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On the regulation of repetitive firing in lumbar motoneurones during fictive locomotion in the cat

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Summary. Repetitive firing of motoneurones was examined in decerebrate, unanaesthetised, paralysed cats in which fictive locomotion was induced by stimulation of the mesencephalic locomotor region. Repetitive firing produced by sustained intracellular current injection was compared with repetitive firing observed during fictive locomotion in 17 motoneurones. During similar interspike intervals, the afterhyperpolarisations (AHPs) during fictive locomotion were decreased in amplitude compared to the AHPs following action potentials produced by sustained depolarising current injections. Action potentials were evoked in 10 motoneurones by the injection of short duration pulses of depolarising current throughout the step cycles. When compared to the AHPs evoked at rest, the AHPs during fictive locomotion were reduced in amplitude at similar membrane potentials. The post-spike trajectories were also compared in different phases of the step cycle. The AHPs following these spikes were reduced in amplitude particularly in the depolarised phases of the step cycles. The frequency-current (f-I) relations of 7 motoneurones were examined in the presence and absence of fictive locomotion. Primary ranges of firing were observed in all cells in the absence of fictive locomotion. In most cells (6/7), however, there was no relation between the amount of current injected and the frequency of repetitive firing during fictive locomotion. In one cell, there was a large increase in the slope of the f-I relation. It is suggested that this increase in slope resulted from a reduction in the AHP conductance; furthermore, the usual elimination of the relation is consistent with the suggestions that the repetitive firing in motoneurones during fictive locomotion is not produced by somatic depolarisation alone, and that motoneurones do not behave as simple input-output devices during this behaviour. The correlation of firing level with increasing firing frequency which has previously been demonstrated during repetitive firing produced by afferent stimulation or by somatic current injection is not present during fictive locomotion. This lends further support to the suggestion that motoneurone repetitive firing during fictive locomotion is not produced or regulated by somatic depolarisation. It is suggested that although motoneurones possess the intrinsic ability to fire repetitively in response to somatic depolarisation, the nervous system need not rely on this ability in order to produce repetitive firing during motor acts. This capability to modify or bypass specific motoneuronal properties may lend the nervous system a high degree of control over its motor output.

Key words: Afterhyperpolarisation – Motoneurone – Fictive locomotion – Repetitive firing – Cat

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Introduction

It has been known since 1929 that motoneurones can discharge repetitively in response to sustained asynchronous synaptic bombardment provided by sensory stimulation (Adrian and Bronk 1929; Denny-Brown 1929), with the rate of the discharge of the motoneurones dependent on the strength of the sensory stimulus. The ability to record intracellularly from mammalian neurones enabled further study of the repetitive discharge of motoneurones by affording the opportunity to stimulate the cells by the controlled injection of current through the microelectrodes. Investigators using this approach have elucidated the intrinsic properties of the motoneurone membrane responsible for the regulation of the autonomous repetitive discharge of alpha-motoneurones and have established that the post-spike hyperpolarising after-potential, or afterhyperpolarisation (AHP), regulates the repetitive firing behaviour seen under these conditions (Baldissera and Gustafsson 1971b; Calvin and Schwindt 1972; Granit et al. 1963a, b).

Few studies have been done to determine if the repetitive firing seen during sustained synaptic excitation is also regulated by the AHP (Granit et al. 1966; Kernell 1965a;

Schwindt and Calvin 1973a; Shapovalov 1972; Shapovalov et al. 1966). Schwindt and Calvin (1973a) suggested that the mechanisms of action of the two forms of excitation (synaptic and intracellular) are similar and additive, and that ". . . only the net driving current . . ." is important to produce repetitive firing in motoneurones, whose firing would then be regulated by the AHP. Although this was in agreement with some studies (Granit et al. 1966), examples of differences between the two methods of excitation in that they were not additive have also been demonstrated (Kernell 1965a; Shapovalov 1972). Thus, it would seem that in some situations 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.

The firing behaviour of motoneurones during one form of natural activation can be examined in the precollicular-postmammillary decerebrate cat induced to walk by stimulation of the mesencephalic locomotor region (see Shik et al. 1966). When the neuromuscular junctions of these cats are blocked, recordings from their peripheral muscle nerves display rhythmic activity similar to that observed during treadmill locomotion (Jordan et al. 1979). This pattern of neural activity which occurs in the absence of muscular contractions and thus without phasic afferent input is termed fictive locomotion (see Perret 1983). Using this controlled fictive locomotion preparation one can record intracellularly from lumbar motoneurones, which reveal rhythmic alternating periods of relative depolarisation and hyperpolarisation, known as locomotor drive potentials (Jordan 1983). These locomotor drive potentials have been shown to be the result of alternating excitatory and inhibitory synaptic inputs (see Jordan 1983). When a motoneurone repetitively fires during fictive locomotion, this firing occurs during the depolarised phases of the locomotor drive potentials. If the cell were to fire in response to its net excitatory synaptic current using its intrinsic repetitive firing mechanisms, then one would expect the repetitive firing behaviour to be the same as that observed during intracellular current injection. Indeed, Zajac and Young (1980) observed that the firing patterns of ventral root filaments during treadmill locomotion induced by stimulation of the mesencephalic locomotor region were consistent with the firing being ". . . controlled by the mechanism that produces afterhyperpolarisation . . . ." Similarly, Hoffer et al. (1987) showed that motoneurone firing patterns during locomotion can be mimicked by intracellular current injection. However, direct evidence demonstrating that motoneurones respond as these relatively simple input-output devices during a motor activity such as walking is lacking. The following experiments were performed to examine the nature of motoneurone repetitive firing during fictive locomotion induced by stimulation of the mesencephalic locomotor region. Preliminary data have been presented (Brownstone et al. 1986, 1987; Jordan and Shefchyk 1984; Brownstone 1989). The results demonstrate that during fictive locomotion the AHP is reduced in amplitude, there is no relation between frequency of firing and current injected into the soma, that expected changes in action potential firing level are not seen, and therefore that the repetitive firing in motoneurones is not produced by simple somatic depolarisation.

Methods

Preparation

The data were obtained from motoneurones of cats of either sex weighing between 2.0 and 4.0 kg. Each animal was anaesthetised with an oxygenated mixture of nitrous oxide and halothane, the blood pressure monitored through common carotid cannulae, at least one vein cannulated for delivery of fluids and drugs intravenously, and the trachea 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 both sciatic nerves were dissected free 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 with soleus, flexor digitorum longus, and tibialis anterior. In addition, branches of the femoral nerve on one side were 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 procedure 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, each cat was suspended in a stereotaxic headframe with their limbs pendant. A back pool was formed, filled with mineral oil, and maintained near 38°C 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 by sectioning the brainstem from just rostral to the superior colliculus dorsally to just caudal to the mammillary bodies ventrally. The tissue rostral to this transection was removed from the animal, and the anaesthesia 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 around 4%. Periodic injections of gallamine were used throughout the experiments to ensure a state of flaccid paralysis. Dextran was administered intravenously to maintain plasma volume. If an animal became hypotensive during the experiments, noradrenaline was infused intravenously at a rate titrated to maintain a reasonable mean arterial pressure.

Locomotion was initiated by cathodal stimulation (50–200 μA, 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 et al. 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 MΩ, tip diameter less than 2 μm) were obtained from lumbar alpha-motoneurones identified by antidromic stimulation and/or time of activity in 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; A.R. Vetter Co.) 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 adequately preserve the action potential waveforms, the d.c. traces were digitised 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 (10 or 20 ms time constant); these signals were then digitised with a sampling rate of 200 Hz.

Sustained current injection

Sustained depolarising 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 depolarised phase of the locomotor drive potential. By using the brainstem stimulus as a trigger for capturing a window of motoneurone activity, the post-stimulus membrane potential could be studied for each stimulus independently. In some cells, the short-latency excitatory post-synaptic potentials (e.p.s.p.s) seen following the brainstem stimulus during the depolarised phase of the locomotor drive potentials (Shefchyk and Jordan 1985) would elicit action potentials routinely. In other cells, few action potentials were seen immediately following each stimulus; by far, the majority of action potentials were not directly time-locked with the stimulus (see Severin, Shik and Orlovskii 1967). These action potentials will be referred to as "locomotor" spikes. Only cells in which these locomotor action potentials predominated were considered to be repetitively firing and used for further analysis. In some cases, post-stimulus time histograms were constructed to further demonstrate that the action potentials seen were not a result of the short-latency e.p.s.p.s.

