the observed high firing rate.

A.h.p. trajectory changes

The results presented here demonstrate that the a.h.p.s in lumbar motoneurones are reduced in amplitude and duration during fictive locomotion. It was demonstrated that these post-spike trajectories are clearly depressed compared to those observed during repetitive firing at similar rates induced by the sustained injection of depolarizing current (Figures 5, 6).

Often, a brief duration, small amplitude post-spike hyperpolarization remains following each locomotor action potential (eg. Figures 6, 11, 16). The conductance responsible for this potential appears not to be affected during fictive locomotion, and repolarizes the membrane to a very constant potential in any given cell, both following locomotor spikes (unpublished observations) and short pulse current evoked spikes (Figure 10A; cf. Nelson & Burke, 1967). It was first shown by Coombs, Eccles, & Fatt (1955) that there were two distinct post-spike hyperpolarizing potentials: an short-lasting potential (about 5 ms), and a longer-lasting potential (about 100 ms). These potentials were thought to result from two distinct potassium conductances. Kolmodin & Skoglund (1958) verified these results, and showed that the short-lasting potential remained very constant regardless of the method of spike initiation. It is likely that this short-lasting post-spike hyperpolarization reflects a high conductance state of the motoneurone membrane due to the action of the delayed rectifier potassium conductance responsible for the falling phase of the spike (Krnjević et al, 1978; Nelson & Burke, 1967). There is no evidence for a change in this conductance during fictive locomotion.

The long lasting hyperpolarizing after-potential, or the a.h.p., which has been shown to be due to a potassium conductance dependent on the intracellular activity of cal-

cium by Krnjević et al (1978), is modified during fictive locomotion. It is conceivable, therefore, that the a.h.p. trajectory could be decreased either by an interference with the usual increase in intracellular calcium activity which immediately follows action potentials, or by a more direct block of this potassium conductance itself.

Short pulse current evoked spikes were elicited throughout the locomotor step cycles in order to distinguish between the following possibilities: (a) there is a tonic reduction of the a.h.p. conductance lasting for the duration of the locomotor episode; (b) the reduction is related to the depolarized phase of the locomotor drive potentials; or (c) the depression of the a.h.p. is associated with the actual production of the action potentials.

In distinguishing between these possibilities, consider these data: (i) The a.h.p.s following these current evoked action potentials were usually reduced in amplitude during fictive locomotion at a voltage comparable to resting membrane potential. This supports a possible tonic or long-lasting decrease in the a.h.p. (ii) In cells which are repetitively firing during fictive locomotion, the action potentials with the smallest a.h.p.s tend to occur during the active phase of the step cycle (Figures 7B-2, 7D, 8, 9). One would expect that during this phase, the a.h.p. would be larger in amplitude because the membrane potential is further from the potassium reversal potential.

These data cannot be explained on the basis of an increased depolarization because it has been shown that even large depolarizations which reduce the action potential amplitude and thus likely reduce calcium entry have been shown to have no effect on the a.h.p. (Coombs, Eccles, & Fatt, 1955). When there was no repetitive firing, the modulation of the a.h.p. seemed to reflect a relationship consistent with a passive dependence on the membrane potential (Figure 7A-1, C). These data support the suggestion that the a.h.p.s are not only tonically reduced during fictive locomotion, but that the reduction may be modulated such that the a.h.p.s are further reduced during the active phases of the locomotor drive potentials, particularly when the motoneurones are repetitively firing. (iii) The a.h.p.s following the current-evoked spikes often appeared larger in amplitude than those following locomotor spikes (see Figure 8). These data lead to the suggestion that the a.h.p. may be maximally reduced immediately following the locomotor spikes and that the mechanism of its reduction may in fact be coupled to the mechanism of spike initiation.

The a.h.p. amplitudes following short pulse current evoked action potentials are measured from the pre-spike potential to the peak amplitude of the a.h.p. The observed decrease in the amplitude during fictive locomotion can therefore be either because of a true reduction in the

a.h.p. amplitude or a deceptively more hyperpolarized pre-spike potential. That is to say that in the case of these action potentials being evoked in the midst of repetitive firing, the pre-spike voltage could possibly reflect the summation of a.h.p. conductances from the preceding repetitive firing, thus bringing this conductance closer to its saturation. If this were the case, then the a.h.p. resulting from the evoked spike would appear to have a lower amplitude. Note, however, that in light of the evidence presented which reflects a reduction in a.h.p. conductance following the repetitive spikes (and the lack of adaptation), this approach to a.h.p. conductance saturation would not be a likely explanation.

The fact that the modulation of the a.h.p. appears to be coupled with the generation of the locomotor activity in the motoneurones indicates that there is a coupling between the mechanism which is responsible for reducing the a.h.p. and the spinal cord circuitry subserving the production of locomotion. Although there is no direct evidence regarding the location in the neuraxis of the mechanism which acts on the motoneurones to reduce their a.h.p., this apparent coupling leads to the suggestion that it is a spinal mechanism. It has been noted that in isolated neonatal rat spinal cords induced to locomote by application of neurotransmitters to the bath, the a.h.p. is significantly reduced during locomotor activity (B. Schmidt, personal

communication), further indicating that the a.h.p. may be modulated by a spinal mechanism.

Potassium accumulation

An important question to resolve is the possible role of extracellular potassium accumulation in the reduction of the a.h.p. (see Syková, 1981; Wallén, Grafe, & Grillner, 1984; Webber & Pleschka, 1981). It has been shown here that potassium accumulation likely is not a significant factor contributing to the regulation of repetitive firing during fictive locomotion in the cat. When motoneurones in a spinal segment were rhythmically active, as was apparent by the e.n.g. activity, it might have been expected that there would be an accumulation of extracellular potassium in that segment, with a resultant decrease in the a.h.p. amplitude in motoneurones from that segment. However, the reduction of the a.h.p. in the motoneurone depicted in Figure 11 was shown to be dependent upon that motoneurone exhibiting locomotor activity, increasing in amplitude once the locomotor activity in that cell ceased, even though the e.n.g. activity from that segment persisted. Furthermore, the post-spike repolarization potential thought to be due to the delayed rectifier potassium conductance does not depolarize toward the end of a step cycle, which it would be expected to do if potassium had accumulated in the extracellular space. Finally, it is possible for action potentials occurring at the end of a burst to have a large

a.h.p. (see Figure 8C). Had potassium accumulation been responsible for the decreased a.h.p., this would not have been expected. The fact that potassium accumulation does not play a significant role in the control of repetitive firing during fictive locomotion is not a surprising finding because one would not expect the mammalian central nervous system to rely on the accumulation of potassium in the extracellular space in order to produce a required motor output just as one would not expect skeletal muscle to rely on local increases in lactate concentration in order to function appropriately.

Frequency-current relations

The lack of the usual frequency-current relation in most motoneurones during fictive locomotion (see Figures 12, 13) is evidence not only that the a.h.p. conductance is decreased during locomotion, but also that the "net driving current," i.e. the net amount of synaptic current which the motoneurone receives (see Schwindt & Calvin, 1973a), is not the sole determining factor in the regulation of repetitive firing during this task. The excitatory synaptic drive is likely important in that it depolarizes the motoneurones and, acting together with a decrease in a.h.p. conductance, allows for the production of repetitive action potentials by relatively low amplitude superimposed synaptic events. The fact that a change in f-I slope occurred in the one cell which maintained a f-I relation suggests that the

reduction in a.h.p. conductance may be graded, and not an all-or-none effect (Figure 14; see also Figure 16C and below). In that cell, when the quality of locomotion as judged by the e.n.g. activity was poor (Figure 14B), the f-I relation was parallel to that obtained at rest (Figure 14A, line a). However, when the quality of locomotion improved (Figure 14C), the f-I slope increased dramatically (Figure 14A, line c). Note that these data preclude the possibility that the a.h.p. conductance is simply shunted by the high membrane conductance during fictive locomotion and therefore would simply appear to be reduced. If this were the case, then one would likely not expect any change in the f-I slope (Kernell, 1969; Schwindt & Calvin, 1973b). Another possibility might be that there would be a decrease in the slope of the f-I relation due to the fact that the injected current would be less effective in producing a given increase in the firing frequency. The witnessed increase in f-I slope is precisely the change one would predict if the a.h.p. conductance was reduced, and indicates that the motoneurone is more sensitive to small increments in synaptic input. Note that the observed reduction in the a.h.p. parallels the improvement in the quality of the locomotion.

The motoneurones in which frequency-current relations were studied displayed only primary ranges of firing. The slopes of the f-I relations were in the lower range of

slopes seen by Kernell (1965b). The reasons for these findings are unclear, but it has been noted by Baldissera and Gustafsson (1971a) that motoneurones of spinalized cats do not exhibit secondary ranges during steady state firing, whereas anæmically decorticated cats, like intact pentobarbitone-anæsthetized cats, do. These investigators suggested that this could be explained if there was a descending input to motoneurones in the anæmically decorticated cats which decreased the a.h.p. conductance in either duration or magnitude and which was abolished by spinalization. The f-I relations in the mesencephalic cats discussed here looked very much like the steady state relationship in spinalized cats such as that shown by Baldissera and Gustafsson (1971a, their Figure 1). Granit, Kernell, & Lamarre (1966) also did not find secondary range firing in motoneurones in unanæsthetized decerebrate cats. The preparation-dependent nature of secondary range firing raises questions about the physiologic significance of secondary range firing.

Could the repetitive firing seen during fictive locomotion represent primary range firing with synaptic noise superimposed, thus accounting for the large standard deviations in firing rate seen (see Figures 12, 13, 16B)? This would appear to be unlikely because motoneurones firing in the primary range are thought to be "... impervious to the effect of synaptic noise.... " (Schwindt, 1973). It

has been shown that synaptic input, or noise, which causes even up to a fifty percent increase in membrane conductance affects neither the a.h.p. trajectories (Schwindt & Calvin, 1973b) nor the f-I relation (Schwindt & Calvin, 1973a). Kernell (1969) also showed that a doubling of the membrane conductance did not affect the slope of the f-I relation. In addition, Shapovalov et al (1966) have shown that depolarizing synaptic current produced in motoneurones from stimulation of the vestibular nerve added to the current injected through the microelectrode, not changing the slope of the f-I relation. Calvin (1975) stated that during repetitive firing in motoneurones, it is not synaptically-induced changes in voltage which alter the firing rate, but rather the increases or decreases in synaptic current. Presented with this evidence, one would expect that if the repetitive firing behaviour seen during fictive locomotion were indeed primary range firing similar to that produced by depolarizing current alone, any added synaptic input would not alter the firing behaviour or the f-I slope. As this is clearly not the case during fictive locomotion, it seems unlikely that the repetitive firing seen in motoneurones represents primary range firing with superimposed synaptic noise.

Deviations from this pattern of synaptic current summating with injected current have been noted before but have remained largely unexplained. Shapovalov (1972) found

that excitatory current produced in motoneurones by stimulation of reticulospinal cells did not add to the current injected through the microelectrode. Kernell (1965a) found similar results produced by stimulation either of the brain stem in the region of the ipsilateral red nucleus, or of peripheral hindlimb nerves. One must conclude from these and the present studies that there are times when the nervous system does alter the frequency-current relations of motoneurones. That is, the response of a motoneurone to a given stimulus, or its input-output relation, is not consistent in different situations. It appears that fictive locomotion is one such circumstance.

It is not surprising that the classical relation between frequency of firing of the motoneurones and the amount of current injected cannot be demonstrated during fictive locomotion. The sustained injection of depolarizing current has been used to study the repetitive firing behaviour of motoneurones since the early 1960s (Granit, Kernell, & Shortess, 1963a), and has elucidated many important intrinsic properties of motoneurones (as have been discussed here) since that time. However, there is no reason to believe that the central nervous system relies on these properties in the production of repetitive firing during movements in general; it has been shown here that it certainly does not rely on these properties during fictive locomotion. Caution must be exerted, therefore, when

extrapolating to the behaving animal from studies which use intracellular current injection to examine the repetitive firing properties of motoneurones.

Adaptation and firing level

Other properties seen during repetitive firing induced by sustained current injection are also not seen during the repetitive firing in fictive locomotion. When current is injected into a motoneurone in the absence of fictive locomotion, the cell adapts in two stages, an early (Granit, Kernell, & Shortess, 1963a) and a late (Granit, Kernell, & Shortess, 1963b; Kernell & Monster, 1982) phase. The time course of late adaptation (tens of seconds; Kernell & Monster, 1982) makes this phenomenon less interesting to the study of repetitive firing during fictive locomotion when there is repetitive firing for less than one second per step cycle. Early adaptation has been attributed to the algebraic summation of the a.h.p. conductances (Baldissera & Gustafsson, 1971b; Ito & Oshima, 1962). The summation of the a.h.p. conductances is thought to reach a plateau after several action potentials in a train and thus steady state firing ensues (Baldissera & Gustafsson, 1974). During fictive locomotion, adaptation of firing rate does not occur. Many cells are seen which exhibit an increase in firing in the middle or even toward the end of a locomotor burst (see Figure 5A). It follows that one method that the nervous system could use to

increase the firing rates within a train would be to simply further decrease the a.h.p. conductance. By reducing the a.h.p. and thus decreasing the tendency for motoneurones to adapt, the motoneurones may fire at increased rates at any time in the step cycle without having to overcome the overall slowing effects of the a.h.p.s. If it is necessary, for example, to increase the force production in a muscle toward the end of its active phase or to reduce the effects of motor unit fatigue, it is only necessary to further reduce the amplitude of the a.h.p. In Figure 16C, it can be seen that there is a small amplitude a.h.p. at firing rates greater than about fifty spikes per second. However, the post-spike trajectory is quite flat at the faster rates of firing. It should be noted that these trajectories do not resemble the post-spike trajectories of either primary or secondary range firing seen during sustained current injection (Schwindt & Calvin, 1972).

It could be thought that another possible mechanism leading to increases in firing rate in the middle or toward the end of a burst, as is often seen in fictive locomotion, would be simply for the nervous system to increase the "net synaptic current" to the motoneurone. If this were the case, then one would expect to see a drastic increase in firing level concomitant with the increase in firing frequency (Kolmodin & Skoglund, 1958; Schwindt & Crill, 1982). For example, Schwindt & Crill (1982) saw an increase in

firing level of 0.2 mV per impulse sec^{-1}. In the present study, it was shown that there was no relation between firing level and firing frequency during fictive locomotion (Figure 16E). Applying the slope of Schwindt & Crill (1982) to the data presented in Figure 16E would show an increase in firing level of 10 mV between the frequencies of 25 and 75 impulses sec^{-1}. Applying any slope to any of the firing level versus firing frequency data during fictive locomotion would be dubious; applying this particular slope would be inappropriate.

It is interesting, though, that the firing level is not absolute; that is, there is no absolute voltage threshold in any given motoneurone. There is often a three to four millivolt range in which action potentials are initiated. It seems likely that other factors also contribute to the initiation of action potentials, such as the rate of change of the membrane potential (see Gustafsson & McCrea, 1984). Supporting this is the fact that on sharply rising locomotor drive potentials (such as in the cells shown in Figures 5A, 17A), the first spikes of the active phases are very often initiated at more hyperpolarized voltages (lower firing levels) than subsequent spikes (see Figures 16D, 17B).

