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CENTRAL SYNAPTIC COUPLING OF WALKING LEG MOTOR NEURONES IN THE CRAYFISH: IMPLICATIONS FOR SENSORIMOTOR INTEGRATION

BY PETER SKORUPSKI AND KEITH T. SILLAR*
Department of Physiology, University of Bristol, Park Row, Bristol BS1 5LS

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Summary

We present electrophysiological evidence for the presence of central output synapses on crayfish walking leg motor neurones. The effect of these central outputs is that a motor neurone can exert tonic graded control over other motor neurones without the requirement for spiking. Excitatory interactions among synergists and inhibitory interactions among antagonists are described. This central coupling among leg motor neurones profoundly affects their responses to afferent input from an identified stretch receptor, the thoracocoxal muscle receptor organ (TCMRO). Injecting current into a motor neurone can change the gain of TCMRO reflexes in other motor neurones. Some motor neurones are also capable of reversing the sign of TCMRO reflexes by inhibiting reflex firing of antagonists and facilitating reflex activity in synergists. The implications of these central interactions of motor neurones in motor control are discussed.

Introduction

In the crayfish the isolated thoracic nervous system is capable of producing a rhythmic motor output resembling that underlying walking (Skorupski, 1985; Sillar & Skorupski, 1986). This motor output consists of rhythmic bursts of spikes in the promotor nerve alternating with bursts of spikes in the remotor nerve. Typically, levator motor neurones fire in phase with the activity in the promotor nerve. This motor pattern thus resembles that during forward but not backward walking. A second motor pattern, less frequently observed, is characterized by antiphasic bursting in promotor and levator motor neurones, and therefore resembles that underlying backward walking in the intact animal (Skorupski, 1985). The thoracic ganglia therefore contain central rhythm generators that coordinate the activity of ipsilateral motor neurones in each segment (Sillar *et al.* 1987) in a rhythmical manner resembling that underlying locomotion in the intact animal. However, the rhythm recorded *in vitro* is often rather slow and irregular, with a period that may vary from 5–30 s on a cycle-by-cycle basis. During intense

* Present address: Department of Zoology, University of Bristol, Woodland Road, Bristol BS8 1UG.

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bursting an average period of about 10 s is typical. This implies that sensory feedback, or other central nervous system (CNS) elements, are of very considerable importance in generating the normal behaviour in the intact animal.

One source of feedback that is implicated in the control of locomotion is the thoracocoxal muscle receptor organ (TCMRO) (Alexandrowicz & Whitear, 1957; Bush, 1981). This proprioceptor signals posterior displacement (i.e. remotion) of the most basal joint of the leg, and during walking, therefore, must monitor the protraction and retraction cycle of the entire leg. The TCMRO is innervated by two nonspiking afferent fibres, which conduct receptor potentials electrotonically to the CNS and evoke graded reflex effects in basal limb motor neurones (Cannone & Bush, 1980; Skorupski & Sillar, 1986). Cyclically stretching and releasing the TCMRO in a preparation that is otherwise isolated from the periphery can entrain a spontaneous motor rhythm to the imposed movement (Sillar *et al.* 1986). Under experimental conditions, then, the TCMRO can alter the timing of the central rhythm and must therefore have direct access to the rhythm-generating circuitry. However, the neural elements that comprise the central rhythm generator have not yet been identified and it is not known at what level the TCMRO afferent neurones address the rhythm-generating circuitry.

Neurophysiological analysis of a variety of behaviours in the Crustacea has revealed that some motor neurones are not simply passive output elements of a central rhythm-generating network but are themselves, by virtue of their central connections, integral parts of the rhythm generator. For example, the pyloric feeding rhythm of lobsters is generated within the stomatogastric ganglion by a network of 14 central neurones, all but one of which are motor neurones (Miller & Selverston, 1985). Many of the paired segmental appendages of decapods participate in rhythmic movements and some of their motor neurones, where examined, can modulate various parameters of the neural rhythm that is correlated with the behaviour (Heitler, 1978; Simmers & Bush, 1983). Walking, however, is more complex and flexible than the simple rhythms referred to above, and one would not necessarily expect that motor neurones of the walking legs should be coupled in the same way as those involved in the rather stereotyped behaviours of feeding and ventilation.