Digitised action potentials were averaged to facilitate comparing the inter-spike trajectories seen during sustained current injection with those seen during fictive locomotion. In order to compare the AHPs during similar interspike intervals in the two situations, action potentials were sorted and averaged based on their succeeding interspike intervals. Due to the fact that there are no identifiable baselines during repetitive firing in either fictive locomotion or current injection, accurate quantification of AHP amplitude differences was impossible. The AHPs were visually compared using the firing level of the action potential as a reference point.

Short pulse current-evoked action potentials

Short pulses (0.5 ms) of depolarising current were injected into 10 motoneurones to elicit action potentials every 100 to 200 ms 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 AHPs that may be related to the phase of the step cycle in which the spike occurred. If a locomotor spike appeared either during the AHP of the evoked action potential or just prior to the evoked action potential, the evoked spike was eliminated from further analysis. The action potentials could be sorted and averaged by two different schemes (Shefchyk and Jordan 1985b) in order to determine patterns in the observed changes in AHPs. 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. The action potentials within each bin were averaged, and comparisons made between the AHPs in the depolarised and hyperpolarised 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 AHP were related to changes in the membrane potential, or whether changes were associated 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 depolarised and hyperpolarised phases, while for the latter type, they will be called active and inactive phases. The AHP amplitude measurements were then made on d.c. recorded waveforms. For the AHPs following the short pulse current-evoked spikes, the amplitudes were determined in the standard method by measuring the amount of deflection below the prespike membrane potential (Coombs et al. 1955).

Frequency-current relation

By injecting various amounts of sustained depolarising current, the frequency-current (f-I) relation of motoneurones could be determined in the absence of locomotor activity (Granit et al. 1963a). 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 examined during fictive locomotion. To do this, various currents (up to 24 nA) were injected into the motoneurones during fictive locomotion, sustained throughout several step cycles, and the instantaneous firing rates (in the active phases only) measured. The maximum current injected was that which evoked a large degree of accommodation of the motoneurone, as judged by the action potential amplitude (24 nA in the presence of locomotor activity, and 50 nA in its absence). This f-I relation during fictive locomotion could then be compared with that obtained at rest.

Firing level

To assess the firing levels of action potentials in a motoneurone, the intracellular signal was digitally differentiated. This differentiated signal was used as a trigger 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 (i.e. a depolarisation of one millivolt in one 100 microsecond sampling interval). All triggered events were examined in order to ensure that only action potentials were discriminated. When calibration pulses (2 mV, $dV/dt = 20\ Vs^{-1}$) were present, slightly higher trigger levels (not more than $25\ Vs^{-1}$) were used in order to exclude triggering from these events. If the trigger then 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 digitised point 100 to 300 ms 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.

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 flexor and extensor activity in a rhythmic pattern similar to that seen during overground locomotion (see Fig. 1A).

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

Although during any particular trial the duration of the step cycle was very constant, this duration varied considerably between trials in different animals, ranging from 442 to 2730 ms (average: 1037 ms) per step cycle. During recording sessions in 18 of the cells, step cycle duration was less than one second. This compares favourably with stepping in the intact animal.

If one examines the rates at which motoneurones discharge during fictive locomotion, it is clearly seen that motoneurones fire very rapidly 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 (e.g. Fig. 7) the average rate of firing during fictive locomotion was 45 impulses s^{-1} with 6 of the 7 motoneurones firing faster than 40 impulses s^{-1}. Conversely, in response to large amounts of sustained depolarising current injection (28 to 50 nA), the maximum rates at which these motoneurones discharged were 21 to 40 impulses s^{-1} (mean: 30 impulses s^{-1}). Even with these high amplitude current injections, the 6 of 7 motoneurones would not fire at the rates seen in fictive locomotion.

In sharp contrast to the large amounts of depolarising current injection required to produce high rates of repetitive firing in motoneurones in the absence of fictive locomotion, it was noted in many motoneurones that very small amounts of sustained hyperpolarising current injection (less than 6 nA) would completely eliminate the repetitive firing during fictive locomotion. In the cell shown in Fig. 1, for example, it can be seen that the injection of only 4 nA of hyperpolarising current completely abolished its repetitive firing during fictive locomotion.

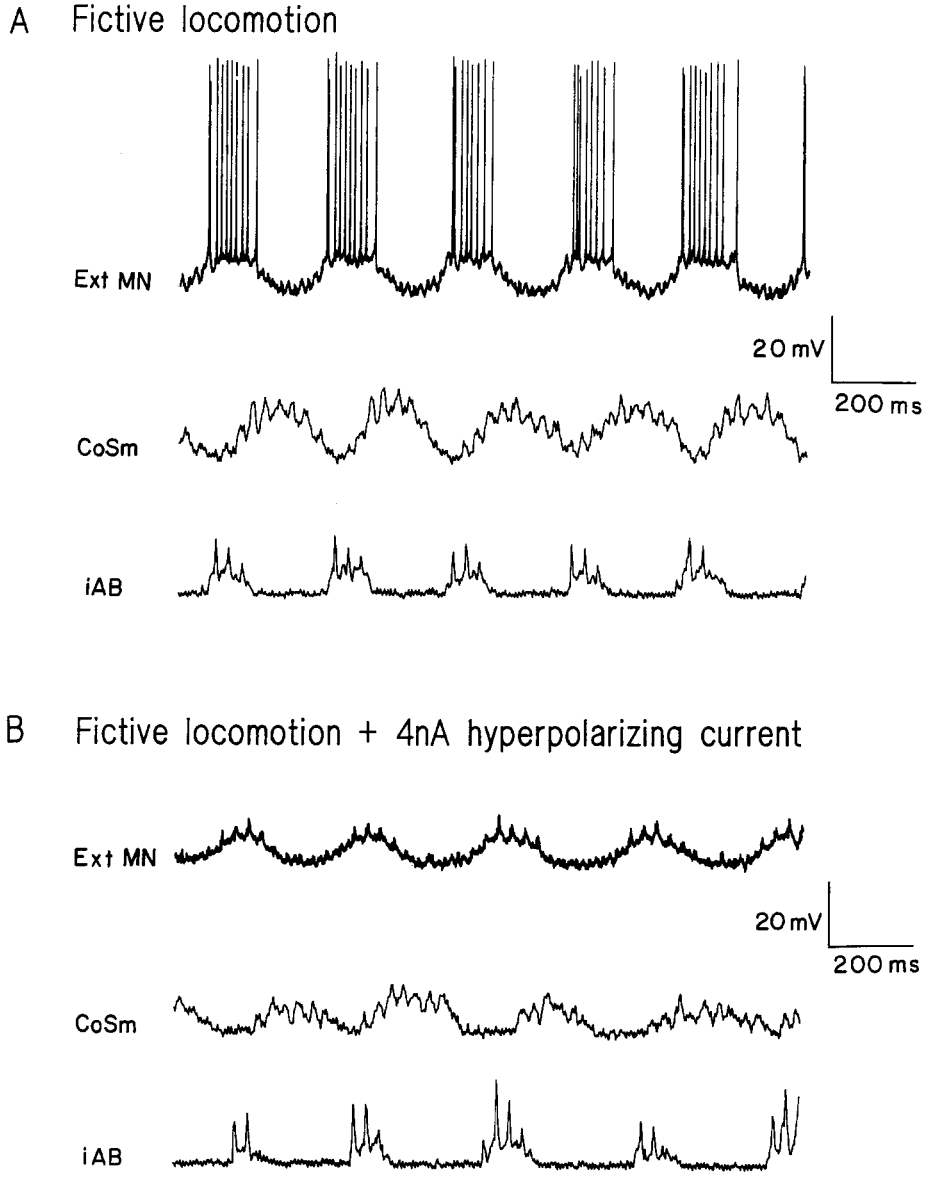

Fig. 1A, B. Effects of small amounts of hyperpolarising current injection on the repetitive firing of motoneurones during fictive locomotion. Depicted is an extensor motoneurone (Ext MN) recorded during fictive locomotion **A**, and subsequently with 4 nA sustained hyperpolarising current injection **B**. Note the complete disappearance of repetitive firing in **B**. Also note that the large e.p.s.p.s seen in **B** are evoked by the stimulation of the brainstem (Shefchyk and Jordan 1985) and are not related to the production of repetitive firing. The electroneurograms shown are the contralateral semimembranosus (CoSm) and the ipsilateral anterior biceps (iAB)

Changes in inter-spike membrane potential trajectories during fictive locomotion

The inter-spike membrane potential trajectories during fictive locomotion were compared to those seen during sustained depolarising current injection in the absence of locomotion in 17 cells. The intracellular record from one such cell is shown in Fig. 2A during 35 nA of repeated depolarising current injections. Figure 2B illustrates the repetitive firing of this motoneurone during fictive locomotion. 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 inter-spike trajectories observed during fictive locomotion are very different from those observed during sustained current injection and that during fictive locomotion similar interspike intervals often display completely different interspike trajectories (arrows). Differences in the AHPs during fictive locomotion compared to those during sustained depolarising current injection were observed in all of the 17 motoneurones examined. It is important to note, however, that although the inter-spike trajectories are different, it remains to be seen whether the AHP conductance has changed or if the AHP trajectory is simply masked by excitatory synaptic current.