Deviations from the normal pattern of adaptation have been noted before. For example, Gustafsson, Lindström and Zangger (1978) noted that when some dorsal spinocerebellar

tract neurones were induced to fire at high frequencies by intracellular injection of current, they exhibited a period of "negative" adaptation. This consisted of a first inter-spike interval intermediate between a shorter second and longer third interval, and was usually seen at very high rates of firing only. Baldissera, Campadelli, & Piccinelli (1982) injected ramp and hold currents intracellularly into motoneurones of pentobarbitone-anæsthetized cats, and found patterns of firing which differed from the usual adaptive firing patterns seen with rectangular pulse current injection. They found that the instantaneous firing frequency of the motoneurones increased during the ramp stimulus, and seemed to be dependent both on the current intensity and the rate of rise of the ramp current. During the hold portion of the current, the firing leveled off to a steady state firing rate, which was lower than the rate attained during the ramp current. These investigators proposed that the inherent ability of motoneurones to encode for both dynamic and static input signals depends on the a.h.p. conductance; they showed that previous models of a.h.p. conductance summation accounted well for their findings. Baldissera, Campadelli, & Piccinelli (1984) showed that there was sinusoidal modulation of the firing frequency which was parallel to the modulation of the sinusoidal current between 2 and 9 cycles per second. Although they did not provide data regarding any cyclic modulation of the instantaneous firing frequencies in the

range of the rate of stepping (less than 2.5 cycles per second; Figure 3), they stated that for current modulation below 1 cycle per second, the motoneurone response was related only to the intensity of the current, and not to its rate of change.

The seeming lack of adaptation during fictive locomotion can then be explained by one of several possibilities. Firstly, if the a.h.p. is indeed of key significance in the regulation of repetitive firing during fictive locomotion, then one could explain the observed firing pattern as being produced by the synaptic current having a time course different than a square wave depolarization. Alternatively, it could be that there is negative adaptation during fictive locomotion, such as that reported by Gustafsson et al (1978). Thirdly, there could be a fluctuating step-like synaptic current during the locomotor drive potential which summates with the negative afterpotential to reduce the effect of the a.h.p. Finally, a decrease in the a.h.p. conductance would reduce the a.h.p. summation, and therefore would change or eliminate the adaptive firing pattern seen.

In the first three cases, it would be thought that the synaptic current acts on the motoneurone in a manner similar to current injected through a microelectrode. Because there is no consistent pattern to the repetitive firing seen during fictive locomotion, including no such "negative

adaptation" nor a consistent increase in instantaneous firing frequency early in the depolarized phases of the locomotor drive potentials, it is unlikely that the data from those current injection studies would be applicable here (Baldissera et al, 1982; Gustafsson et al, 1978). (Note that the onset of the depolarized phases of the locomotor drive potentials appears quite similar from step to step, yet there is a high variability in the instantaneous firing frequencies.) If the synaptic current consisted of fluctuating steps of current moving the motoneurone to the right and left along a primary range relation and thus obscuring the pattern of adaptation, one would still expect to see characteristic changes, on the average, in the inter-spike trajectories related to the corresponding inter-spike intervals. That is, primary range trajectory changes ought to be seen (as mentioned above, secondary ranges have not been seen in these cells). These changes consist of a constant sloped rise to the succeeding spike with a shallower, shorter hyperpolarized portion of the a.h.p. as the interval becomes shorter (Schwindt & Calvin, 1972). When the action potentials are sorted and averaged depending on their succeeding inter-spike interval, such trajectory changes are not seen (Figure 16C). The most likely explanation in light of the presented data is that there is a decrease in a.h.p. conductance and therefore in a.h.p. conductance summation, thus eliminating the process of adaptation.

Accommodation

The process of accommodation of motoneurones has been studied using linearly rising ramp currents by many investigators (eg. Bradley & Somjen, 1961; Burke & Nelson, 1971; Sasaki & Otani, 1961; Schlue, Richter, Mauritz, & Nacimiento, 1974a,b). Accommodation has been defined to be the phenomenon where the threshold current intensity for the initiation of an action potential (or the firing level) in a neurone will increase as the rate of rise of the current stimulus decreases. Several signs are seen concomitant with this process, including a decrease in action potential overshoot and a decrease in the rate of rise of the action potential (Bradley & Somjen, 1961; Richter & Heyde, 1975). In motoneurones, accommodation is more often seen in some cell types than in others (Burke & Nelson, 1971). The mechanism underlying accommodation is thought to be a combination of partial sodium channel inactivation as well as an increase in potassium conductance (Schlue et al, 1974b). As such, accommodation can be considered to be a function of the net synaptic input; that is, it is not a function of the interspike interval.

Do motoneurones accommodate during fictive locomotion? It is unlikely that motoneurones form an intrinsic part of the spinal circuitry subserving the production of locomotion, because the pharmacologic blocking of their recurrent collateral systems does not lead to a change in the

locomotor pattern (Noga, Shefchyk, Jamal, & Jordan, 1987). Therefore, there is no central need for motoneurones to accommodate; that is, "fatigue" of motoneurones is not necessary for the production of the rhythmic alternating movements of locomotion, as has been suggested (but largely discounted) for other rhythmic movements such as respiration (see Feldman, 1986, p. 489, for discussion). Nevertheless, some classical signs of neuronal accommodation are seen in the repetitive firing of fictive locomotion. These signs include decreased spike overshoot and decreased rate of rise of the action potentials. Most of these changes occur between the onset of the locomotor episode and the first four or five step cycles. Some changes occur within each step regardless of the timing of the step in the locomotor episode (see Figure 17A).

Another sign of accommodation which is seen in current injection studies is an increase in firing level with progression of a burst (Schwindt & Crill, 1982). This increase in firing level is likely due to accommodation of the initial segment, which is thought to have similar accommodative properties to axons (Schwindt & Crill, 1982). This increase is seen during fictive locomotion (Figures 16D, 17B) suggesting that the initial segment, or zone of action potential initiation, does indeed accommodate to a degree. Note that the relationship discussed above between firing level and rate of firing is said to break down once

the initial segment completely accommodates, at which time action potentials are said to be initiated in the soma (Schwindt & Crill, 1982). However, if the initial segment were to completely accommodate during fictive locomotion, then there would be no initial segment spikes seen at that time. This is not the case in fictive locomotion where initial segment spikes can be seen preceding the soma-dendritic spikes (see Figure 16F). Moreover, because the spike overshoot decreases and because spike width increases without the delay between the initial segment and soma-dendritic spikes being accentuated, it seems likely that the soma also accommodates. These signs of accommodation can be explained on the basis of combined initial segment and somatic sodium channel inactivation, with a possible increase in potassium conductance (Schlue et al, 1974b).

It is difficult to quantify the degree of accommodation which occurs during fictive locomotion. If a step depolarization were governing the repetitive firing of the motoneurones, then it is likely that a great deal of synaptic current would be necessary to cause the motoneurone to fire at the high rates seen during fictive locomotion (however, see Figure 4). If such large amounts of synaptic currents were present, one might expect a large degree of accommodation. Note that irrespective of the accommodation taking place, the output of the motoneurone is not altered (Figure 17C). Perhaps by decreasing the a.h.p. conductance

and thus making the motoneurone more sensitive to the inputs it receives (see below), the central nervous system enables the motoneurone to fire at these high rates in response to a lower net synaptic input, and thus not succumb to the counterproductive effects of accommodation.

Delayed depolarization

The underlying mechanism of a.h.p. reduction in motoneurones during fictive locomotion has not been established. That is, the reduction in the calcium dependent potassium conductance underlying the a.h.p. may be secondary to a decrease in intracellular calcium activity, or it may be secondary to a blockade of the calcium dependent potassium channels themselves. Can changes in the delayed depolarization during fictive locomotion shed light on the mechanism of a.h.p. reduction?

The neuronal event(s) responsible for the delayed depolarization trajectory have yet to be firmly established. Some investigators have attributed the voltage trajectory of this potential to reflect the influx of calcium which occurs during the preceding action potential (eg. Barrett & Barrett, 1976; Krnjević et al, 1978). Conversely, Krnjević, Lamour, MacDonald, & Nistri (1978) later demonstrated that ions which block calcium activity do not affect the delayed depolarization, and concluded that the potential is likely predominantly sodium dependent. Also,

some investigators believe that the delayed depolarization in motoneurones is the result of an active spike process invading the dendrites (Kernell, 1964; Nelson & Burke, 1967; Traub & Llinás, 1977). Harada & Takahashi (1983) studied the action potentials of motoneurones in isolated neonatal rat spinal cords. They reported that the delayed depolarization is likely a somatic rather than a dendritic effect which results from the calcium component of the action potential. They suggested that its amplitude is usually reduced by the voltage-dependent potassium conductance responsible for the falling phase of the action potential. Its appearance would then reflect a shift in the balance between the calcium conductance and the voltage-dependent potassium conductance. It appears that the only issue which is resolved concerning the origin of the delayed depolarization is that it is not understood.

The variations in the delayed depolarization seen during fictive locomotion (Figure 10A) very much resemble those following action potentials evoked during membrane potential displacements caused by segmental inhibitory mechanisms (Figure 10A; cf. Figure 4B in Nelson & Burke, 1967), or passive membrane potential displacements (Figure 6 in Nelson & Burke, 1967). If the delayed depolarization is solely the result of an increase of intracellular calcium activity, then the increased size of the hump during the active phases of the locomotor drive potentials during

fictive locomotion would indicate that there is an increase in intracellular calcium activity; the a.h.p. reduction would have to then be attributed to a blockade of the calcium-dependent potassium channel itself. However, there is no reason to exclude the possibility that during fictive locomotion, the increase in the delayed depolarization during the active phase of the step cycle is not simply related to the degree of invasion of an active spike process into the dendrites (Nelson & Burke, 1967) due to the increased membrane conductance at that time (Shefchyk & Jordan, 1985b). Furthermore, the increase in its amplitude may simply be secondary to a decrease in the calcium-dependent potassium, or a.h.p., conductance. Finally, Hultborn, Murakami, Tsukahara, & Gustafsson (1984) present a model which demonstrates that an increase in the current underlying the delayed depolarization can in fact lead to a decrease in the measured a.h.p. amplitude. The modulation of the delayed depolarization thus does not at this point resolve the issues of the mechanism of delayed depolarization production in general nor that of a.h.p. reduction during fictive locomotion.

Evidence that the decrease in a.h.p. amplitude is due to a decrease in a.h.p. conductance

It has been demonstrated that there is a clear reduction in the a.h.p. amplitude in most cells during fictive locomotion, either as compared to the a.h.p.s in the absence of locomotor activity or as compared to the a.h.p.s

in the different phases of the step cycle (Figures 5 through 9, 11). The proposition that this reflects a true reduction in the a.h.p. conductance and not simply an apparent reduction in a.h.p. amplitude has been supported above by several observations. Firstly, it was shown that the usual f-I relation of the motoneurone is not seen during fictive locomotion, and that very low amplitude injections of hyperpolarizing current can abolish the repetitive firing. Secondly, it was demonstrated that in one cell, there was an increase in the f-I slope during fictive locomotion. This increase in slope was explained by there being a decrease in the a.h.p. conductance. Thirdly, the lack of adaptation is also consistent with a decrease in the conductance of the calcium dependent potassium channels, leading to a decrease in a.h.p. summation. A proposal which accounts for this evidence is that there is a decrease in the a.h.p. conductance; however, the conductance associated with the a.h.p. has not been directly measured in the studies presented here. An alternative explanation to this might be that there is a lesser degree of accommodation during fictive locomotion than during sustained depolarizing current injection. In that case, the threshold current intensity for the initiation of action potentials would be lower, and small amounts of synaptic current may initiate spikes. Whether the accommodative properties of the sodium channels are regulated during fictive locomotion by as yet undisclosed mechanisms cannot be

determined by the current studies.

Possible mechanisms of a.h.p. reduction

Several studies have described the actions of many agents which alter the a.h.p. conductance in many different cell types; this list is rapidly expanding (Aldenhoff, Gruol, Rivier, Vale, & Siggins, 1983; Benardo & Prince, 1982; Dinan, Crunelli, & Kelly, 1987; Grafe, Mayer, & Wood, 1980; Haas & Konnerth, 1983; Haas & Rose, 1987; Holz, Shefner, & Anderson, 1986; Madison & Nicoll, 1982; North & Tokimasa, 1983; van Dongen et al, 1986; Weinreich, 1986; Yoshimura, Polosa, & Nishi, 1986). Fung & Barnes (1987) found that stimulation of the locus coeruleus reduced both the current threshold for initiation of repetitive firing in lumbar motoneurones and the a.h.p. trajectory, thus providing evidence that a central nervous system structure can influence the input-output relations of motoneurones. Several possible mechanisms could account for the reduction in the a.h.p. seen during fictive locomotion. Firstly, the calcium-dependent potassium conductance responsible for the a.h.p. could be blocked directly. This could result from the action of a neuroactive substance acting directly on these channels from the external surface of the membrane, or from the action of, for example, cyclic nucleotides at the inner surface of the membrane. This latter case is the mechanism of noradrenaline depression of the a.h.p. in hippocampal pyramidal cells (Madison & Nicoll 1986b).

Secondly, the activity of intracellular calcium could be disrupted, either by decreased calcium entry during the action potential or by an interference with the calcium activity within the motoneurone. There is evidence that the former mechanism accounts for the serotonin-induced a.h.p. reduction in dorsal root ganglia cells of the bullfrog (Holz et al, 1986). The latter mechanism could result from the decrease in the binding of calcium to its receptor on the calcium dependent potassium channel as is thought to occur with the activation of muscarinic acetylcholine receptors in myenteric neurones of the guinea pig (North & Tokimasa, 1983), or conceivably by increasing calcium sequestration within motoneurones.

It has been demonstrated that serotonin facilitates the response of rat facial motoneurones to glutamate, prompting the suggestion that it acts as a gain setter to enhance the effects of excitatory synaptic input in these cells (McCall & Aghajanian, 1979). White & Neuman (1980) demonstrated similar results on cat and rat lumbar motoneurones. Neither of these studies used intracellular recording techniques, so the effects of serotonin on the a.h.p. were not demonstrated. However, it has been reported that application of serotonin decreases the amplitude of the a.h.p. in lamprey motoneurones (van Dongen, Grillner, & Hökfelt, 1986). It has also been shown that serotonin increases the firing frequency of motoneurones during fic-

tive swimming in the lamprey (Harris-Warrick & Cohen, 1985). Because the lamprey has a spinal serotonergic system into which motoneurone dendrites project, it was proposed that this system is used during lamprey swimming to increase the firing rate of the motoneurones (Grillner, Wallén, Dale, Brodin, Buchanan, & Hill, 1987). Taking these above data into account, one must consider the possibility of a role of a serotonergic system in enhancing the excitatory input to motoneurones, possibly by effecting a reduction in the a.h.p. conductance.

Although there is some evidence for a spinal serotonergic system in mammals, this system is quite limited (a total of 3 to 9 serotonergic cells per rat spinal cord; Newton & Hamill, 1988). There is, however, evidence for descending serotonergic projections from the nuclei raphe obscuris and pallidus as well as from the nucleus interfascicularis hypoglossi to the area of lumbar motoneurones (Martin, Jordan, & Willis, 1978; Steinbusch, 1984). Whether any of the cells in these nuclei are active during mammalian locomotion remains to be demonstrated.