In this paper we show that crayfish leg motor neurones are extensively coupled and that this coupling has important implications for sensorimotor integration: motor neurones are capable of participating in the central reflex modulation that we previously demonstrated to be a property of the central rhythm generator (Skorupski & Sillar, 1986). We argue that crayfish leg motor neurones must be regarded as integral components of the neural circuitry, both central and reflex, that generates rhythmic leg movements during walking.

Materials and methods

Animals

Experiments were performed on adult male and female crayfish, *Pacifastacus*

leniusculus, measuring 8–10 cm from rostrum to telson. Animals were purchased from a local supplier (Riversdale Farm, Dorset) and kept in aquaria at 12°C. Experiments were carried out at room temperature.

Preparation

A crayfish was decapitated and the abdomen, legs and dorsal carapace were removed. The remaining ventral thorax was then pinned out on Sylgard and a cannula was inserted into the sternal artery. The preparation was perfused *via* the cannula with oxygenated crayfish saline of the following composition (mmol l^{-1}): NaCl, 210; KCl, 2·5; $MgCl_2$, 2·5; $CaCl_2$, 14; Tris, 10; maleic acid, 4·5; pH 7·55. The isolated ganglion–TCMRO preparation was identical to that described previously (Skorupski, 1985; Sillar & Skorupski, 1986). The fourth ganglion and TCMRO complex were removed from the animal's body, with the third and fifth ganglia remaining attached by the central connectives, and pinned out on Sylgard. The fourth ganglion was desheathed and fatty tissue surrounding the neuropile aspirated away.

Recording and data analysis

Suction electrodes were used to record extracellularly from the cut distal ends of up to four motor nerves innervating the promotor, remotor, anterior levator and depressor muscles. Glass microelectrodes, filled with 2 mol l^{-1} potassium acetate or 5% Lucifer Yellow (Stewart, 1978) in 1 mol l^{-1} LiCl, were used to record intracellularly, singly or in pairs, from processes of motor neurones in the neuropile. Data were stored on a seven-channel FM tape recorder for later analysis. In some experiments a computer-based signal-averaging system was used. The computer was also used to plot continuous rate histograms of spiking activity from prediscriminated, extracellularly recorded spikes.

Identification of motor neurones

Promotor, remotor, levator and depressor motor neurones were identified by correlating intracellularly recorded action potentials with spikes recorded extracellularly in one of the above nerves. This physiological identification was usually confirmed anatomically after ionophoresing Lucifer Yellow from the recording micropipette and subsequent histological processing. Motor neurones with axons that left the ganglion *via* the large anterior or posterior ventral nerves could not be classified by physiological criteria. These nerves mainly innervate the sense organs and muscles of the distal limb, although the ventral heads of the basipodite levator and depressor muscles are innervated by branches of the anterior nerve (Skorupski, 1985).

Histology

Cobalt backfills were made by dipping the cut end of a motor nerve in 200 mmol l^{-1} $CoCl_2$ for 12–16 h at room temperature. The cobalt was precipitated with a few drops of ammonium sulphide in approximately 10 ml of crayfish saline

and the preparation fixed in 4% formaldehyde. After dehydration in a graded alcohol series, the preparation was cleared in methyl salicylate and drawn with a *camera lucida*. Lucifer Yellow, when used, was ionophoresed from the recording microelectrode, following electrophysiology, with 0·5 s, 10 nA pulses of hyperpolarizing current at 1 Hz. The preparation was then fixed, dehydrated, cleared, viewed as a whole mount under a fluorescence microscope and drawn as above.