Systematic comparison of AHP amplitudes in these 17 cells revealed a consistent decrease in AHP amplitude during fictive locomotion when compared to sustained current injection. To facilitate this comparison, action potentials were sorted and averaged based on their succeeding interspike intervals so that the trajectories compared represented similar intervals. In the semimembranosus motoneurone shown in Fig. 3, near-maximal rates produced by current injection (Fig. 3A) are compared with some of the slower rates during fictive locomotion (Fig. 3B), all with interspike intervals of 35 to 37 ms. When the averages of these traces are superimposed using the mean action potential firing level as a point of reference, it

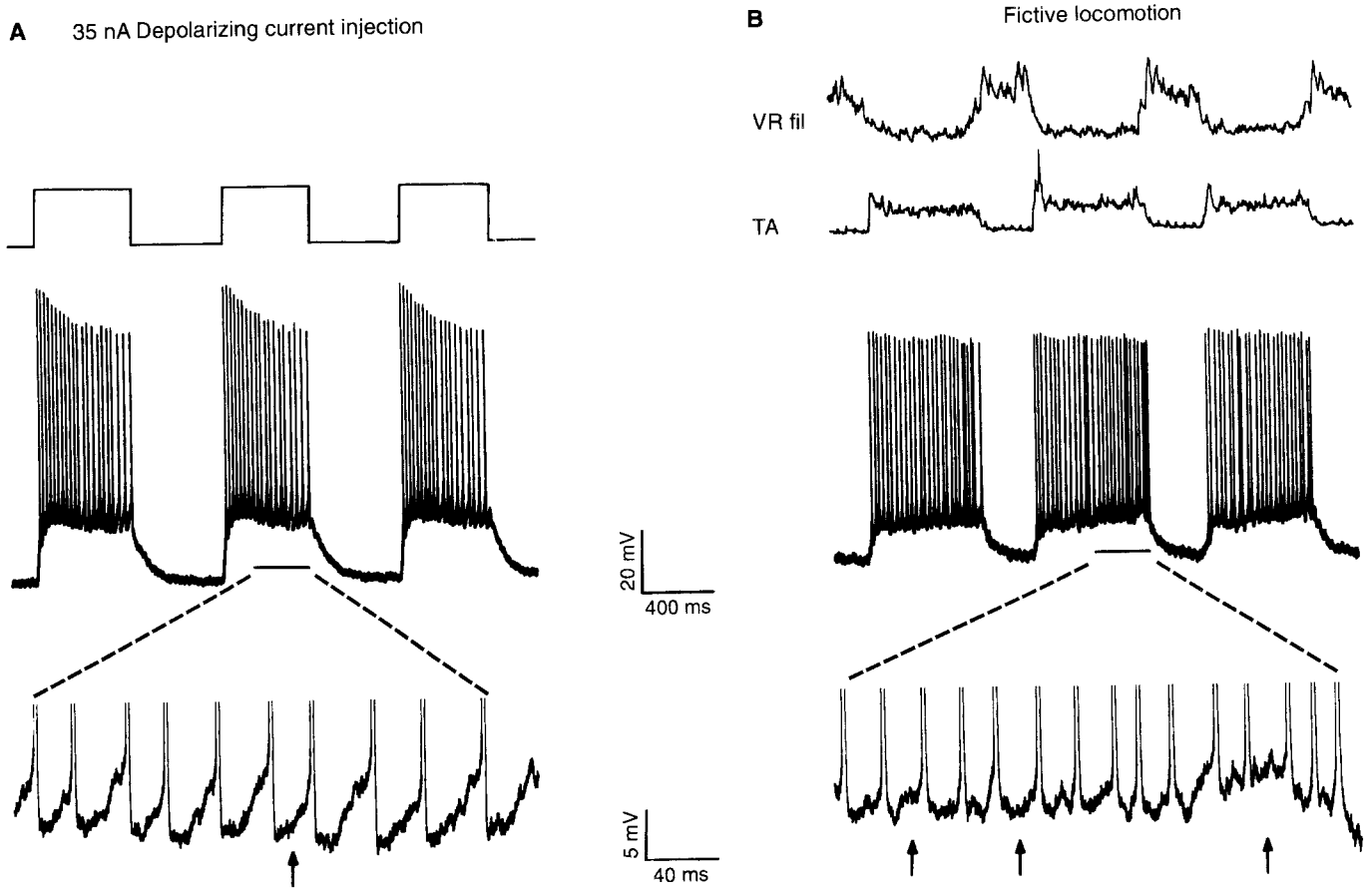

Fig. 2A, B. Comparison of repetitive firing during square wave depolarising current injection *A* and during fictive locomotion *B* in a motoneurone. **A** The top trace shows the time of injection of repeated square wave depolarising current (35 nA). The next trace is the intracellular recording, a portion of which is amplified in voltage and expanded in time in the lower trace. The membrane potential deflection in the middle trace of *A* does not represent the true membrane potential deflection, due to electrode rectification. Mean firing rate is 40 (s.d. 8) impulses s^{-1}. **B** Fictive locomotion. The top two traces are a left seventh lumbar segment ventral root filament (VR fil) and the left sided tibialis anterior e.n.g. (TA) digitised as explained in text. The next two traces are the intracellular records as in *A*. Mean firing rate is 51 (s.d. 27) impulses s^{-1}. Note that interspike trajectories can be quite variable even when inter-spike intervals are of similar duration (arrows)