Hounsgaard, Hultborn, & Kiehn (1986) have shown that serotonin can contribute to the production of plateau potentials in turtle motoneurones. One might therefore suggest serotonin as a candidate neuroactive substance involved in fictive locomotion, contributing both to plateau potentials in the depolarized phases of the locomotor

drive potentials, and to a reduction in the a.h.p. conductance. Zhang & Krnjević (1987b) reported that iontophoretic application of serotonin onto cat motoneurones had no effect on the a.h.p. trajectory; White & Fung (1988), on the other hand, reported a decreased a.h.p. amplitude with a similar application of serotonin. However, in light of the paucity of intraspinal serotonergic cells and the reduction of the a.h.p. during fictive locomotion in the neonatal rat in vitro spinal cord preparation (B. Schmidt, personal communication), it would seem unlikely that serotonin leads either to the production of the depolarized phase of the locomotor drive potentials or to the repetitive firing behaviour observed in cat motoneurones during fictive locomotion.

Production of action potentials

The locomotor drive potentials are caused by alternating excitatory and inhibitory synaptic input to the motoneurones (Shefchyk & Jordan, 1985b). The excitatory synaptic input likely results from asynchronous input from interneurones onto motoneurones, thus creating the depolarized phase of the locomotor drive potentials. It has been shown in this study that the net synaptic current does not determine the repetitive firing behaviour seen during fictive locomotion. The action potentials would therefore be caused either by the synaptic noise of the asynchronous excitatory bombardment, or by large superimposed synaptic

inputs from the synchronous activity of interneurones. The latter condition cannot be ruled out by this study, but the likelihood is low because large e.p.s.p.s are rarely seen prior to action potential initiation (unpublished observations). If the former condition is the case, then it would be presumed that the synaptic noise would cause the membrane to initiate an action potential, dependent likely on the state of sodium channel inactivation (accommodation) of the motoneurone, plus a combination of the rate of change of the membrane potential and the absolute membrane potential (Gustafsson & McCrea, 1984).

It has been noted in this study that the a.h.p. is seen to be reduced in amplitude and time course particularly when the motoneurones are repetitively firing (Figure 7). Furthermore, indirect evidence has been presented which indicates that this reduction is due to a reduction of the a.h.p. conductance (eg. Figures 12, 13, 14). It is conceivable that this reduction in the a.h.p. conductance enhances the ability of the motoneurones to fire repetitively during fictive locomotion. The calcium-dependent potassium conductance responsible for the a.h.p. is suspected of contributing in part (about 20% on the average) to motoneurone resting conductance (Krnjević et al, 1978). By decreasing this conductance it is possible that motoneurones are more responsive to small amplitude excitatory synaptic inputs due to both the ensuing depolarization

resulting from the closing of potassium channels and the increase in resting membrane input resistance. It would be thought that this change in the resting input conductance would primarily affect the d.c. component of excitatory synaptic inputs, as the peaks of unitary Ia e.p.s.p.s are little affected by such changes (McCrea, Shefchyk, & Carlen, 1989). However, by increasing the d.c. component of the excitatory synaptic input, and hence the depolarization, e.p.s.p.s would be more effective in the production of action potentials. The motoneurone would therefore best be induced to fire repetitively when this conductance is maximally blocked.

This notion is consistent with the hypothesis of Madison & Nicoll (1986a) who note the number of substances which interfere with the a.h.p. conductance, and state:

> "... that such a diverse array of putative neurotransmitters can block this potassium conductance, and that this blockade has such a profound effect on the responsiveness of the affected neurone, implies strongly that the calcium-activated potassium channel plays a central role in the regulation of neuronal excitability ...."

This leads to two questions: (1) Does the decrease in the a.h.p. conductance lead to an increase in the amplitude of the locomotor drive potentials by increasing the input resistance of the motoneurone? and (2) Can the selective activation of the mechanism which reduces the a.h.p. conductance play a role in controlling which motoneurones will

repetitively fire?

With regard to the first question, it should be noted that, in general, there is an increase in membrane conductance during both the depolarized and hyperpolarized phases of the locomotor drive potentials (Shefchyk & Jordan, 1985b). These conductance changes are thought to be due to the classical actions of neurotransmitters in the production of excitatory and inhibitory postsynaptic potentials leading to the locomotor drive potentials. Also, when a cell is repetitively firing during fictive locomotion, the amplitude of the membrane potential variations generally appears greater than when it is not (compare Figure 7C, D). It must be stressed that the magnitude of the underlying locomotor drive potentials cannot be established when a cell is repetitively firing; therefore this increase in amplitude is strictly qualitative. An examination of input resistance when motoneurones are repetitively firing is equally difficult, yet an approximation could be made by measuring the membrane potential deflections in response to small hyperpolarizing current injections during the sustained injection of hyperpolarizing current into the cell to block the spiking activity. A comparison between input resistance changes in a given cell when it is not firing with the same cell when it is repetitively firing would be necessary to determine if the decrease in a.h.p. conductance can contribute to increased locomotor drive potential

amplitudes. By so doing, small excitatory synaptic inputs which would otherwise be ineffective in producing action potentials would be able to cause the motoneurone to fire during fictive locomotion.

The second question above regarded the ability of the central nervous system to selectively decrease the a.h.p. conductance in specific motoneurones, and thus have a degree of control over which motoneurones will repetitively fire. The data presented here do not shed any light on this matter, but it is certainly an interesting possibility. For instance, if one defines the term "functional recruitment" to mean the initiation and sustenance of repetitive firing in a motor unit (after all, it is the repetitive firing of motor units which will initiate a particular motor task), then it is clear from the above discussion that a selective decrease in a.h.p. conductance in a motoneurone will increase the probability that that motoneurone will fire repetitively. In order to explain different recruitment patterns of motor units during walking, trot, and gallop (see Hodgson, 1983), one would simply have to postulate that the central nervous system has control over the a.h.p. conductances in the different types of motor units. For example, if it is disadvantageous to recruit type S motoneurones during gallop, the nervous system could simply decrease the a.h.p. conductance in type FF (and FR) units more than it does in type S units. This

postulate provides the nervous system with another degree of control over its motor units, in addition to fixed synapses (i.e. the number, density, localization, and efficacy of synapses on a given motoneurone) and fixed circuital elements. Further experiments are necessary to determine if this type of control mechanism indeed occurs during fictive locomotion.

Motoneurone output

It has been demonstrated here that motoneurones fire at high rates during fictive locomotion. No consistent pattern of firing was seen. The pattern of firing seen in fictive locomotion rarely parallels the optimal pattern of stimulation of muscle nerves for the production of steady state tension in skeletal muscle fibres (Stein & Parmiggiani, 1979). Those investigators found that stimulation of the soleus nerve with a short first interval, a longer second interval, and intermediate subsequent intervals was the most effective pattern of stimulation for the production of steady state force in the soleus muscle. In accordance with this, Zajac & Young (1980) noted an initial firing doublet in ventral root filaments during treadmill walking. Contrary to these studies, however, an initial firing doublet was rarely seen in the intracellular records from the motoneurones examined in this study. Hoffer, Sugano, Loeb, Marks, O'Donovan, & Pratt (1987) also rarely found initial doublets in ventral root filaments in intact

cat treadmill locomotion. Of the 30 cells included in these experiments, only two consistently began a large proportion of their step cycles with an initial doublet (see Figure 4). This difference could be related to the preparation and the fact that during fictive locomotion the motoneurones do not receive any rhythmic afferent input. Alternatively, there could be a difference between what is recorded in the soma and what is recorded in the axon. Gogan, Gustafsson, Jankowska, & Tyc-Dumont (1984) elucidated states in which a second action potential could occur in the axon without being witnessed by the recording electrode in the soma. They found that this re-excitation is initiated in the axon, possibly at the first node of Ranvier, during conditions which increase the delay between the initial segment and soma-dendritic action potentials (Gogan et al, 1984). In fictive locomotion, a large increase in membrane conductance (see Shefchyk & Jordan, 1985b) at the start of the active phase at the time of the initiation of the first action potential might tend to favour an increase in this delay and would therefore favour re-excitation, providing an initial firing doublet in the axon. The larger a.h.p. often seen following the first spike in these cells could then provide the longer second interval required for near optimal conditions of initial motor unit activation (Stein & Parmiggiani, 1979).

The determination of the factors regulating repetitive

firing in motoneurones during motor acts has important consequences for understanding the mechanisms regulating motoneurone output. It has been demonstrated here that although motoneurones have the intrinsic capability to fire repetitively in response to current injected into them, the central nervous system has the ability to alter these intrinsic properties. Whereas the motoneurone uses its action potential afterhyperpolarization to regulate its firing in response to current injection, it has been shown here that the nervous system can alter this a.h.p. conductance thereby reducing the motoneurone's intrinsic control of repetitive firing. Although very important facts about the intrinsic properties of motoneurone membranes have been learned by the sustained injection of depolarizing current, the ability of the central nervous system to modify these properties must be taken into consideration when interpreting these findings. It is clear from this study that the a.h.p. reduction during fictive locomotion plays an important role in regulating motoneurone output and that the input to motoneurones cannot be assumed to be simply equivalent to current injected through a microelectrode (see Hoffer et al, 1987; their Figure 12). With a decreased a.h.p. conductance, motoneurones can fire at high rates; without adaptation, they can maintain these high rates. The capacity to regulate the a.h.p. conductance and thereby the sensitivity of its motoneurones provides the nervous system with the ability to exercise a high degree

of control over its motor output.

Figure 1: Measurements of afterhyperpolarizations. Depicted is the a.h.p. following an action potential produced by injection of a short duration (0.5 msec) depolarizing current pulse into the cell shown in Figure 8, in the absence of fictive locomotion. Abbreviations are: amplitude (**Amp**), duration (**Dur**), half-decay (**HD**), half-width (**HW**), time to half-decay (**TTHD**), and time to peak (**TTP**). The marked points indicate the beginning of the action potential (**A**), the peak of the a.h.p. (**B**), the point at which the a.h.p. decays to one-half its amplitude (**C**), and the end of the decay phase of the a.h.p. (**D**). Note that during fictive locomotion, points **B** and **D** are very difficult to accurately obtain, while **A** and **C** are far more reliable. Hence, the measurements used for analysis of a.h.p. duration during fictive locomotion were the half-width and the time to half-decay. In some cells, the half-width is not an accurate indicator of a.h.p. duration because it will vary depending on the presence of a delayed depolarization.

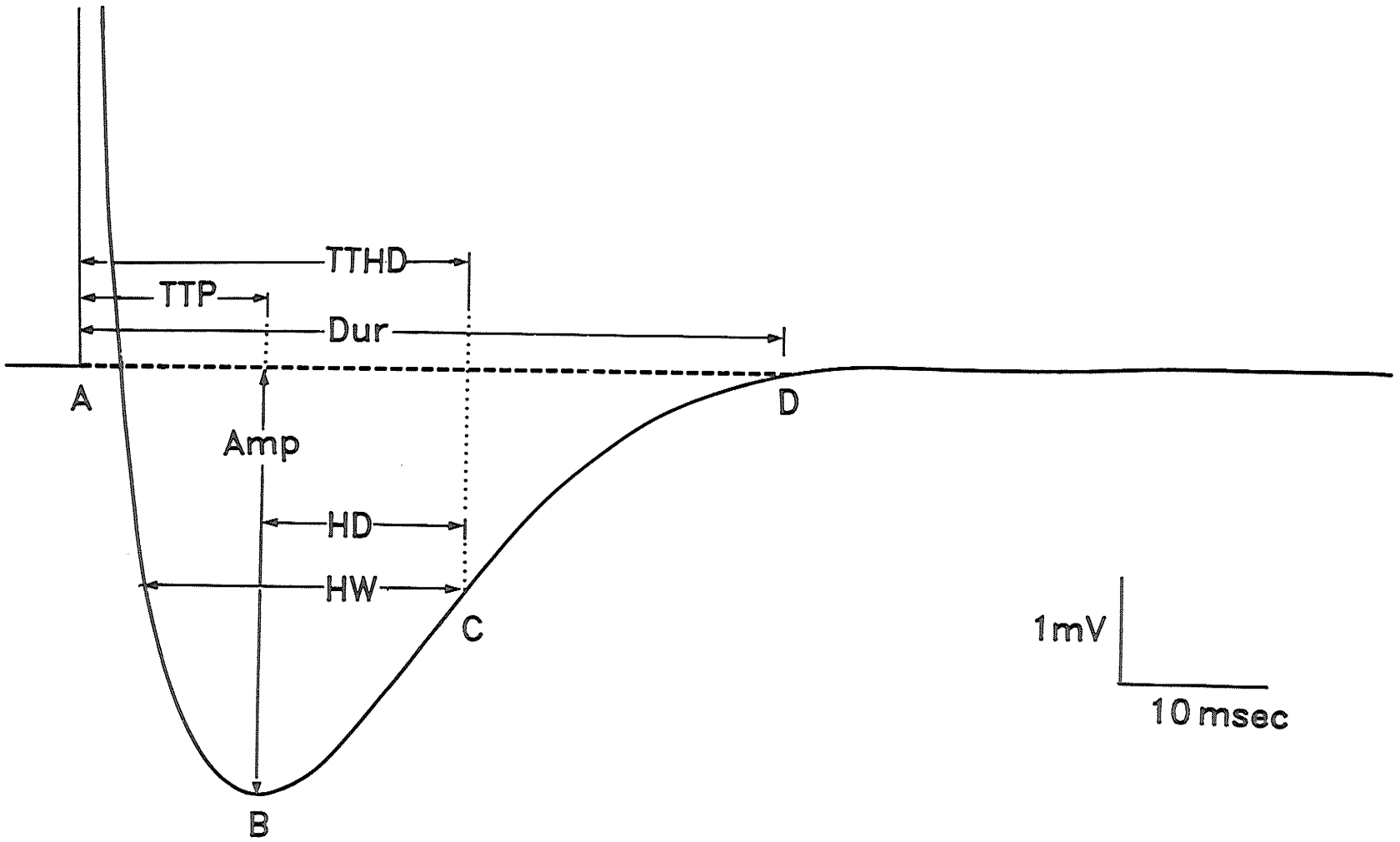

TTHD
TTP
Dur
A
Amp
D
HD
HW
C
B
1mV
10 msec

Figure 2: Fictive locomotion produced by stimulation of the mesencephalic locomotor region with 220 uA, 20 Hz, 1 ms pulses. All traces were digitized simultaneously. Top trace: d.c. intracellular record from a left tibialis anterior (TA) motoneurone, digitized at 5 kHz. Note that this low rate is not sufficient to capture the full excursion of the action potentials. The next five traces are rectified, low pass filtered electroneurograms, digitized at 200 Hz. They are all from the left side of the cat and from top to bottom are: anterior biceps (AB), posterior biceps (PB), medial gastrocnemius (MG), flexor digitorum longus (FDL), and tibialis anterior (TA). The scale bar is 10 mV for the intracellular record and 200 ms for all traces.