Results

Morphology of crayfish leg motor neurones

The morphology of crayfish leg motor neurones conforms to the general arthropod scheme (Burrows & Hoyle, 1972; Evoy, 1977). Their somata, which form a rind at the anterior and posterior ventral margins of a thoracic ganglion, range in diameter from 30 to 100 μm (Fig. 1). A single slender neurite arises from the soma, which is otherwise devoid of any processes, and ascends into the main dorsolateral neuropilar region. Here the primary neurite expands considerably in diameter and gives off numerous side branches. However, the dendritic field is always restricted to the hemiganglion of origin; no processes of a leg motor neurone have ever been observed to cross the midline.

Cobalt backfills of the promotor nerve reveal a maximum of 13 somata, the majority of which are clustered at the anterior ventral margin of a ganglion (Fig. 1A), and backfills of the nerve supplying remotor and depressor muscles reveal a cluster of about 20 cell bodies located at the ventral posterior margin of the ganglion (Fig. 1B).

Selective staining of individual neurones by injecting Lucifer Yellow from the recording micropipette show that levator motor neurone somata are located anteriorly, like those of promotor motor neurones (Fig. 1A,C), whereas motor neurones of the distal limb have their somata located in either the anterior or the posterior ventral rind of the ganglion (P. Skorupski, K. T. Sillar & R. C. Elson, unpublished observations).

In about 20% of preparations where a motor neurone was ionophoretically stained with Lucifer Yellow following electrophysiological recording, histological processing of the ganglion revealed the presence of two or more stained neurones with similar morphology (Fig. 1D).

Central interactions of crayfish leg motor neurones

Injecting current through the recording microelectrode into the neuropilar processes of a leg motor neurone frequently influenced the spiking activity of neurones other than the one penetrated. Depolarizing a motor neurone often increased the firing frequency of its synergists and decreased that of its antagonists, whereas hyperpolarization had the opposite effect. These effects were due neither to extracellular spread of current, since they disappeared immediately on withdrawing the microelectrode, nor to reafferent reflex effects, since the ganglion