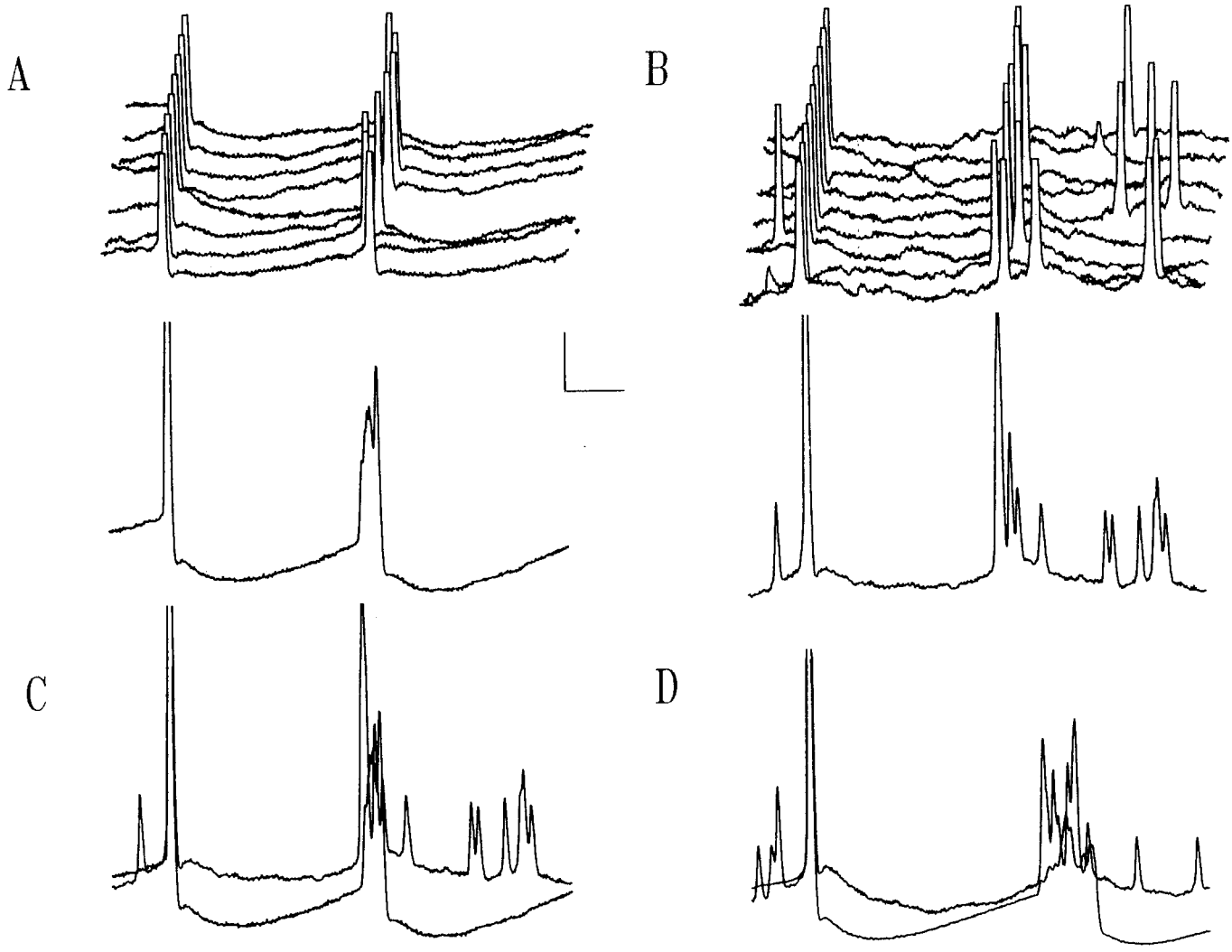

Fig. 3A–D. Comparison between post-spike trajectories of similar interspike intervals in repetitive firing in a semimembranosus motoneurone during sustained depolarising current injection and fictive locomotion. **A** Action potentials with post-spike intervals of 35 to 37 ms produced by the sustained injection of 28 to 50 nA depolarising current. The upper portion shows 8 action potentials which have been separated and overlaid. The lower portion shows the average of these 8 spikes. **B** Repetitive firing during fictive locomotion in the same cell. The upper part shows 9 spikes as in *A*. The lower portion is the average of these 9 spikes. **C** The two averages overlaid, using the firing level as a reference voltage point. **D** Similar superimposed averages from the same motoneurone but with post-spike intervals of 45 to 50 ms. The calibration is 10 mV for the top of *A* and *B* and 5 mV for the remaining sweeps; 10 ms for all sweeps

is quite clear that the AHP amplitude is significantly decreased during fictive locomotion (Fig. 3C). This was seen at all comparable interspike intervals; the averages from the 45 to 50 ms intervals are shown superimposed in Fig. 3D.

AHP changes in short pulse current-evoked action potentials

The observed reduction in the AHP amplitude may occur uniformly throughout the locomotor episode, may occur only during the depolarised phases of the locomotor drive potentials, or 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) depolarising current pulses were injected into 10 cells to elicit action potentials every 100 to 200 ms 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 AHPs compared to those seen following action potentials evoked at rest. In 5 of these 7 cells, the average AHPs 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 to 59%). One such average from an anterior biceps motoneurone is shown in Fig. 4, where the membrane potential during the inactive phase of the step

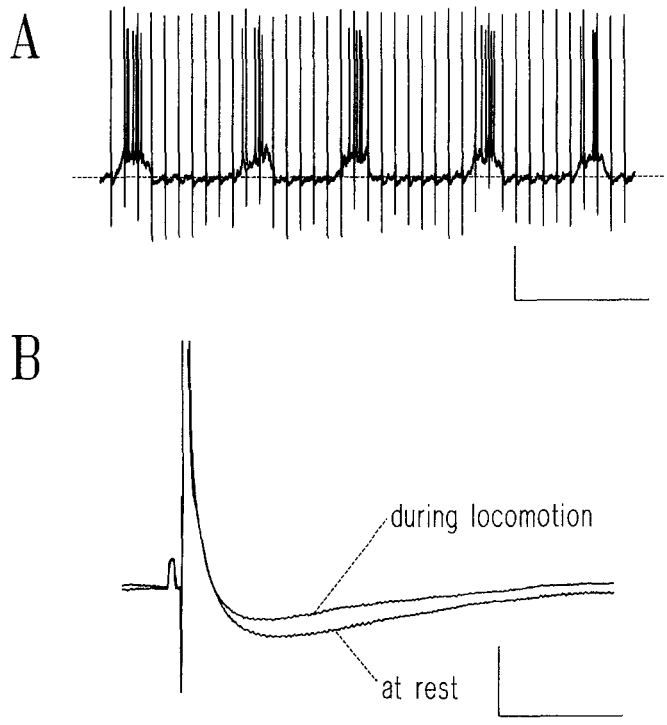

Fig. 4A, B. Comparison of AHP following spikes evoked at rest and at a similar voltage during fictive locomotion. **A** Intracellular record showing 5 step cycles with evoked action potentials throughout. The dotted line indicates resting potential. **B** Averages of these action potentials showing a reduction in AHP amplitude during fictive locomotion (99 spikes) compared with at rest (58 spikes). There is a 2 mV 1 ms calibration pulse preceding the averaged spikes. The calibration for *A* is 20 mV, 1 s and for *B* is 5 mV 20 ms

cycle was near the resting potential (Fig. 4A). The average AHP following spikes evoked at this membrane potential are superimposed in Fig. 4B, where it can be seen that the AHP is decreased in amplitude during fictive locomotion. However, in the two remaining cells, the mean AHPs were increased in amplitude at membrane voltages similar to resting potential during fictive locomotion (18% and 38%).

In 3 motoneurones, the effects of stimulation of the mesencephalic locomotor region on the AHP amplitude was examined in the few seconds following the start of brainstem stimulation and before the onset of fictive locomotion. In these motoneurones, there was little or no change in AHP amplitude prior to the onset of locomotion. However, once the locomotor activity began, the AHP amplitude decreased (cf. Fig. 6, where the AHP returns despite continued brainstem stimulation).

Effects of membrane potential on AHP amplitude modulation. In order to examine any modulation of the AHPs during fictive locomotion, the evoked action potentials were sorted into six bins depending on the prespike membrane potential. Although the AHPs were usually decreased in amplitude during fictive locomotion, they did show a progressive increase in amplitude as the membrane potential became more depolarised (Fig. $5A_1$). This modulation of AHP amplitude would be expected because the membrane potential is further from the AHP reversal potential during the depolarised phase than during the hyperpolarised phase (Coombs et al. 1955). Three of the 10 cells showed minor variations in this, with the AHP amplitude decreasing in one bin as the cell depolarised, then increasing again with further depolarisation.

AHP 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 8 cells had a decrease in the average AHP amplitude during at least a portion of the active phase as compared to the inactive phase of the step cycle (Fig. $5A_2$, B_2). This decrease had no consistent timing within the active phase of the fictive step cycle from cell to cell. The decreased amplitude was not apparent when the averaging was based on pre-spike membrane potential (see above) because it was not decreased for the whole of the active phase. The fact that the AHP amplitude is decreased at a point when the membrane potential is further from the potassium equilibrium potential during only a portion of the active phase of the step cycle is consistent with there being a modulation of the AHP amplitude within each single step cycle rather than it being uniformly reduced throughout the locomotor episode. It should be noted that the decrease in AHP amplitude shown here does not necessarily reflect a decrease in the AHP conductance. There may be a masking of the AHP outward current by concomitant inward current (such as excitatory synaptic current or a voltage-dependent non-inactivating conductance) or perhaps by an increase in the membrane input conductance (shunting). This point will be discussed further below.

Effects of locomotor spiking on AHP amplitude modulation. A difference was noted in the modulation of the amplitudes of the AHPs 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 in Fig. 5. It can be seen that when this cell was not exhibiting locomotor spiking (Fig. 5A, C), the average AHP amplitude varied passively with the membrane potential (Fig. $5A_1$). This modulation can also be seen when the action potentials are averaged dependent on their timing in the fictive step cycle (Fig. $5A_2$), being largest during the active phase of the cycle, at which time the membrane was most depolarised. However, when the same cell was repetitively firing during fictive locomotion (Fig. 5B, D), there was no obvious relation between AHP amplitude and membrane polarisation (Fig. $5B_1$). In this case, the averaged AHP following short pulse current-evoked spikes in the active phase of the step cycle had the smallest amplitude (Fig. $5B_2$, trace 2).