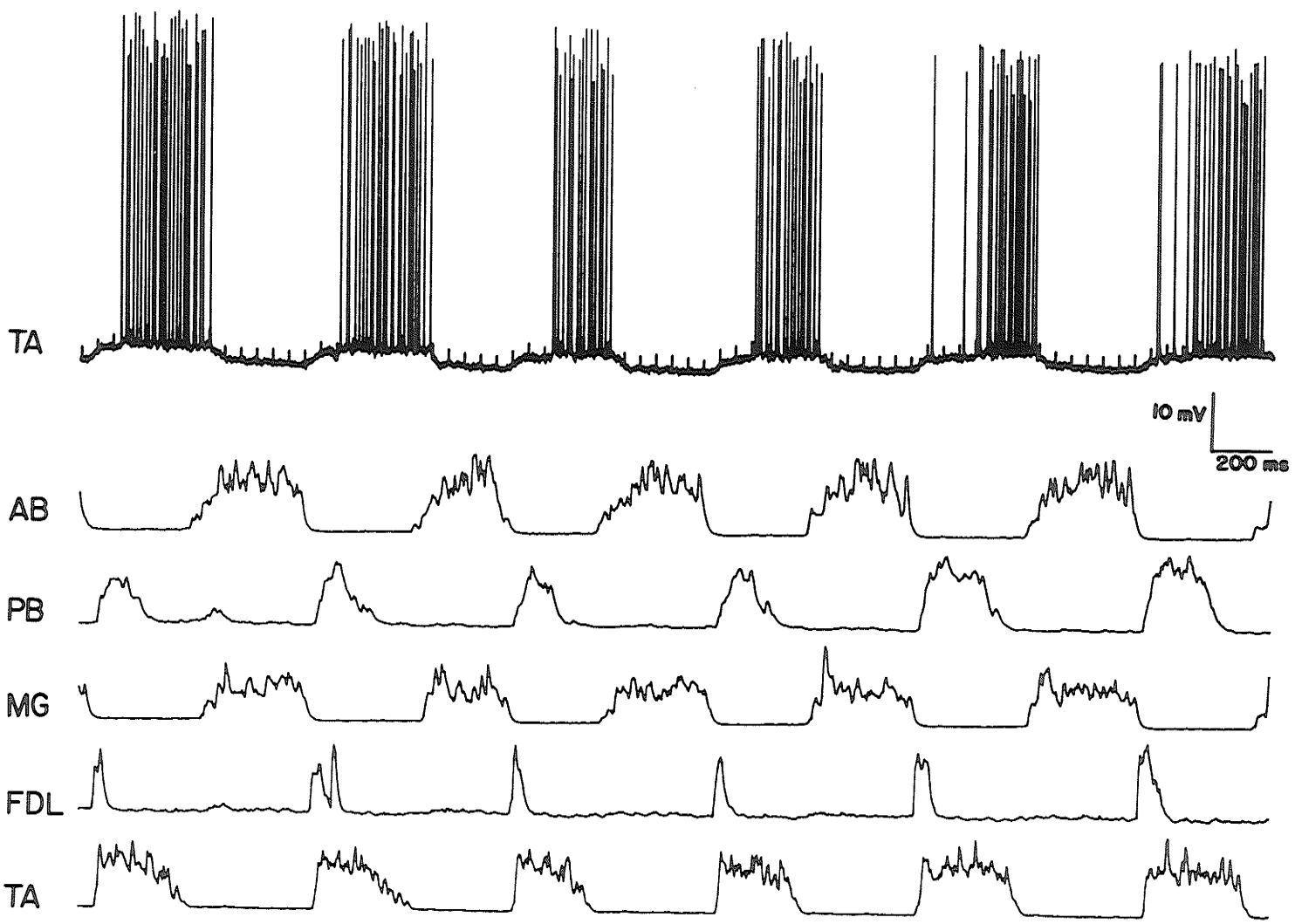

TA
10 mV
200 ms
AB
PB
MG
FDL
TA

Figure 3: Histogram showing the durations of the fictive step cycles in the motoneurones used in this study. The step cycle duration was defined as the time from the onset of one depolarized phase of the fictive step cycle to the onset of the next depolarized phase, averaged through the locomotor episode. Note that most step cycles are less than 1400 msec in duration. See text for further explanation.

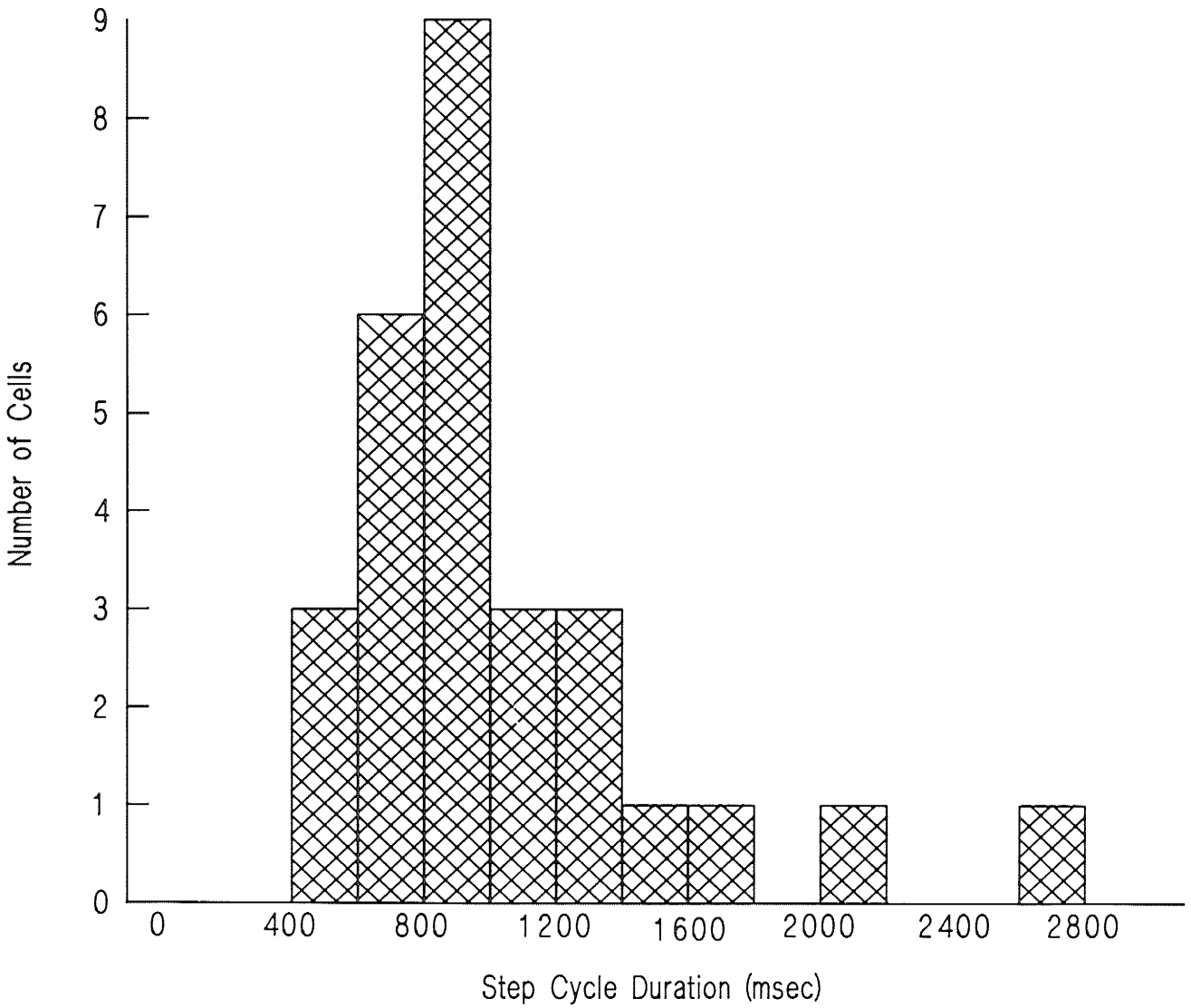

Number of Cells
0
1
2
3
4
5
6
7
8
9
0
400
800
1200
1600
2000
2400
2800
Step Cycle Duration (msec)

Figure 4: Effects of small amounts of hyperpolarizing current injection on the repetitive firing of motoneurones during fictive locomotion. Depicted is an extensor motoneurone recorded during fictive locomotion (**A**), and subsequently with 4 nA sustained hyperpolarizing current injection (**B**). Note the complete disappearance of repetitive firing in **B**. The electroneurograms shown are the contralateral semimembranosus (coSm) and the ipsilateral anterior biceps (iAB).

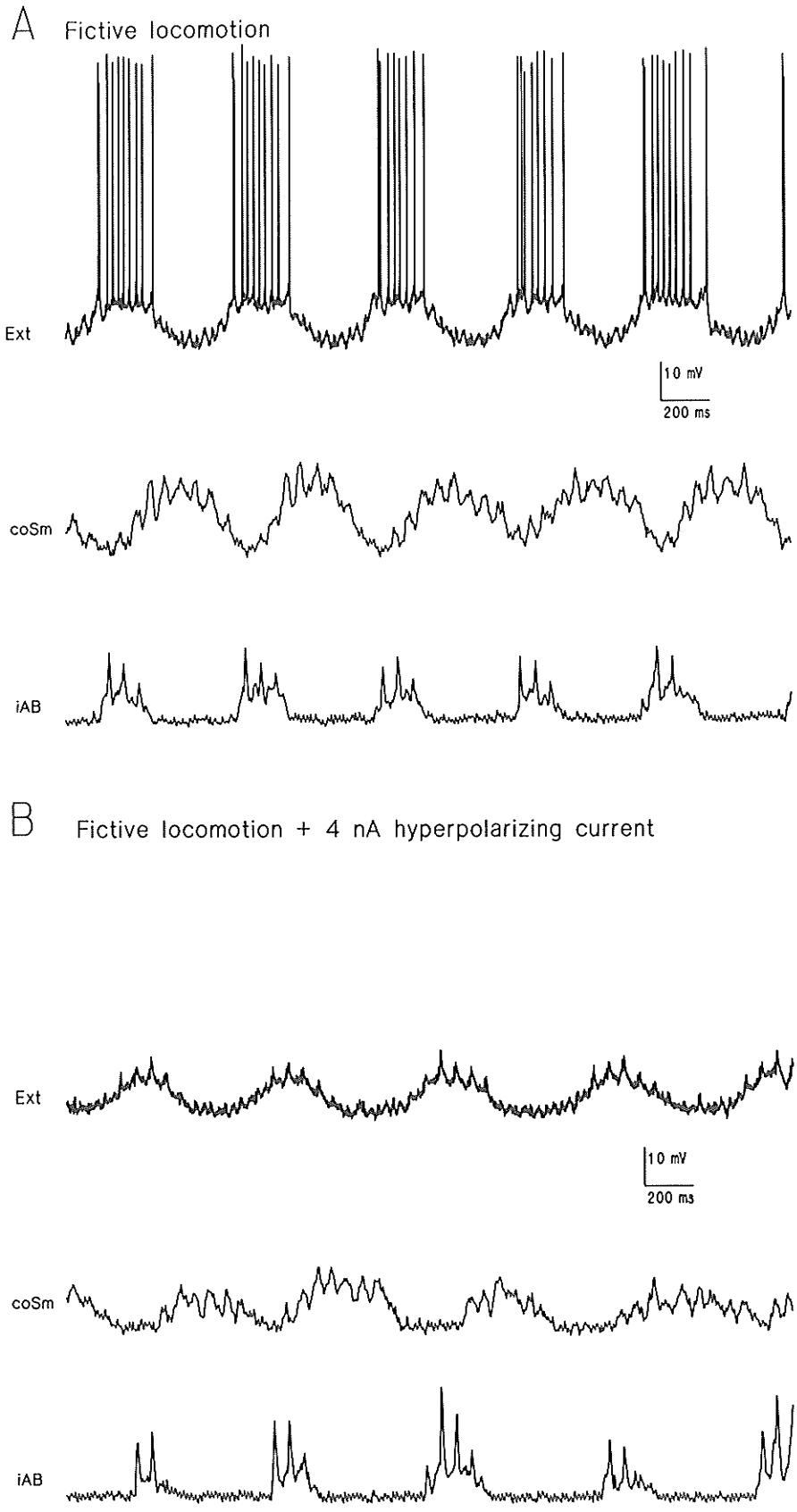

A
Fictive locomotion
Ext
10 mV
200 ms
coSm
iAB
B
Fictive locomotion + 4 nA hyperpolarizing current
Ext
10 mV
200 ms
coSm
iAB

Figure 5: Comparison of repetitive firing during fictive locomotion (**A**) and during square wave depolarizing current injection (**B**) in a motoneurone. **A:** Fictive locomotion. The top two traces are a left seventh lumbar segment ventral root filament and the left sided tibialis anterior e.n.g. digitized as explained in text. The next trace is the intracellular recording, a portion of which is amplified in voltage and expanded in time in the lower trace. Mean Firing rate is 51 (s.d. 27) impulses sec^{-1}. **B:** Square wave depolarizing current injection. The top trace shows the current injection (35 nA), with the next two traces being the intracellular records as in **A.** Note that the membrane potential deflection in the middle trace of **B** does not represent the true membrane potential deflection due to the fact that the bridge circuit was not in perfect balance. Mean Firing rate is 40 (s.d. 8) impulses sec^{-1}. Note that inter-spike trajectories can be quite variable even when inter-spike intervals are of similar duration (arrows). The upper scale bar is 20 mV, 400 ms; the lower is 5 mV, 40 ms.