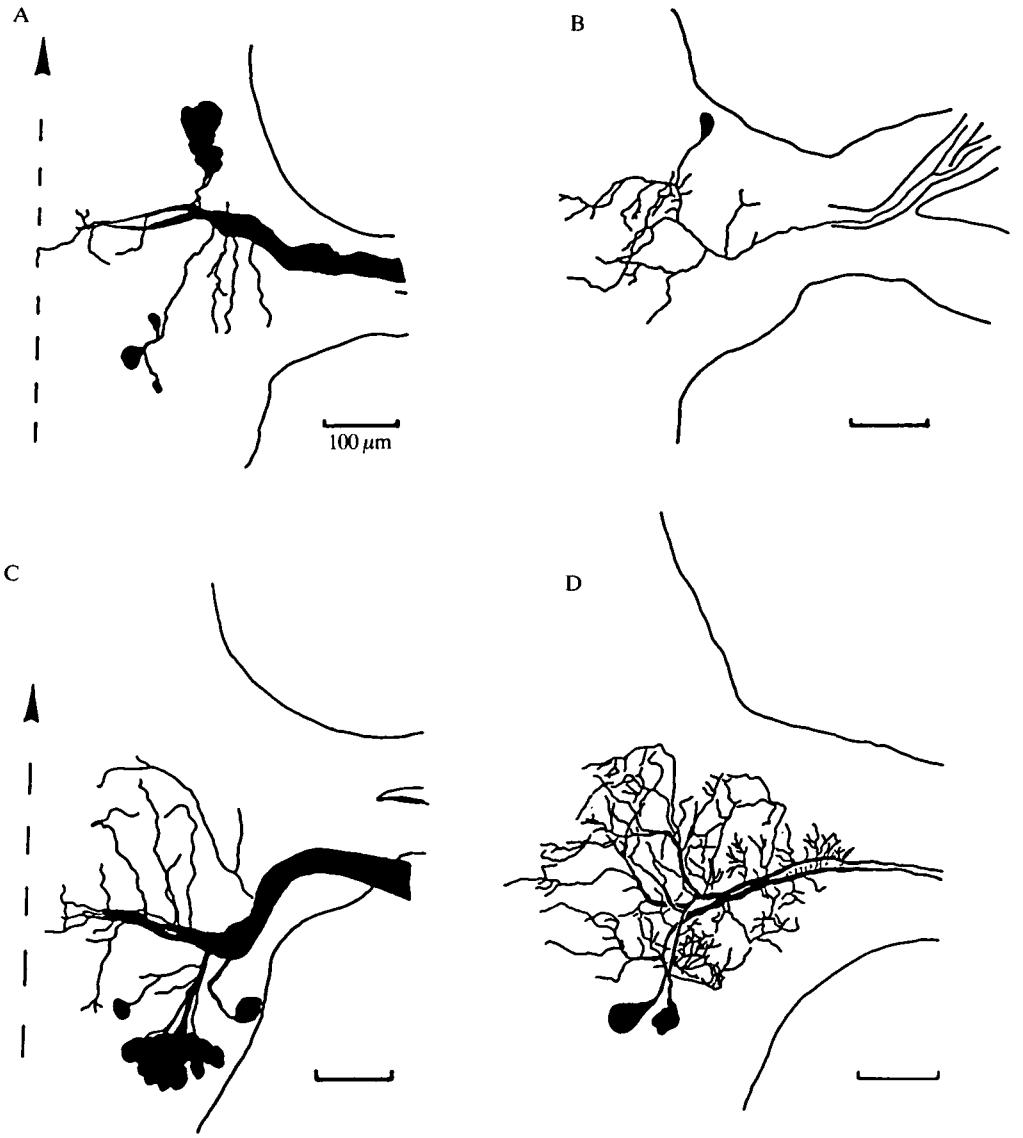

Fig. 1. Morphology of basal limb motor neurones. (A) Cobalt backfill of the promotor nerve. 13 cell bodies were counted in this preparation. (B) Cobalt backfill of the combined remotor and depressor nerve. 20 cell bodies were counted in this preparation. (C) A single promotor motor neurone stained by ionophoresis of Lucifer Yellow. (D) A pair of dye-coupled remotor motor neurones revealed after a single motor neurone had been injected with Lucifer Yellow. Outline of the right fourth thoracic hemiganglion, viewed dorsally, is shown in A–D. Rostral is at the top (arrowhead), dashed line represents the midline of the ganglion.

was isolated from the periphery. We conclude that crayfish leg motor neurones possess central output synapses that are affected by membrane polarization.

Excitatory coupling of synergistic motor neurones

Evidence for excitatory coupling of promotor motor neurones is shown in Figs 2 and 3. Similar excitatory coupling among synergists was also observed in other classes of motor neurone. Intracellular penetration of a promotor motor neurone was confirmed by two criteria: (1) an intracellularly recorded action potential was followed 1:1 at a short and constant latency by an extracellularly recorded spike (Fig. 2A); (2) stimulation of the promotor nerve evoked an antidromic spike in the neurone (Fig. 2B). When the intensity of antidromic stimulation was reduced to just below spike threshold for this neurone a smaller, all-or-none depolarizing potential was recorded at the same latency (Fig. 2B). A possible explanation for this potential is that it was a central synaptic input from the antidromic spike of a second promotor motor neurone that was activated by electrical stimulation at a lower threshold. This type of coupling potential was often recorded in a motor neurone on stimulation of its motor nerve.

Central synaptic output of the promotor motor neurone was demonstrated by passing about 4 nA of current through the recording electrode. This was not sufficient to generate action potentials in this particular neurone, but nevertheless increased the firing rate of a second, tonically active promotor motor neurone recorded extracellularly in the promotor nerve (Fig. 2C). Hyperpolarizing the neurone decreased the spike frequency of the tonically active motor neurone (Fig. 2D). When the spikes of the tonically active motor neurone were used to trigger a signal averager a small depolarization was observed in the intracellularly recorded motor neurone (Fig. 2E). This depolarization occurred with a somewhat variable latency about 5 ms prior to the extracellularly recorded spikes. This suggests that the depolarization was not due to a reciprocal, constant-latency input from the tonically active motor neurone. Instead, the variable onset and duration of the depolarization suggest that it probably arises from common presynaptic input to the two motor neurones.