To judge the significance of this modulation of amplitude, the AHP amplitude and motoneurone membrane potential were both plotted versus time in the step cycle (Fig. 5C, D). When the motoneurone was not repetitively

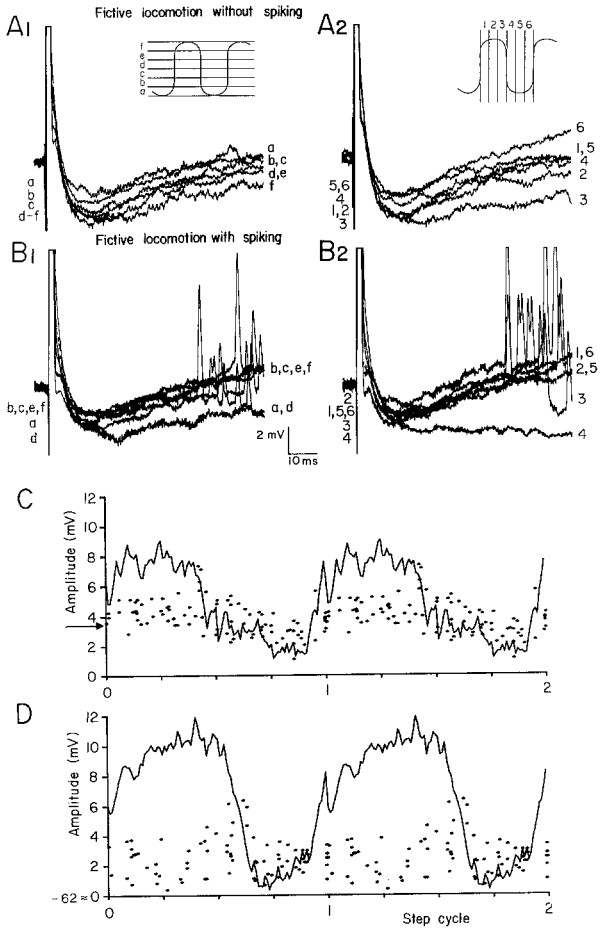

Fig. 5A–D. Short pulse current-evoked action potentials throughout the step cycle in an extensor motoneurone. **A** and **C** are data from 9 step cycles when the motoneurone was not firing repetitively during fictive locomotion. During a later fictive locomotor trial shown in **B** and **D** (data from 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 *A1* and *B1*, and based on time in the step cycle in *A2* and *B2*. Note that the traces have been vertically shifted to align the pre-spike voltages in order to facilitate comparisons of AHP amplitude. The letters and numbers to the left of the averages correspond to the traces at the peaks of the AHPs 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 AHP amplitudes throughout the locomotor episode (dots). The membrane potential is averaged based on the step cycle, and then plotted twice to illustrate its cyclic nature during fictive locomotion. The ordinates in these graphs are for the absolute AHP amplitudes as well as for the relative membrane potential. The arrow in **C** indicates the resting AHP amplitude prior to the locomotor runs. 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. Note that some AHP amplitudes measured at the end of the active phase of the step cycle may be artefactually high because of the repolarisation of the membrane at this time. Also note that although the amplitude of the locomotor drive potential appears larger in **D** than in **C**, the presence of repetitive spiking in **D** precludes accurate determination of the amplitude of this potential. Therefore the average depolarisation of the membrane potential will be exaggerated

firing (Fig. 5C), the changes in AHP amplitude paralleled the changes in membrane potential as would be expected for purely a passive dependence on the membrane potential. However, when the motoneurone was repetitively firing (Fig. 5D), the AHP amplitudes did not follow the changes in membrane potential, but rather were very often reduced during a portion of the depolarised phase.

Effect of locomotor activity on AHP amplitude

Some motoneurones do not fire rhythmically during fictive locomotion. In one such cell, shown in Fig. 6, constant 20 nA intracellular depolarising bias current was injected throughout the illustrated time period. The rhythmic depolarisations of the membrane potential stopped following the first locomotor drive potential shown (Fig. 6A), yet repetitive firing was sustained due to the bias current. During this last locomotor drive potential, there was, on the average, no obvious AHP following the locomotor spikes (Fig. 6B). However, following this, the AHP returned (Fig. 6C) even though fictive locomotion persisted with no apparent change in the rhythmic activity of the other seventh lumbar segment motoneurones represented in the ventral root filament and electroneurographic records. It appears that the reduction in AHP amplitude is related to the presence of locomotor drive potentials in the motoneurone.

Frequency-current relation

Although the AHP trajectory is clearly depressed during fictive locomotion, the contribution of the AHP conductance to the production of repetitive firing can be further established by testing the frequency-current (f-I) relationship of motoneurones. If the AHP conductance is not affected during locomotion and the repetitive firing is produced by membrane depolarisation, 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 and Calvin 1973a; Shapovalov et al. 1966). Alternatively, if the AHP conductance were reduced, one might expect an increase in the slope of the f-I relation. However, *if the AHP conductance is not involved in the regulation of repetitive firing, then there should be no relation between the frequency of firing and the current injected during fictive locomotion.* The f-I relation was therefore examined in 7 motoneurones; a typical result is illustrated in Fig. 7. In the absence of fictive locomotion, when the steady-state mean frequency was plotted versus the current injected (Fig. 7A), these cells displayed only primary ranges of firing (see Baldissera and Gustafsson 1971a). The f-I relations had slopes averaging 0.74 impulses s^{-1} nA^{-1} (range 0.44 to 1.3). These slopes are comparable to the lower end of the range found by Kernell (1965b) in pentobarbitone-anaesthetised 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 to 0.94). The minimum current

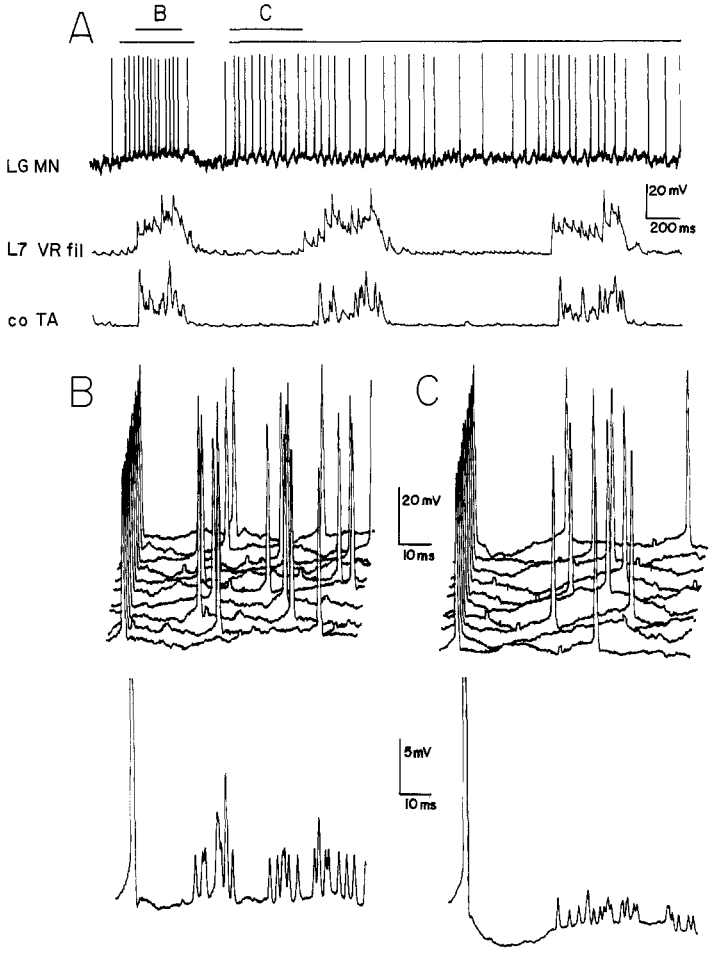

Fig. 6A–C. **A** A lateral gastrocnemius motoneurone (LG MN) which stopped showing locomotor activity, even though fictive locomotion persisted, as is evident from the accompanying e.n.g. activity (ipsilateral seventh lumbar segment ventral root filament [L7 VR fil] and contralateral nerve to tibialis anterior [TA]). The motoneurone continues to fire repetitively as 20 nA of depolarising 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 Fig. 3. 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 AHP once the locomotor activity in this motoneurone stops