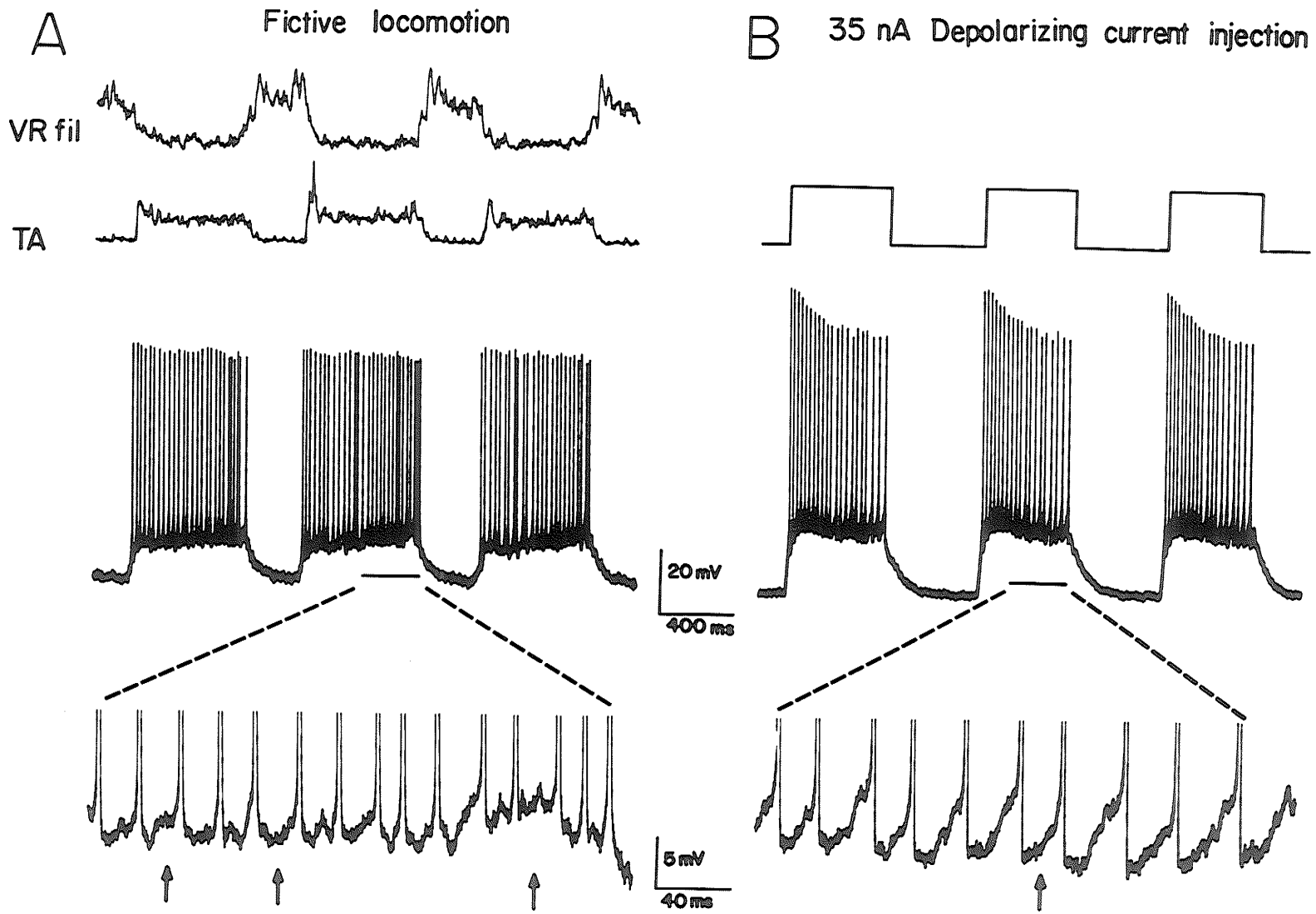

A
Fictive locomotion
VR fil
TA
20 mV
400 ms
5 mV
40 ms
B
35 nA Depolarizing current injection

Figure 6: Comparison of post-spike trajectories in repetitive firing in a semimembranosus motoneurone produced during fictive locomotion and during sustained depolarizing current injection. **A:** Repetitive firing produced by the sustained injection of 32 nA depolarizing current. The upper portion shows ten consecutive action potentials which have been separated and overlaid. The lower portion shows the average of 35 such spikes. **B:** Repetitive firing during fictive locomotion in the same cell. The upper part is as in **A.** The lower portion is the average of 46 such spikes. Action potentials with succeeding intervals less than about 30 ms were eliminated from this analysis. **C:** The two averages overlaid, using the firing level as a constant voltage point. **D:** As in **C** but using the post-spike repolarization potential as a constant voltage. The upper scale bar is 20 mV for the upper portions of **A** and **B**, and 5 mV for the lower portions. The time is 10 ms. The lower scale bar is 2 mV, 10 ms.

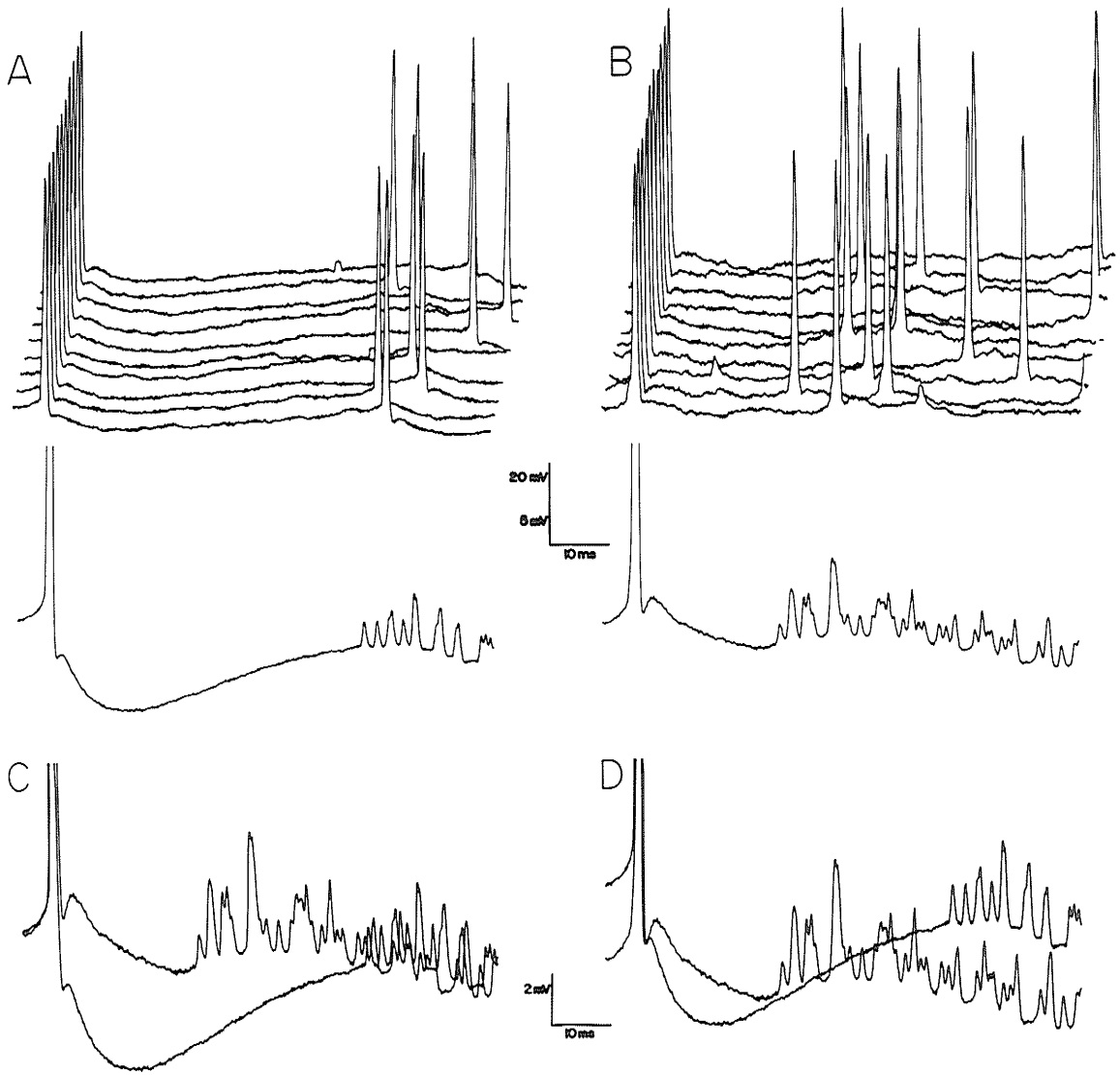

A
B
20 mV
5 mV
10 ms
C
D
2 mV
10 ms

Figure 7: Short pulse current evoked action potentials throughout the step cycle in an extensor motoneurone. **A** and **C** are data from 9 step cycles when the motoneurone was not firing repetitively during fictive locomotion. During a later fictive locomotor trial shown in **B** and **D** (data from 10 step cycles), the motoneurone was firing repetitively. **A** and **B** are averages of the evoked action potentials (truncated), being averaged based on the pre-spike membrane potential in **A-1** and **B-1**, and based on time in the step cycle in **A-2** and **B-2**. The letters and numbers to the left of the averages correspond to the traces at the peaks of the a.h.p.s and to the right correspond to the end of the traces. The insets illustrate locomotor drive potentials and display the corresponding methods of dividing the locomotor episode into bins. **C** and **D** show the average membrane potential in the step cycles (solid line) and the a.h.p. amplitudes throughout the locomotor episode (dots). The ordinates in these graphs are for the absolute a.h.p. amplitudes as well as for the relative membrane potential. The absolute membrane potential is indicated in **D**: 0 mV is approximately equivalent to a membrane potential of -62 mV. Resting membrane potential was -61 mV. The arrow in **C** indicates the resting a.h.p. amplitude prior to the locomotor runs. The membrane potential and the a.h.p. amplitudes are repeated in the graphs for a second step cycle. Note that some a.h.p. amplitudes measured at the end of the active phase of the step cycle may be artefactually high

because of the sudden repolarization of the membrane. See text for complete description. Scale bar is 2 mV 10 ms for **A** and **B**.

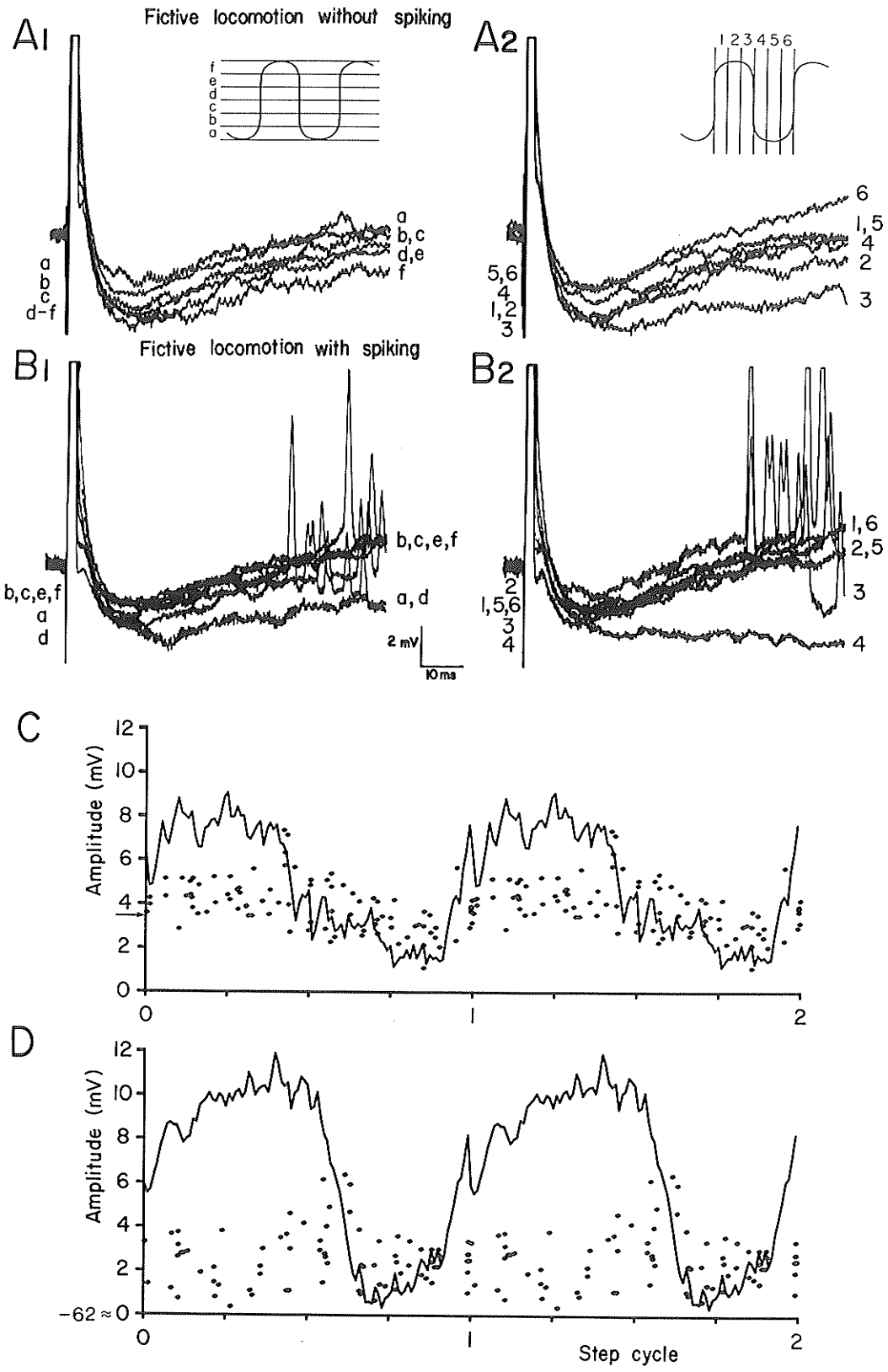

A1
Fictive locomotion without spiking
f
e
d
c
b
a
a
b
c
d-f
a
b,c
d,e
f
A2
1 2 3 4 5 6
5,6
4
1,2
3
6
1,5
4
2
3
B1
Fictive locomotion with spiking
b,c,e,f
a
d
b,c,e,f
a, d
2 mV
10 ms
B2
2
1,5,6
3
4
1,6
2,5
3
4
C
Amplitude (mV)
12
10
8
6
4
2
0
0
1
2
D
Amplitude (mV)
12
10
8
6
4
2
−62 ≈ 0
0
1
2
Step cycle

Figure 8: **A:** Short pulse current evoked action potentials (truncated) during one fictive step cycle in a semimembranosus motoneurone. The arrows indicate the a.h.p.s following the current evoked action potentials. The other action potentials are the locomotor spikes. The two evoked spikes indicated are shown on an expanded scale in **B.** Note that the a.h.p. of the spike evoked in the midst of the locomotor spikes is much smaller in amplitude and shorter in duration than those preceding or succeeding the active phase of the step cycle. **C:** A longer segment of a locomotor episode from this same cell, without the current evoked spikes. The top trace is the intracellular record, and the lower two are e.n.g.s (left semimembranosus and tibialis anterior) digitized as in Figure 2. Note the small amplitude locomotor drive potentials in this cell, the large a.h.p.s at the start and end of each step cycle, and the relative lack of appreciable a.h.p.s otherwise.