Fig. 2. Excitatory interactions of promotor motor neurones. (A) A promotor motor neurone was identified by correlating intracellularly recorded action potentials (top trace) 1:1 with a spike in the promotor nerve following at a short and constant latency (bottom trace). (B) Antidromic stimulation of the promotor nerve (traces superimposed at three different intensities): 1, low-intensity stimulation produces no response; 2, intermediate intensity evokes either no depolarizing potential at a short latency or a small one; 3, higher intensity evokes an antidromic spike at a similar short latency. (C) Depolarizing a promotor motor neurone (mn1) to a level below threshold for spike initiation (lower trace, bridge overbalanced) excites another promotor motor neurone recorded extracellularly (upper trace, mn2). (D) Hyperpolarizing the motor neurone decreases activity of mn2 and reduces the tonic level of background synaptic input. (E) Spikes in mn2 are not associated with discrete postsynaptic potentials in the intracellularly recorded motor neurone: four consecutive averages, 50 sweeps each, triggered by spikes in mn2.

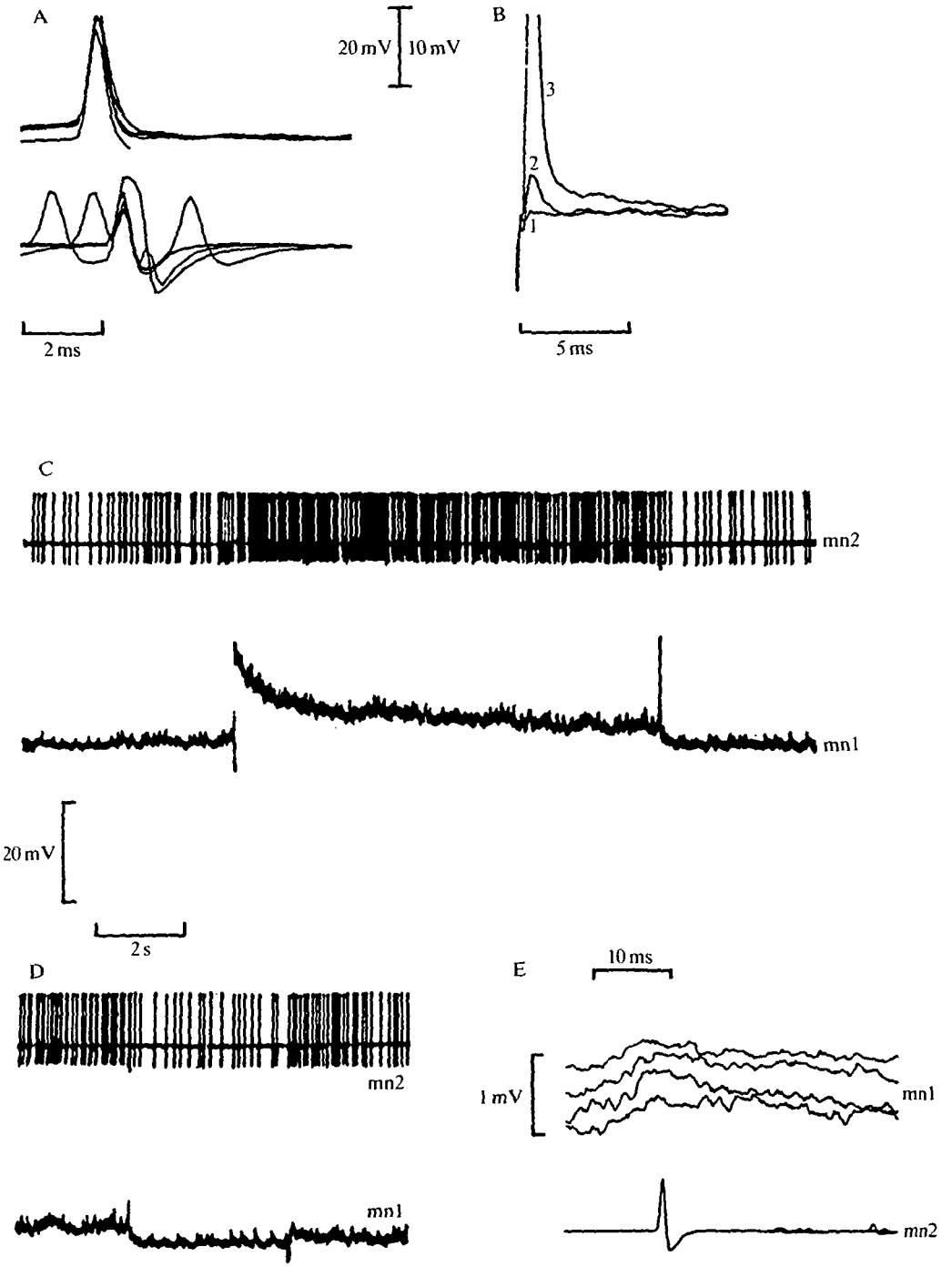
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Fig. 2