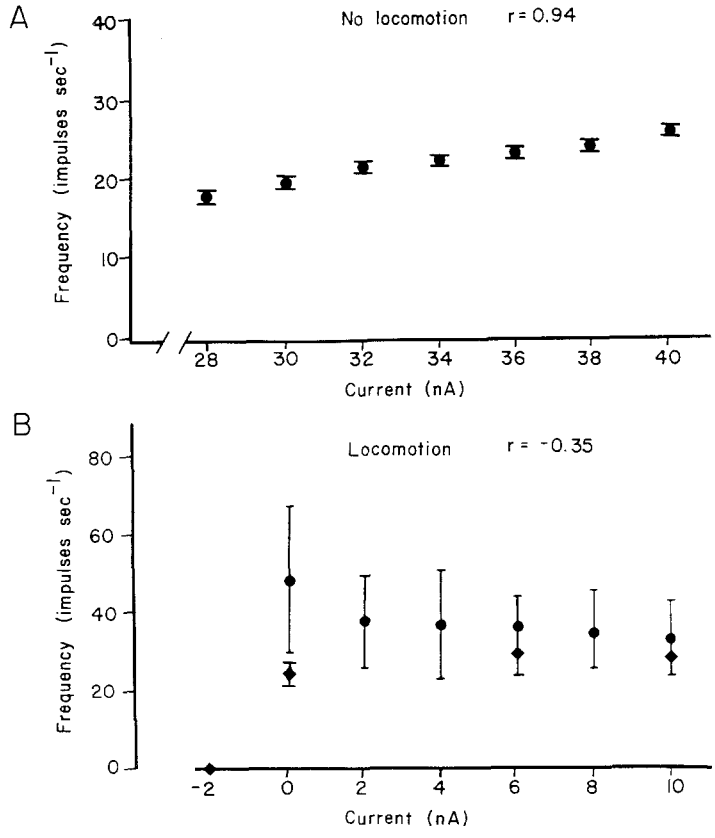

Fig. 7A, B. The frequency-current relations of the anterior biceps motoneurone shown in Fig. 9 prior to (**A**) and during (**B**) fictive locomotion. In *A* the filled circles represent the mean steady state firing frequency. The bars in both **A** and **B** represent the standard deviations. The slope of the relation in A is 0.62 impulses s^{-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. The stimulation of the brainstem was at a rate of 19 Hz; a post-stimulus time histogram revealed that the action potentials were not associated with the stimulus. 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 hyperpolarising current eliminated repetitive firing altogether

needed to induce repetitive firing in a motoneurone averaged 21.7 nA (range: 10 to 32 nA). The f-I relationship was then tested during fictive locomotion. During fictive locomotion, the amount of current injected into motoneurones without blocking the action potentials was always less than or equal to that injected in the control condition. Figure 7B (circles) illustrates that during the repetitive firing produced during fictive locomotion in the same motoneurone as that illustrated in Fig. 7A, there was 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 (Fig. 8), near zero (mean $r = -0.11$, range -0.35 to 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 synaptic current in the motoneurone is of such high magnitude that the motoneurone fires at maximal rates, and would thus be unresponsive to further current provided through the microelectrode. If this were the case, then the injection of hyperpolarising current into the cell should counteract this excitatory synaptic current, decrease the firing frequency, and thus reveal a f-I relation similar to that produced in the absence of fictive locomotion. However, as shown previously (see Fig. 1), in those motoneurones examined, the injection of very small amounts of hyperpolarising current completely eliminates the repetitive firing seen in fictive locomotion. In a second f-I trial during fictive locomotion in the motoneurone shown in Fig. 7 (Fig. 7B, diamonds), 2 nA of hyperpolarising current completely abolished the repetitive firing. This result demonstrates that the repetitive firing of motoneurones during fictive locomotion is not produced simply by somatic depolarisation secondary to excitatory synaptic current.

In one motoneurone, the Pearson correlation coefficient was not near zero during fictive locomotion (Fig. 8A). At first, although the motoneurone was receiving rhythmic input as was evident from the rhythmic bursts of repetitive firing, the e.n.g.s were quite silent (Fig. 8B; left tibialis anterior e.n.g.). During this time, the slope of the f-I relation (0.70 impulses s^{-1} nA^{-1}; $r = 0.653$; Fig. 8A, line a) was similar to that observed in the absence of locomotor activity (0.78 impulses s^{-1} nA^{-1}; $r = 0.892$; Fig. 8A, line b). Subsequently, the quality of locomotion improved (Fig. 8C; left tibialis anterior e.n.g.), the AHP was reduced but not eliminated, and the slope of the f-I line increased dramatically (to 2.9 impulses $s^{-1}nA^{-1}$; $r = 0.738$; Fig. 8A,

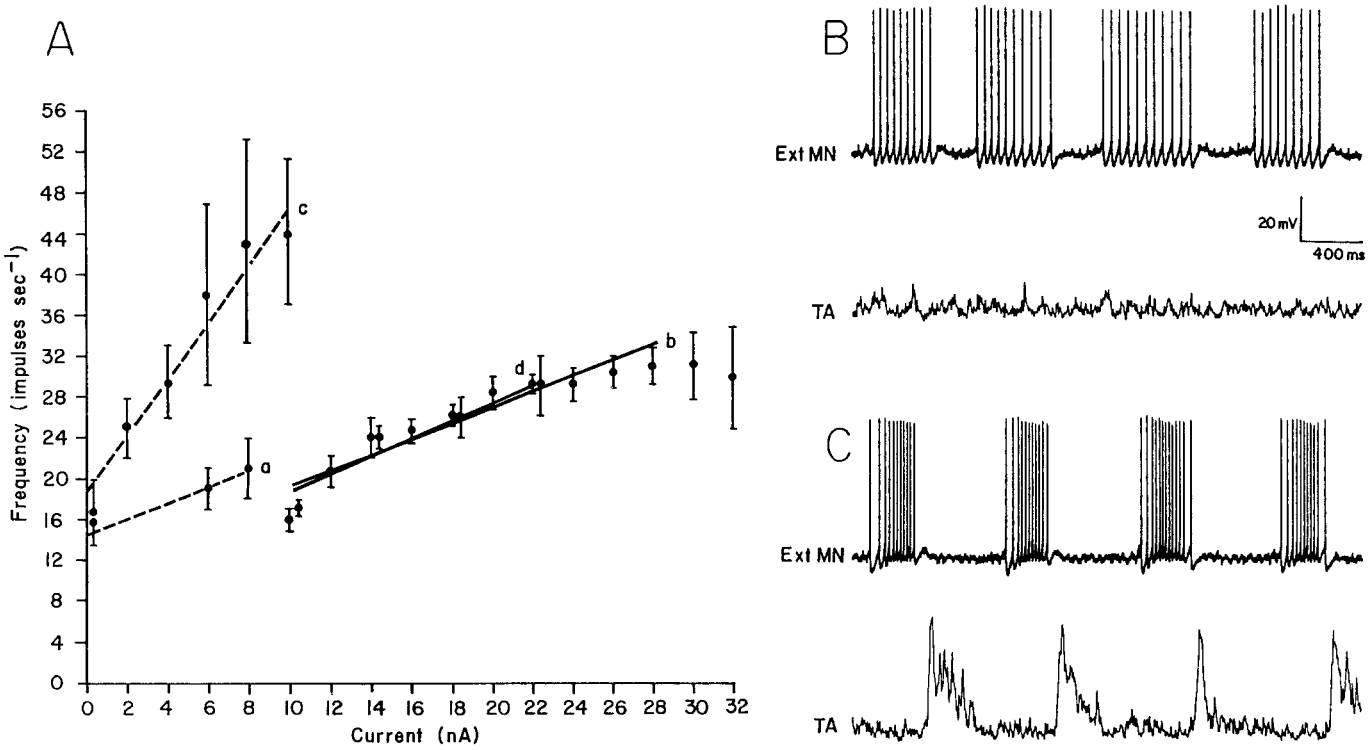

Fig. 8A–C. **A** The frequency-current relations of an extensor motoneurone (Ext MN). As in the previous figure, the data points represent the mean steady state firing frequencies, while the bars represent the standard deviations. The relation during an initial locomotor trial (line a) when locomotor activity was poor, as judged by the e.n.g.s (panel *B*: during 6 nA current injection) has the same slope as the control (line b). With the improvement of locomotor activity (panel *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). Note the regular 2 mV calibration pulses in *B*, fixed in time in relation to the brainstem stimulation. The e.n.g.s shown in **B** and **C** are from the nerve to the left tibialis anterior muscle (TA); and are shown with equal gain. The scale bar for *B* and *C* is 20 mV for the intracellular records, and 400 ms

line c). Following this episode of fictive locomotion, the f-I slope was not appreciably different than the initial control (0.86 impulses s^{-1} nA^{-1}; $r=0.848$; Fig. 8A, line d). This suggests that in this motoneurone, the AHP conductance did play a role in the regulation of repetitive firing during fictive locomotion. However, it must have been reduced to a fraction of its control conductance.

Firing level

Another way to demonstrate that repetitive firing during fictive locomotion is not produced by net somatic depolarisation is to examine variations in action potential firing level. Certain characteristic changes in firing level have been previously observed: the firing level ought to become increasingly depolarised (a) the later in a spike train the action potential occurs, and (b) the shorter the preceding inter-spike interval. This has been shown during repetitive firing produced either by intracellular current injection (Barrett et al. 1980; Schwindt and Crill 1982) or by afferent stimulation (Kolmodin and Skoglund 1958).