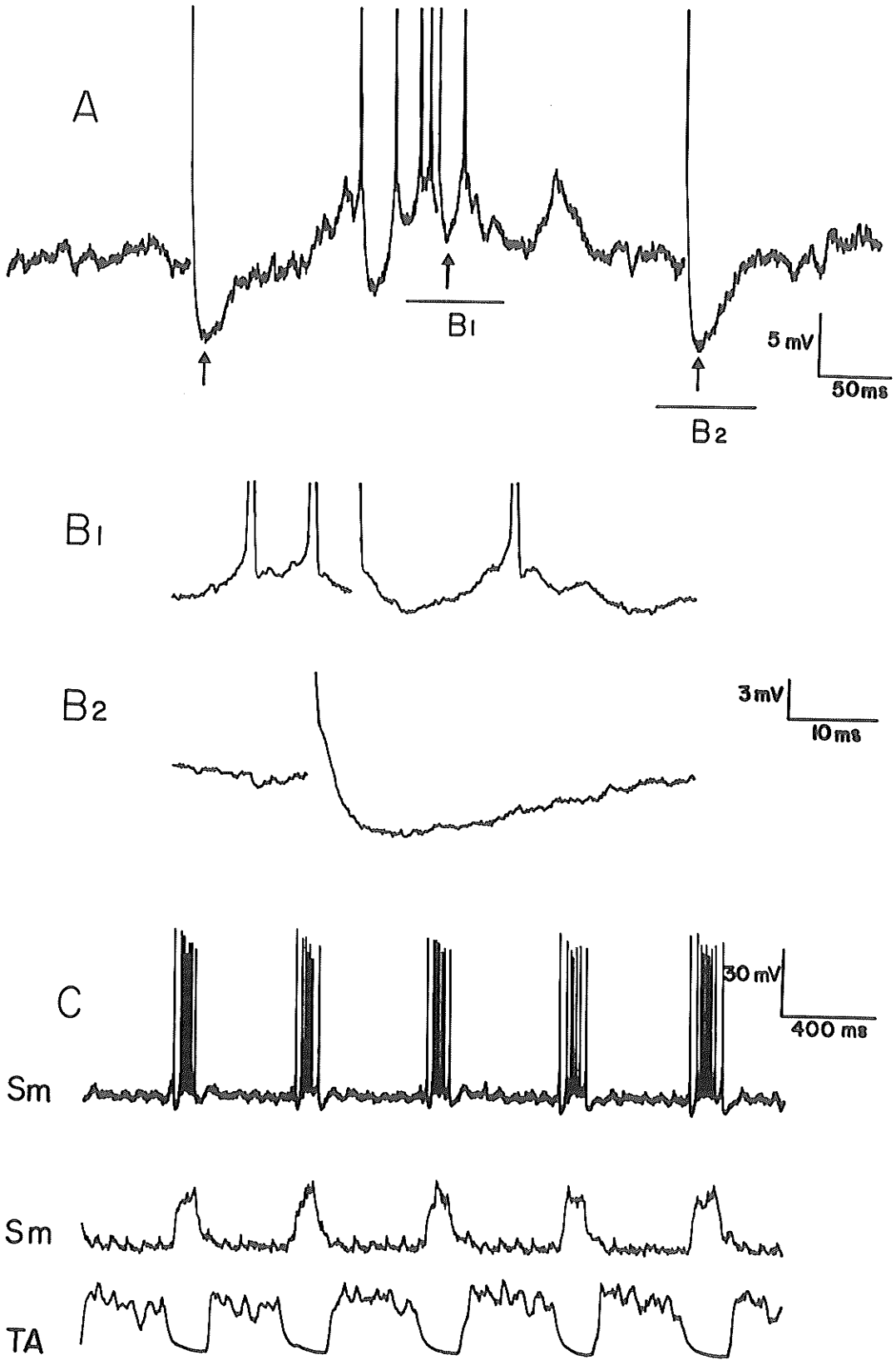

A
B1
B2
5 mV
50ms
B1
B2
3mV
10ms
C
30 mV
400 ms
Sm
Sm
TA

Figure 9: Duration of a.h.p.s following short pulse current evoked action potentials in a medial gastrocnemius motoneurone. **A:** The truncated action potentials have been sorted and averaged into six bins dependent on their pre-spike membrane potentials, and overlaid with a common baseline as in Figure 7. The smallest amplitude and shortest duration a.h.p. is from the most depolarized potential. **B:** The average membrane potential in the step cycle is plotted for two cycles (solid line) with the amplitudes of the a.h.p.s following the evoked spikes (dots) and the time to half decay (i.e. time from spike onset to the point when the a.h.p. has decayed half-way to baseline) for each of the six bins in **A** (open circles). Note that the a.h.p. amplitudes are smallest in the active phase as are their durations. The long duration seen at the end of the active phase is artefactual, because the membrane potential following spikes elicited at this time in the step cycle often does not return to its baseline. Scale bar in **A** is 2 mV, 10 ms.

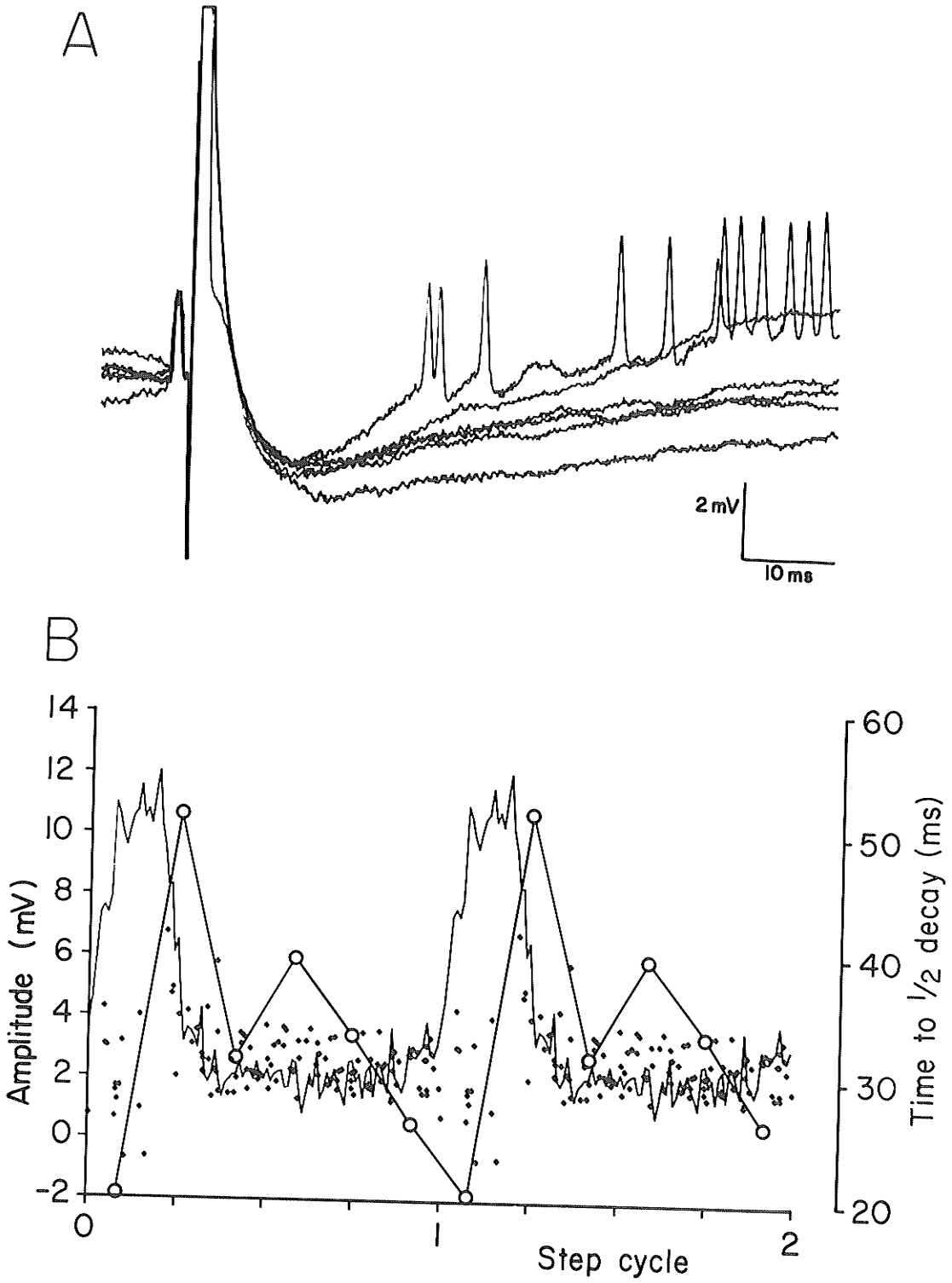

A
2 mV
10 ms
B
Amplitude (mV)
14
12
10
8
6
4
2
0
-2
0
1
2
Step cycle
60
50
40
30
20
Time to 1/2 decay (ms)

Figure 10: The delayed depolarizations following short pulse current evoked spikes during fictive locomotion, averaged in bins dependent on prespike membrane potentials in a semimembranosus - anterior biceps motoneurone (**A**) and a medial gastrocnemius motoneurone (**B**). The cell in **A** has predominantly hump-type delayed depolarizations, which appear to be modulated as would be expected with the changes in membrane voltage (Nelson & Burke, 1967). The cell in **B** shows predominantly smoothly declining delayed depolarizations except at the most depolarized potentials where hump-type delayed depolarizations are seen. The scale bar for both **A** and **B** is 5 mV, 3 ms.

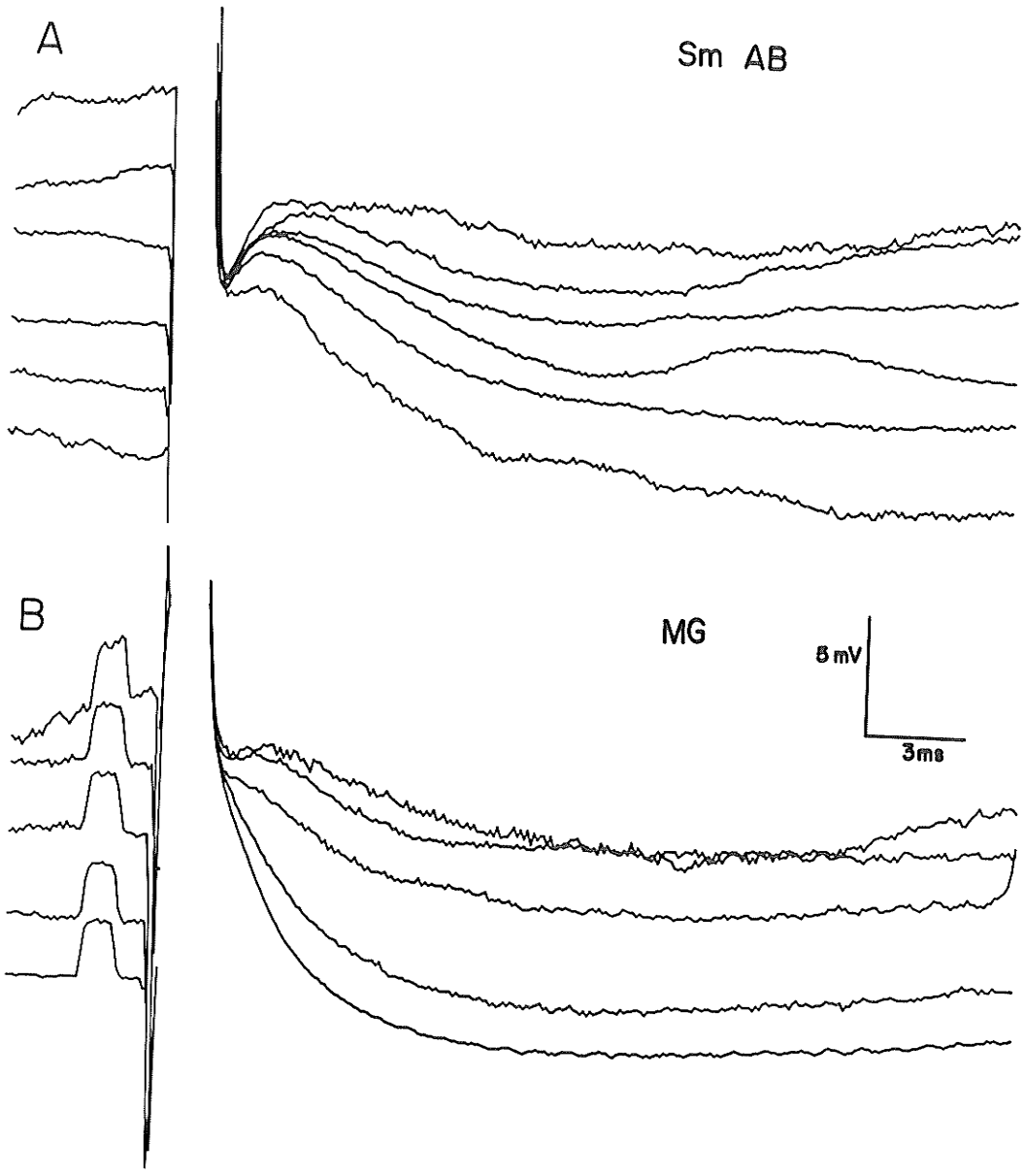

A
Sm AB
B
MG
5 mV
3ms

Figure 11: **A:** A lateral gastrocnemius motoneurone which stopped showing locomotor activity, even though fictive locomotion persisted, as is evident from the accompanying e.n.g. activity. The motoneurone continues to fire repetitively as 20 nA of depolarizing current was being injected into the cell throughout this episode. There is one locomotor drive potential shown at the left hand side of the intracellular record. The action potentials from the indicated short line segments in **A** are shown in **B** and **C** in a similar fashion to those of Figure 6. The averages in the lower portions of **B** and **C** are taken from all the action potentials shown by the corresponding long line segments in **A**. Note the return of the a.h.p. once the locomotor activity in this motoneurone stops.

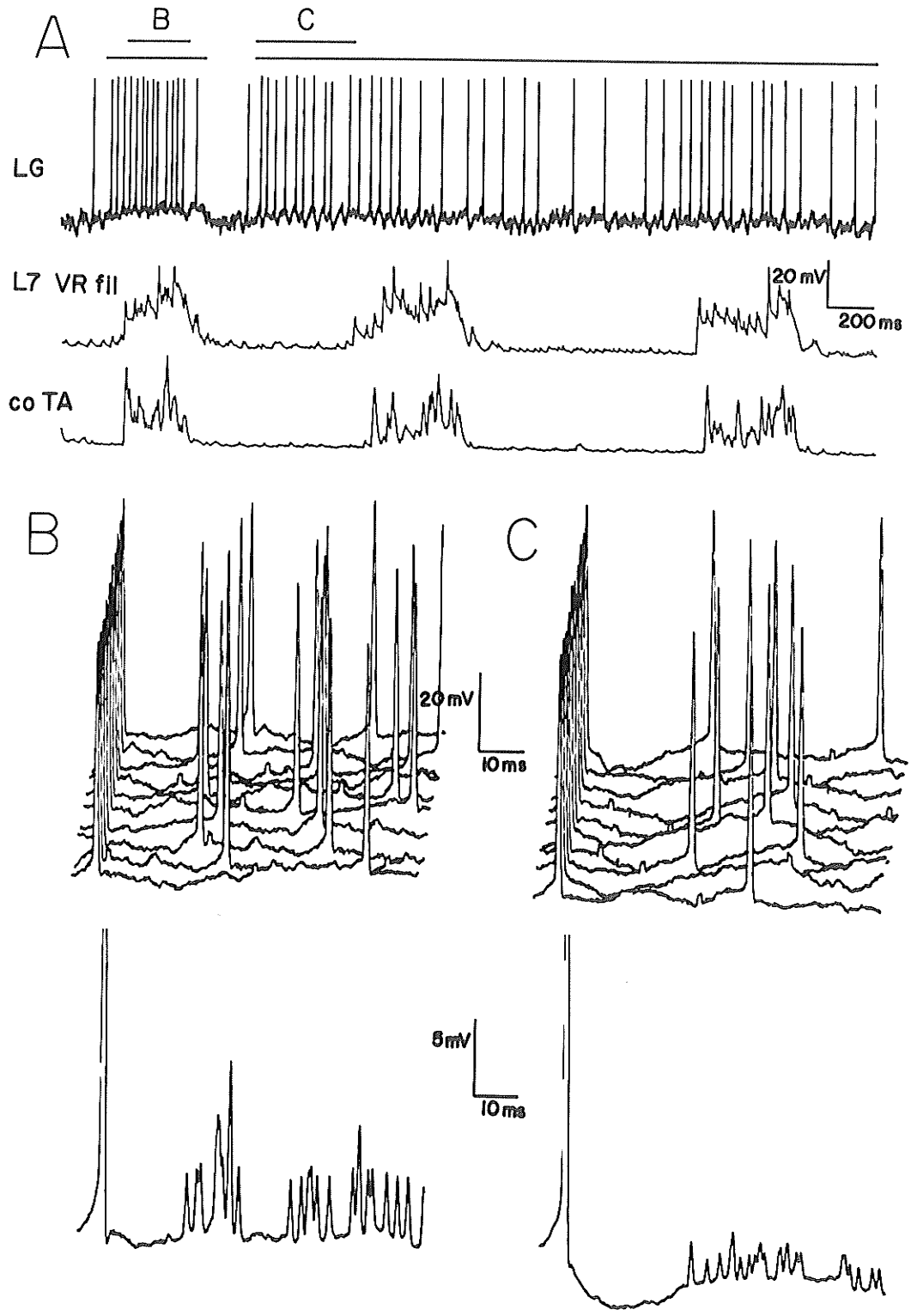

A
B
C
LG
L7 VR fII
20 mV
200 ms
co TA
B
20 mV
10 ms
C
5mV
10 ms

Figure 12: The frequency-current relations of the anterior biceps motoneurone shown in Figure **16** prior to (**A**) and during (**B**) fictive locomotion. In **A** the filled circles represent the mean steady state firing frequency. The bars in both **A** and **B** represent the standard deviations. The slope of the relation in **A** is 0.62 impulses sec^{-1} nA^{-1} ($r = 0.94$). The current threshold at which repetitive firing was initiated (the rhythmic threshold) was 28 nA. In **B**, the filled circles represent the f-I relation during an initial fictive locomotor run. It can be seen that there is no relation between the amount of current injected and the frequency at which the motoneurone fires during fictive locomotion ($r = -0.35$). In a subsequent fictive locomotor trial, a similar relation was obtained (filled diamonds). Note that during this run, injection of 2 nA of hyperpolarizing current eliminated repetitive firing altogether.