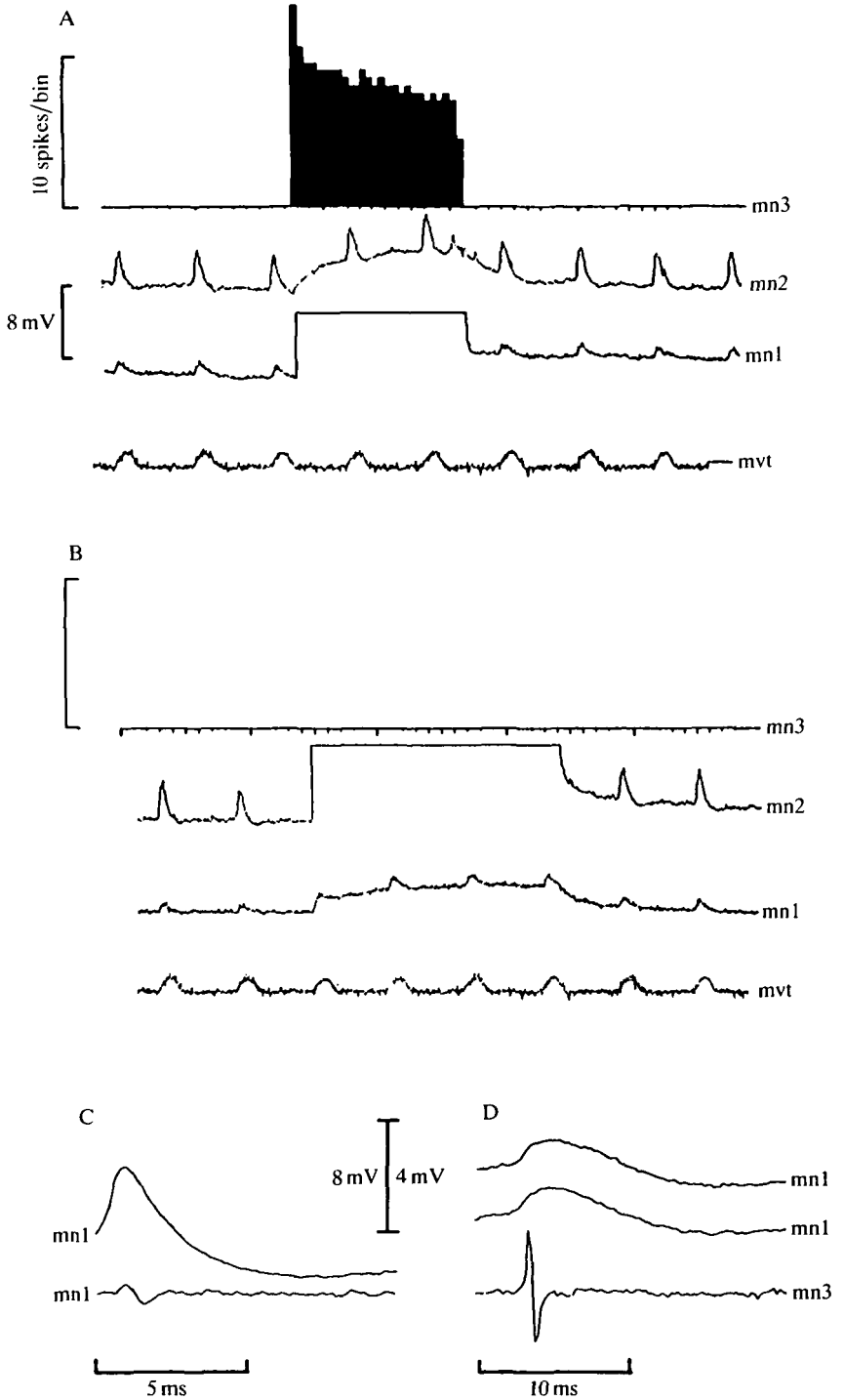
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Fig. 3