Eight motoneurones were studied to determine changes in their firing level during fictive locomotion. One of these motoneurones is depicted in Fig. 9. Indeed, a tendency for the firing level to become more depolarised later in the train was evident (Fig. 9B). However, there was no relation between firing level and instantaneous firing frequency (Fig. 9C). Schwindt and 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 (Fig. 9D). Similar results to those shown in Fig. 9 were seen in all eight motoneurones examined. This lack of relationship between firing level and instantaneous firing frequency therefore further supports the hypothesis that repetitive firing during fictive locomotion is not produced by net somatic depolarisation, such as the repetitive firing either seen during intracellular current injection (Schwindt and Crill 1982) or that induced by afferent stimulation (Kolmodin and Skoglund 1958).

Discussion

Fictive locomotion

Data have been presented which have demonstrated that the repetitive firing of motoneurones during fictive locomotion is not produced by simple somatic depolarisation. The AHP is reduced in magnitude, there is no relation between the firing frequency and the current injected into the soma, and there is no change in firing level with increasing firing frequency.

The rates at which motoneurones discharge during fictive locomotion were usually greater than 40 impulses s^{-1} (mean: 45 impulses s^{-1}; range 22–57 impulses s^{-1}). These rates are slightly higher than the rates reported for motoneurone firing during treadmill locomotion in either intact cats (Hoffer et al. 1987), or decerebrate cats with stimulation of the mesencephalic locomotor

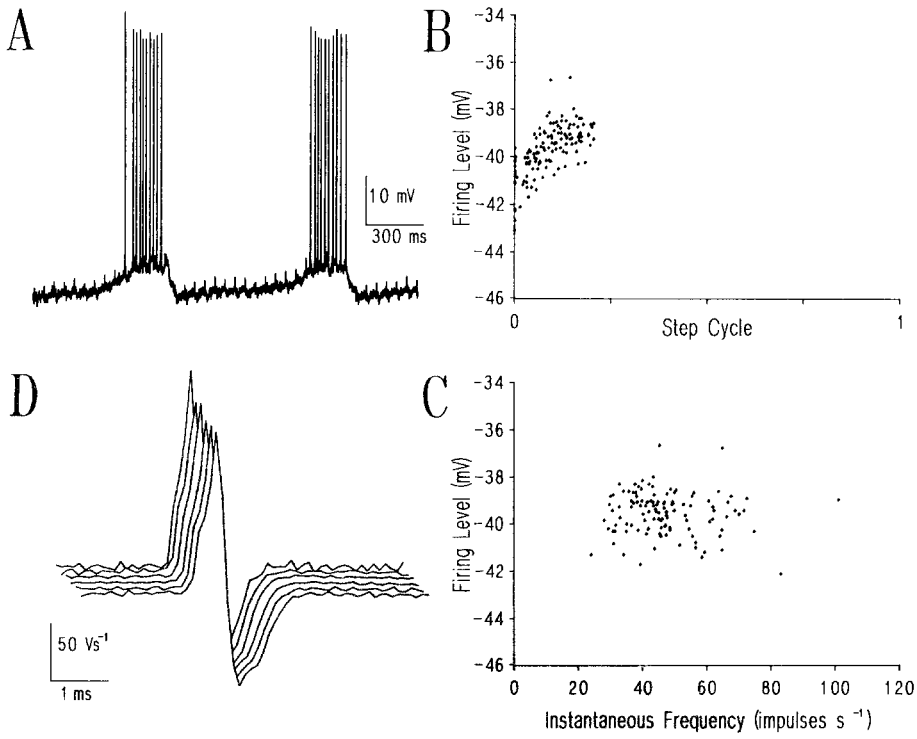

Fig. 9A–D. Same cell as in Figure 7. **A** intracellular record during fictive locomotion for two of the fifteen step cycles used in the subsequent analysis. The 2 mV calibration pulses as described in Fig. 8 can be seen at regular intervals throughout the trace. **B** firing level of each action potential plotted against the time of occurrence in the normalised step cycle. Note the depolarisation of the firing level with time ($r=0.657$). **C** firing level versus the instantaneous firing frequency, showing the lack of any relation ($r=0.114$). **D**: differentiated action potentials averaged based on the post-spike interval. The initial segment spike can be distinguished from the soma-dendritic spike at all firing frequencies

region (Severin et al. 1967; Zajac and Young 1980). These same motoneurones did not discharge at comparable frequencies during large amounts of current injection (up to 50 nA). That the synaptic currents during the depolarised phases of the locomotor drive potentials do not initiate repetitive firing strictly through somatic depolarisation is demonstrated by the fact that small amounts of hyperpolarising current injected into motoneurones through the microelectrode during fictive locomotion completely eliminates the repetitive firing (Figs. 1, 7). In addition, the frequency of firing remains unchanged despite the injection of depolarising current, leading to a very non-linear firing behaviour. It therefore seems likely that the central nervous system regulates the repetitive firing of motoneurones during fictive locomotion through mechanisms distinct from, or in addition to, those previously shown to be in operation during repetitive firing produced by somatic depolarisation. Such mechanisms might include a ligand-mediated reduction in AHP conductance (for example, see Madison and Nicoll 1986; Van Dongen et al. 1986), the production of plateau potentials in the motoneurones (Hounsgaard et al. 1988), and/or voltage-dependent excitation of motoneurones (Brownstone et al. 1991a, b) such as that provided through NMDA receptor activation (see Collingridge and Lester 1989 for review). These mechanisms, all of which may provide the motoneurone with a region of negative slope conductance (see Schwindt and Crill 1980), could therefore contribute to the non-linearity of the firing behaviour.

AHP trajectory changes

The results presented here demonstrate that post-spike trajectories are clearly depressed compared to those observed during repetitive firing at similar rates induced by the sustained injection of depolarising current (Figs. 2, 3). Often, a brief duration (less than 1 ms), small amplitude post-spike hyperpolarisation remains following each locomotor action potential (e.g. Figs. 3, 6). The conductance responsible for this potential appears not to be affected during fictive locomotion, and repolarises the membrane to a very constant potential in any given cell, both following locomotor spikes and short pulse current evoked spikes (unpublished observations; cf. Nelson and Burke 1967). It is likely that this short-lasting post-spike hyperpolarisation reflects a high conductance state of the motoneurone membrane due to the delayed rectifier potassium conductance responsible for the falling phase of the spike (Krnjevic et al. 1978; Nelson and Burke 1967). It is the trajectory of the long lasting hyperpolarising afterpotential, or the AHP (due to a calcium-dependent potassium conductance; Krnjevic et al. 1978), which is modified during fictive locomotion.

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 AHP conductance lasting for the duration of the locomotor episode; (b) the reduction is related to the depolarised phase of the locomotor drive potentials; or (c) the depression of the AHP is associated with the production of action potentials. The fact that the AHPs following these current-evoked action potentials were usually reduced in amplitude during fictive locomotion at a voltage comparable to resting membrane potential, even when this occurred during the inactive phase of the step cycle (Fig. 4), supports a possible tonic or long-lasting decrease in the AHP. However, since this reduction did not appear until locomotor activity began, it seems unrelated to possible non-locomotor effects of the brainstem stimulation. In addition, there is evidence that the AHPs are not only tonically reduced during fictive locomotion, but that the reduction is modulated such that the AHPs are further reduced during the active phases of the locomotor drive potentials, particularly when the motoneurones are repetitively firing (Fig. 5). In cells which are repetitively firing during fictive locomotion, the action potentials with the smallest AHPs tend to occur during the active phase of the step cycle (Fig. 5). One would expect that if all other motoneurone conductances were relatively constant during this phase, the AHP 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 depolarisation because it has been shown that even large depolarisations which reduce the action potential amplitude, and thus likely reduce calcium entry, have been shown to have no effect on the AHP (Coombs et al. 1955). When there was no repetitive firing during fictive locomotion, the modulation of the AHP seemed to reflect a relationship consistent with a passive dependence on the membrane potential (Fig. $5A_1$, C). Finally, it is possible that the AHP is maximally reduced immediately following the locomotor spikes, and that the mechanism of its reduction is in fact coupled to the mechanism of spike initiation, because the AHPs following the current-evoked spikes during the active phase of the step cycle often appeared larger in amplitude than those following locomotor spikes (not illustrated).

The observed decrease in the amplitude of the AHPs following current-evoked action potentials during fictive locomotion could be due to either a reduction in the AHP conductance or to the AHP conductance already being close to saturation from the summation of AHP conductances from the preceding repetitive firing (see Baldissera and Gustafsson 1971b; Ito and Oshima 1962). However, the facts that the AHPs are decreased in amplitude even when the action potentials are evoked early during repetitive firing, and that they are sometimes largest at the end of a train, are more consistent with a reduction in AHP conductance than with AHP conductance saturation.