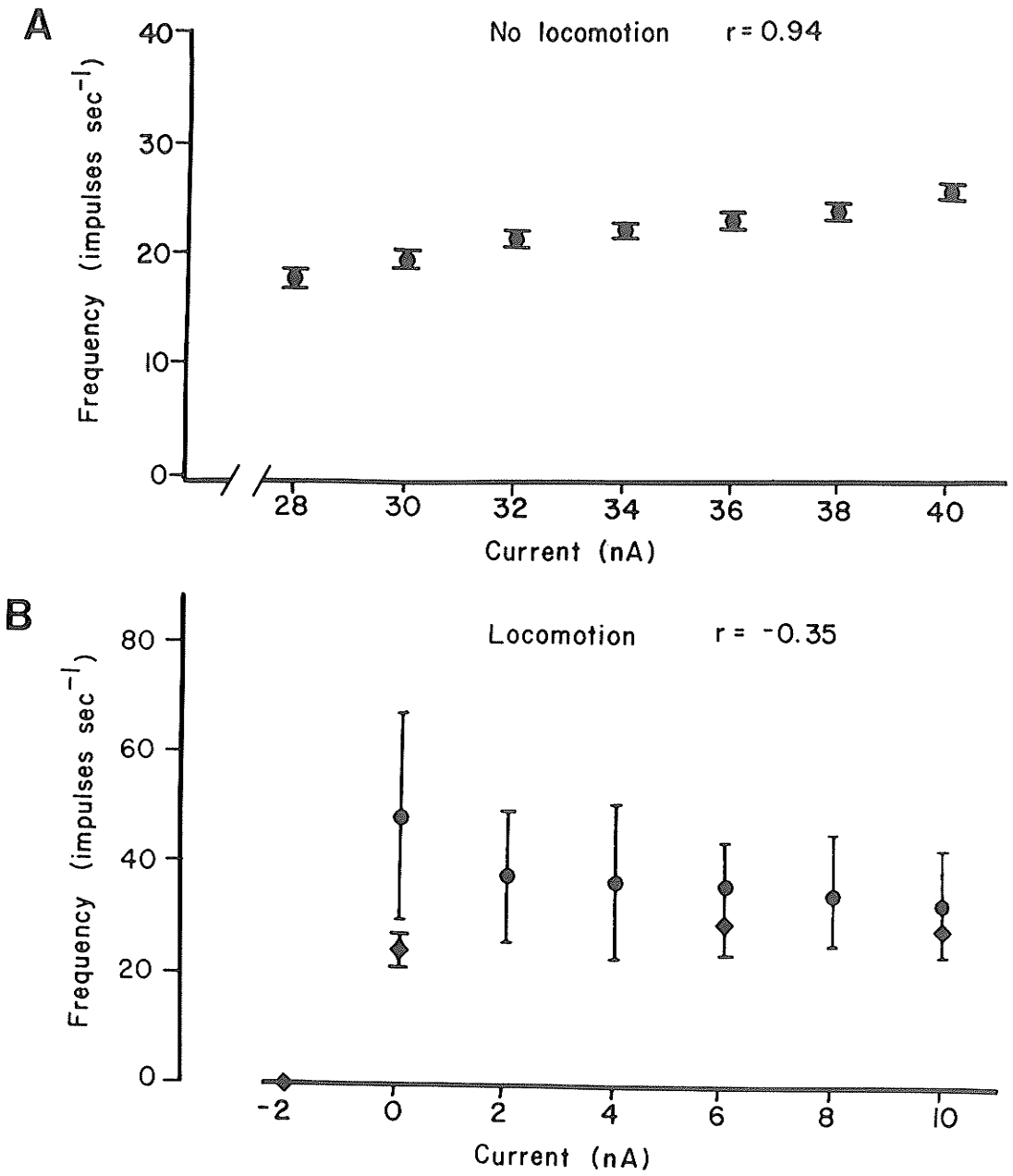
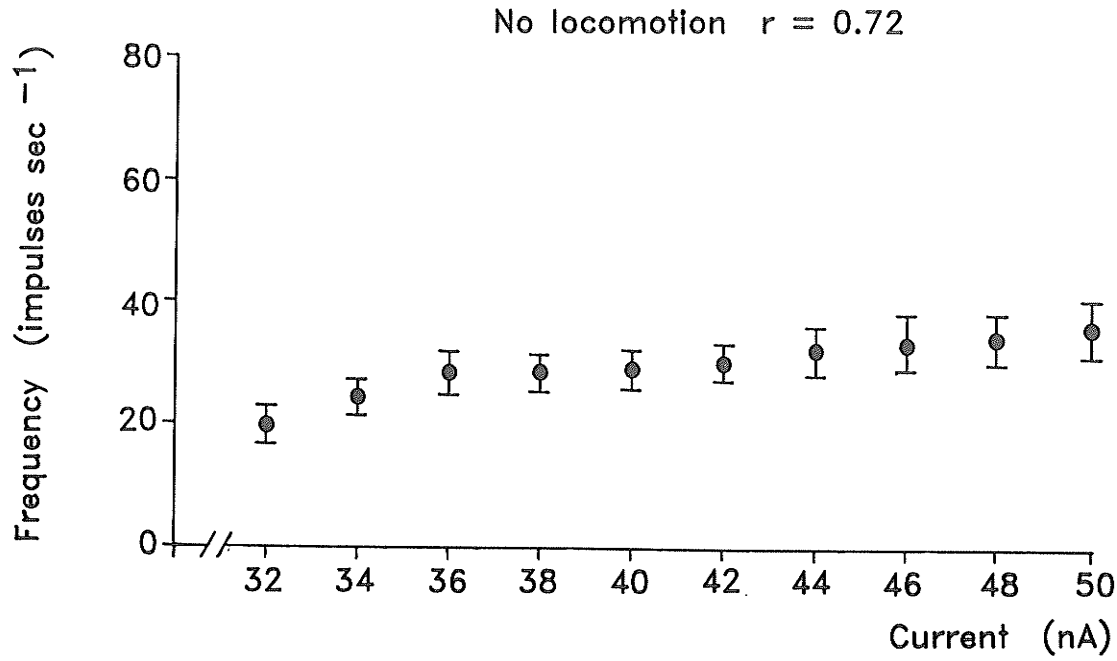

A
No locomotion
r = 0.94
Frequency (impulses sec⁻¹)
40
30
20
10
0
28
30
32
34
36
38
40
Current (nA)
B
Locomotion
r = -0.35
Frequency (impulses sec⁻¹)
80
60
40
20
0
-2
0
2
4
6
8
10
Current (nA)

Figure 13: Frequency-current relation of a tibialis anterior motoneurone shown in a manner similar to Figure 12. The top graph shows the frequency-current relation at rest, while the bottom plot is during fictive locomotion. The rhythmic threshold for this cell was 32 nA. The slope of the control relation is 0.71 impulses sec^{-1} nA^{-1} ($r = 0.72$). There was no significant relation between current injected and firing frequency during fictive locomotion ($r = -0.31$). With the injection of 5.8 nA hyperpolarizing current during fictive locomotion, this cell showed an almost complete abolition of spiking: in some step cycles, one or two action potentials remained.


No locomotion r = 0.72
Frequency (impulses sec −1)
80
60
40
20
0
32
34
36
38
40
42
44
46
48
50
Current (nA)

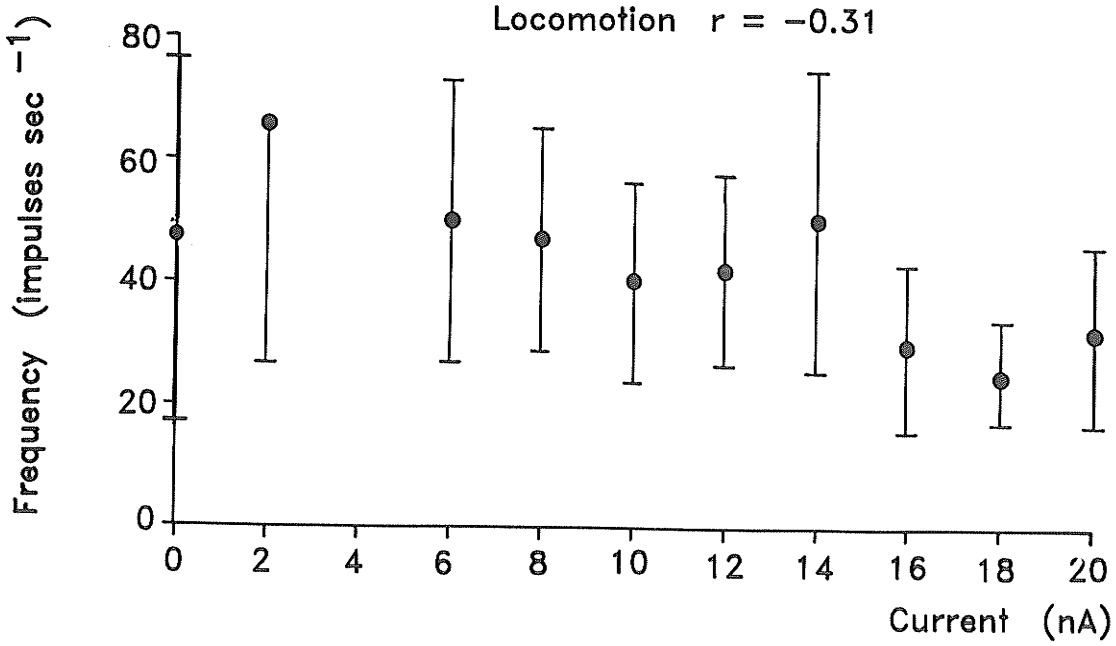

Locomotion r = −0.31
Frequency (impulses sec −1)
80
60
40
20
0
0
2
4
6
8
10
12
14
16
18
20
Current (nA)

Figure 14: **A:** The frequency-current relations of a flexor motoneurone. Line a represents the relation during an initial locomotor trial when locomotor activity was poor, as judged by the e.n.g.s (**B:** during 6 nA current injection). This line has the same slope as the control (line b). With the improvement of locomotor activity (**C:** during 6 nA current injection), the slope of the relation increased (line c). Following this, the control returned to the initial slope (line d). The slopes (Pearson correlation coefficients) are: line a: 0.70 (0.653); line b: 0.78 (0.892); line c: 2.85 (0.738); line d: 0.86 (0.848). The e.n.g.s shown in **B** and **C** are from the nerve to the left tibialis anterior muscle. Note that no locomotor drive potentials are seen in **B** and **C** due to the concomitant current injection. The scale bar for **B** and **C** is 20 mV for the intracellular records, and 400 ms. The e.n.g.s are shown with equal gain.

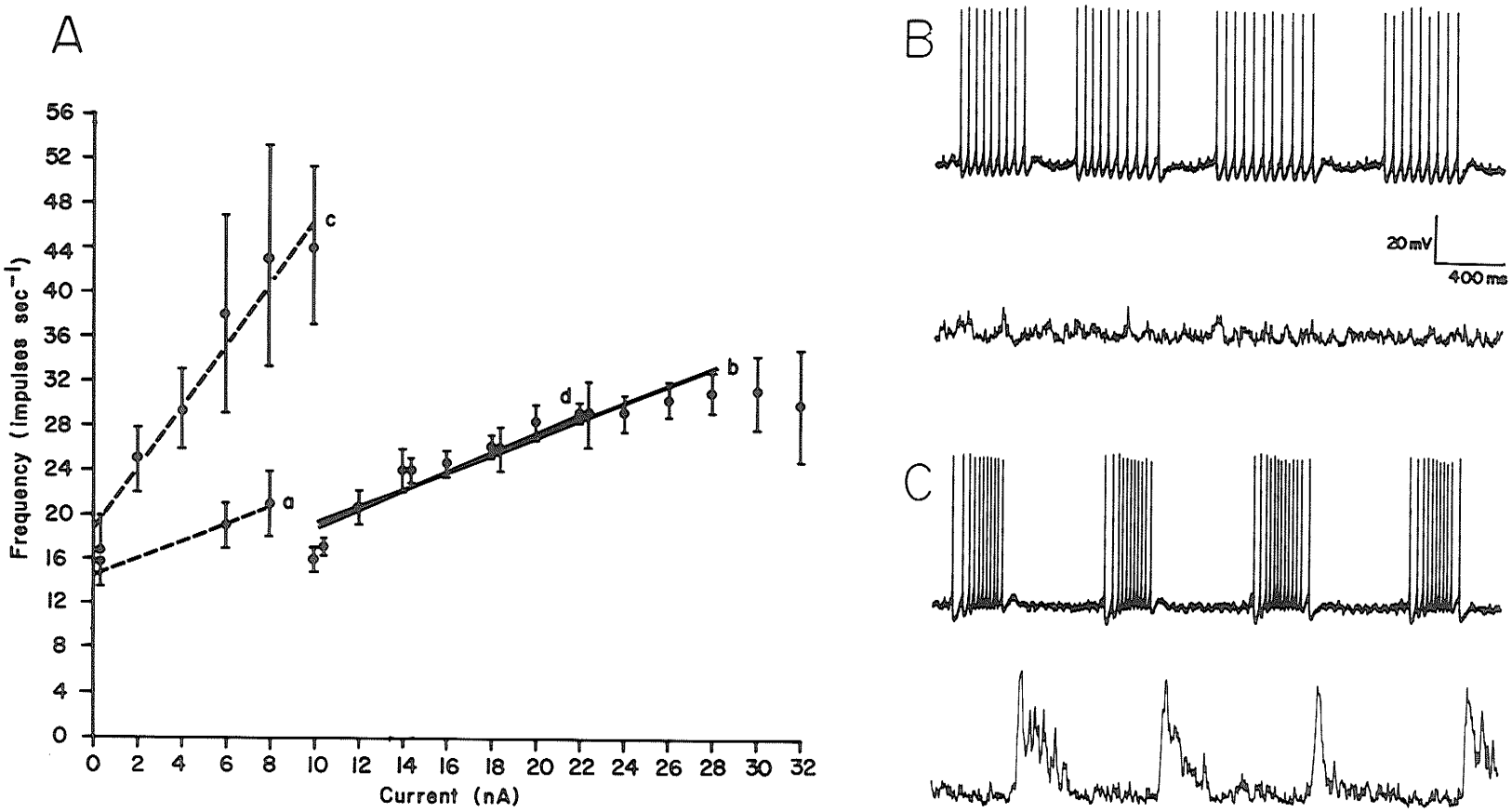

A
B
C
Frequency (Impulses sec⁻¹)
Current (nA)
0
4
8
12
16
20
24
28
32
36
40
44
48
52
56
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
a
b
c
d
20 mV
400 ms

Figure 15: Adaptation. **A:** Instantaneous firing frequencies are shown for this semimembranosus motoneurone during approximately 3 seconds of sustained depolarizing current injection (32 nA). Note the initial high rate of firing. **B:** The top graph depicts the time of occurrence of each action potential in the same motoneurone as in **A** for 8 step cycles during fictive locomotion. The mean step cycle duration was 738 msec (s.d. 184 msec), with the spiking lasting an average of 214 msec (s.d. 86 msec). Each cycle is repeated to show the rhythmic nature of fictive locomotion. The lower graph shows the instantaneous firing frequency plotted versus the time from the onset of the step cycle for the eight step cycles shown above. It can be clearly seen that there is no initial high rate of firing, and no consistent pattern of firing within each step cycle of the fictive locomotor run.