These data also lead to the suggestion that the modulation of the AHP and the generation of the locomotor activity in motoneurones are coupled. In preliminary experiments using isolated neonatal rat spinal cords induced to locomote by application of putative neurotransmitters to the bath, the AHP amplitude is significantly reduced during locomotor activity (Schmidt 1990), indicating that the AHP may be modulated by a spinal mechanism.

Frequency-current relations

The lack of relationship between the frequency of firing of motoneurones and the current injected during fictive locomotion (Fig. 7) is consistent with the view that the AHP is not involved in the regulation of repetitive firing. Another possible explanation for this finding is that motoneurones receive large discrete synaptic inputs which lead to the initiation of action potentials. However, such large synaptic inputs are not seen during fictive locomotion. One further explanation is that the action potentials are initiated on top of a plateau potential, during which time motoneurones are impervious to current injected through the microelectrode. However, this latter explanation does not exclude a reduction in the AHP. In fact, a reduction in potassium conductance, such as the AHP, can lead to the expression of plateau potentials and hence the observed lack of frequency-current relations (Hounsgaard and Kiehn 1989).

The motoneurones in which frequency-current relations were studied displayed only primary ranges of firing at rest. The slopes of the f-I relations were in the lower range of primary range slopes seen by Kernell (1965b) in pentobarbitone-anaesthetised cats. The reasons for these findings are unclear, but it has been noted by Baldissera and Gustafsson (1971a) that motoneurones of spinalised cats do not exhibit secondary ranges during steady state firing, whereas anaemically decorticated cats, like intact pentobarbitone-anaesthetised cats, do. Granit et al. (1966) also did not find secondary range firing in motoneurones in unanaesthetised decerebrate cats. Baldissera and Gustafsson (1971a) suggested that their observations could be explained if there were a descending input to motoneurones in the anaemically decorticated cats which decreased the AHP conductance in either duration or magnitude and which was abolished by spinalisation. The f-I relations in the mesencephalic cats discussed in the present paper resemble the steady state relationship in the spinalised cats shown by Baldissera and Gustafsson (1971a, their Fig. 1).

There are situations in which excitatory synaptic current is able to sustain repetitive firing through somatic depolarisation, in which case the synaptic current summates algebraically with current injected through the microelectrode (Granit et al. 1966; Kernell 1969; Schwindt 1973; Schwindt and Calvin 1973a, b; Shapovalov et al. 1966). This synaptic current does not change either the AHP trajectories (Schwindt and Calvin 1973b), or the f-I relation (Schwindt and Calvin, 1973a). Also, the firing is thought to be ". . . impervious to the effect of synaptic noise . . . ." (Schwindt 1973). In fact, 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. On the other hand, there have been several reports demonstrating synaptic current which does not summate linearly with injected current, revealing changes in the slope of the f-I relation. Kernell (1965a) showed that excitatory synaptic input to motoneurones resulting from stimulation of either the brainstem in the region of the red nucleus, or of hindlimb nerves was not algebraically additive to the injected current. Shapovalov (1972) showed similar results with excitatory synaptic current provided by stimulation of reticulospinal cells. Clearly, the central nervous system can alter the frequency-current relations of motoneurones so that the response of a motoneurone to a given stimulus, or its input-output relation, is not consistent in different situations.

The high rates of firing and the lack of firing frequency regulation in motoneurones during locomotion have been noted in cats induced to walk on a treadmill by stimulation of the mesencephalic locomotor region. Severin et al. (1967) noted that motoneurones fired at higher frequencies during treadmill locomotion than during activation by the stretch reflex. They also noted that any given motoneurone would fire at a certain frequency, regardless of the speed of walking (change of treadmill speed) or stimulation parameters (either frequency or strength). In fact, increasing the strength of stimulation was found to increase the total electromyogram. This was accomplished by the recruitment of additional units rather than by increasing the firing frequency of individual units. These data are certainly consistent with the data presented here demonstrating that a change in input to the motoneurones during locomotion is not necessarily reflected by a change in their output.

Clearly, the regulation of the repetitive firing behaviour of motoneurones during fictive locomotion is different from that seen in the barbiturate-anaesthetised cats, in that the firing is not simply produced by net excitatory current causing somatic depolarisation and thus intrinsically-regulated repetitive firing, with the AHP responsible for the repetitive firing behaviour. One possible factor which may contribute to this difference is voltage-dependent excitation of motoneurones during fictive locomotion (Brownstone et al. 1991a, b), such that small synaptic inputs produce the large excitation to give the depolarised phase of the locomotor drive potentials, and thus allow for small asynchronous synaptic inputs to trigger action potentials. This would account for the fact that when small amounts of hyperpolarising current are given, the motoneurone is taken out of the range of voltage-dependent excitation, and thus stops firing. This voltage-dependent excitation could be reflected as a region of negative slope conductance of the membrane. It has been shown that either topically applied penicillin or changes in potassium channel activity can change the current-voltage relation of a motoneurone such that it gains a region of negative slope conductance (Schwindt and Crill 1980). It could be speculated that, during fictive locomotion, the acquisition of a negative slope conductance is brought about by a decrease in AHP (a potassium) conductance.

Firing level

It has been shown that action potential firing level in motoneurones becomes more depolarised both as a spike train progresses and as firing frequencies increase (Barrett

et al. 1980; Kolmodin and Skoglund 1958; Schwindt and Crill 1982). It was proposed that this depolarisation is secondary to accommodation of the initial segment (Schwindt and Crill 1982). During fictive locomotion, the pattern of firing of any given motoneurone is very consistent, although different cells may fire fastest at the beginning, middle, or end of each active phase. If increases in firing rate were secondary to increases in net excitatory synaptic input, one would expect to see a correlation between firing level and firing frequency. Although the firing level does depolarise with time in a spike train (Fig. 9B), there is no relation between firing level and instantaneous firing frequency (Fig. 9C). In the absence of firing level depolarisation with increasing firing rates, it would seem unlikely that net excitatory synaptic current could be responsible for the production of the action potentials.

Concluding remarks

Contrary to data obtained from ventral root filaments during treadmill locomotion (Zajac and Young 1980), an initial firing doublet was rarely seen in the intracellular records from the motoneurones examined here (2/30 cells; e.g. Fig. 1). This disparity could be related to the lack of rhythmic afferent input during fictive locomotion, or possibly could be explained by there being a difference between what is recorded in the soma and what is recorded in the axon. Gogan et al. (1984) showed that during conditions which increased the delay between the initial segment and soma-dendritic action potentials, re-excitation may occur, possibly at the first node of Ranvier, thus producing a second action potential in the axon which is not observed by the micropipette in the soma. It is conceivable that this occurs during locomotion, when there is perhaps a large increase in membrane conductance at the start of the active phase that would tend to favour re-excitation and thus provide an initial firing doublet in the axon. It should be noted, however, that both Hoffer et al. (1987) and Severin et al. (1967) also rarely found initial doublets in ventral root filaments in intact cat treadmill locomotion.

There has not yet been a systematic study of the control of the repetitive firing behaviour of motoneurones during any other motor activity. Jodkowski et al. (1988) demonstrate an example where the AHP may contribute to the repetitive firing of motoneurones during spontaneous respiration. However, it is neither known whether the AHP conductance is reduced nor whether the AHP regulates the repetitive firing during spontaneous respiration. Kirkwood et al. (1982a, b) demonstrated that synchronisation of intercostal motoneurones during respiration is imposed by descending neuronal activity. This synchronisation limits the possibility that the AHP plays a significant role in regulating the repetitive firing behaviour of the intercostal motoneurones during respiration. The mechanisms underlying repetitive firing activity of respiratory motoneurones remain to be elucidated.

The determination of the factors regulating repetitive firing in motoneurones during motor acts has important consequences for understanding the mechanisms controlling motoneurone output. It has been demonstrated here that although mammalian motoneurones have the intrinsic capability to fire repetitively in response to injected current, the central nervous system need not rely on this intrinsic ability. During fictive locomotion, the motoneurone does not fire repetitively simply in response to the net excitatory synaptic current it receives. Although very important facts about the intrinsic properties of motoneurone membranes have been learned through the sustained injection of depolarising current, the ability of the central nervous system to modify or bypass these properties has now been established. It is clear that the input to motoneurones during motor acts cannot be assumed to be simply equivalent to current injected through a microelectrode (Hoffer et al. 1987). The capacity of the nervous system to alter the responsiveness of its motoneurones to synaptic currents provides it with the ability to exercise a high degree of control over its motor output.

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