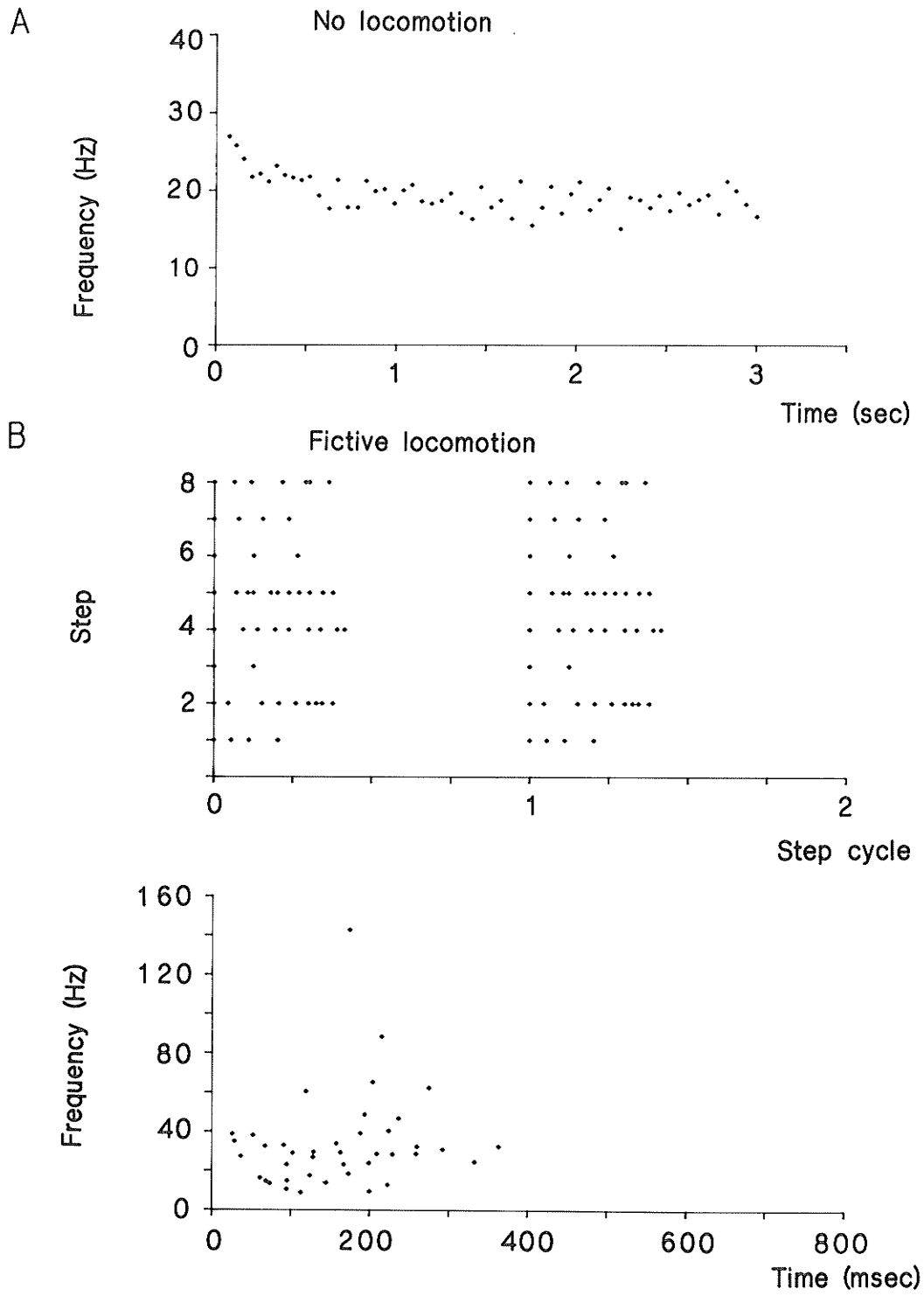

A
No locomotion
Frequency (Hz)
40
30
20
10
0
0
1
2
3
Time (sec)
B
Fictive locomotion
Step
8
6
4
2
0
1
2
Step cycle
Frequency (Hz)
160
120
80
40
0
0
200
400
600
800
Time (msec)

Figure 16: Same cell as in Figure 12. **A** shows the intracellular record during fictive locomotion for four of the fifteen step cycles used in the subsequent analysis. **B** shows the average firing frequency for the first nine inter-spike intervals; the bars indicate the standard deviations. Note the lack of adaptation. In **C-1** the spikes have been averaged dependent on their interspike interval; the gain is increased for these same traces in **C-2**. Note the lack of appreciable afterhyperpolarizations, especially during the shorter intervals. **D** depicts the firing level of each action potential plotted against the time of occurrence in the normalized step cycle. Note the depolarization of the firing level with time ($r = 0.657$). **E** plots the firing level versus the instantaneous firing frequency, and shows the lack of any relation ($r = 0.114$). The differentiated action potentials were averaged in the same method as in **C** and are shown in **F**. The initial segment spike can be distinguished from the soma-dendritic spike at all firing frequencies.

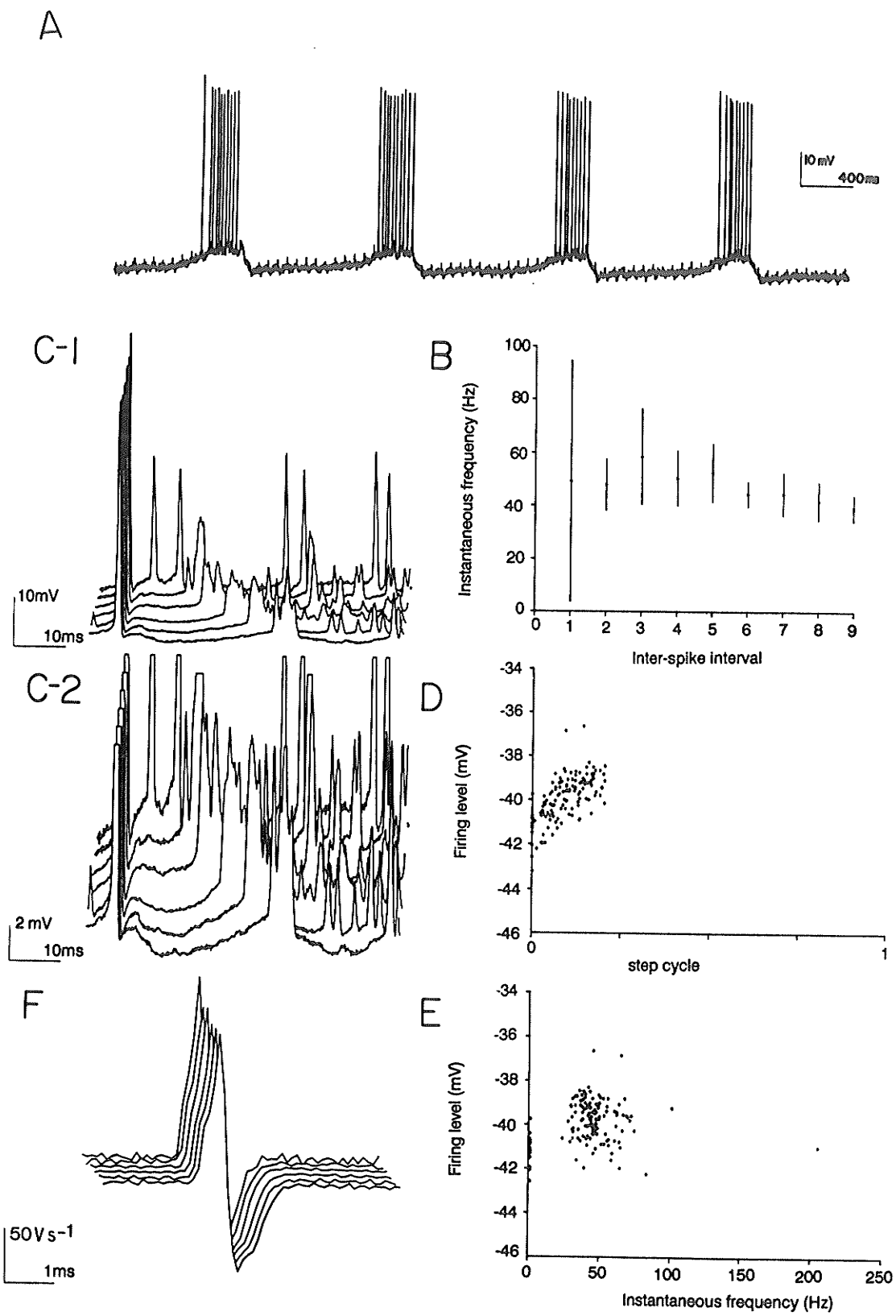
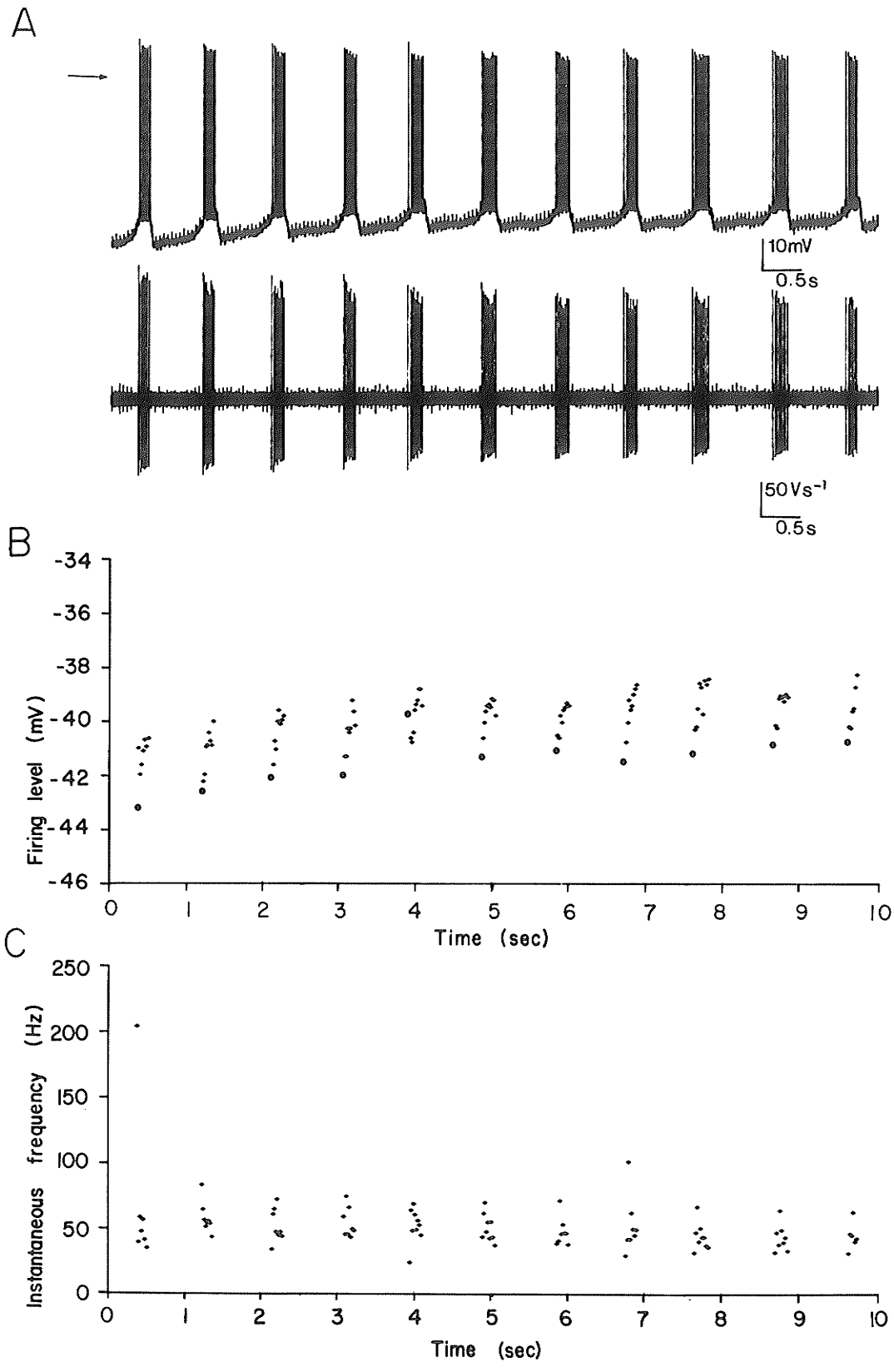

A
10mV
400ms
C-1
10mV
10ms
B
Instantaneous frequency (Hz)
Inter-spike interval
C-2
2 mV
10ms
D
Firing level (mV)
step cycle
F
50V s-1
1ms
E
Firing level (mV)
Instantaneous frequency (Hz)

Figure 17: Motoneurone accommodation. Eleven step cycles from the anterior biceps motoneurone shown in Figure 16. It can be seen in the intracellular record in **A** (top trace) that action potential overshoot decreases within each step cycle as well as throughout the run. The arrow represents a membrane potential of 0 mV. The differentiated intracellular record is shown in the lower portion of **A**, where it can be seen that the rates of rise of the action potentials show a parallel decrease. Plotted on the same time scale in **B** is the firing level of each action potential. The large filled circles represent the firing levels of the first action potential of each step cycle; the small diamonds represent the firing levels of the subsequent spikes. It can be seen that there is a progressive depolarization of the firing level within each step cycle, which in part recovers during the inactive phase. The result is that the firing level depolarizes throughout the run. However, there is no effect of these accommodative changes on the output of the motoneurone, as the instantaneous frequency, plotted in **C**, does not show any significant change throughout this locomotor episode.


A
10mV
0.5s
50Vs⁻¹
0.5s
B
Firing level (mV)
-34
-36
-38
-40
-42
-44
-46
0
1
2
3
4
5
6
7
8
9
10
Time (sec)
C
Instantaneous frequency (Hz)
250
200
150
100
50
0
0
1
2
3
4
5
6
7
8
9
10
Time (sec)

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