Fig. 3 shows the result of one experiment in which simultaneous intracellular recordings were made from a pair of interacting promotor motor neurones (arbitrarily called mn1 and mn2). During this experiment the TCMRO was periodically stretched at 1 Hz, evoking a subthreshold depolarization in each motor neurone by reflex on each cycle of stretch. When mn1 was depolarized with current injection mn2 also depolarized by about 4 mV and spiking activity, recorded extracellularly in the promotor nerve, was elicited in a third promotor motor neurone (mn3, Fig. 3A). The reflex responses in mn2 remained unchanged in amplitude and duration during the current injection, suggesting that the interaction was not associated with a detectable increase in postsynaptic conductance, although it cannot be ruled out that a conductance change was taking place at a remote site. When mn2 was depolarized with current injection a smaller depolarization was recorded in mn1 but no spikes were evoked in mn3. Again, the amplitude of the reflex responses in one neurone were not affected by current injection into the other (Fig. 3B). A possible explanation for this result, then, is that mn1 and mn2 were connected by a moderately rectifying electrical synapse.

The axon spike of mn1, as recorded extracellularly, was very small and signal averaging was required to reveal it (Fig. 3C). When the signal averager was then triggered from the larger spike of mn3, a time-locked depolarizing potential was recorded in mn1 (Fig. 3D). This potential occurred at a constant latency less than 1 ms in advance of the peripheral spike of mn3. It seems likely that this depolarization was an electrical EPSP resulting from the electrotonic conduction of the attenuated neuropilar spike of mn3 across an electrical synapse. It was not always possible, however, to correlate discrete potentials with the spiking activity of single motor neurones, even when the intracellularly recorded neurone was shown to drive one or more of the extracellularly recorded units (see Fig. 2D).

Inhibitory coupling of antagonistic motor neurones

In addition to the synergistic effects described above, many, but not all, motor neurones exerted inhibitory effects upon their antagonists. Thus, depolarizing a remotor motor neurone often decreased or abolished activity in the promotor nerve (e.g. Fig. 4A), whereas depolarization of some promotor motor neurones

Fig. 3. (A,B) Simultaneous intracellular recordings from a pair of interacting promotor motor neurones, arbitrarily called mn1 and mn2. The TCMRO is stretched and released at 1 s intervals throughout the recording (mvt, length monitor). (A) Depolarizing mn1 with 20 nA of current results in a sustained subthreshold depolarization of mn2 and spiking in a third promotor motor neurone (mn3). The spikes of mn3 trigger a window discriminator and are plotted as continuous-rate histogram (100 ms bin width). The spikes of mn1 in the extracellular recording were too small to be resolved by the discriminator. (B) Depolarizing mn2 with 20 nA current causes a subthreshold depolarization of mn1 but does not activate mn3. (C) Spike-triggered averaging from intracellularly recorded spikes (upper trace) in mn1 reveal its small-amplitude extracellularly recorded spike in the promotor nerve (lower trace). (D) Triggering the averager from the large extracellular spike of mn3 (lower trace) reveals a phase-locked EPSP in mn1 (upper traces, two consecutive averages, 50 sweeps each).

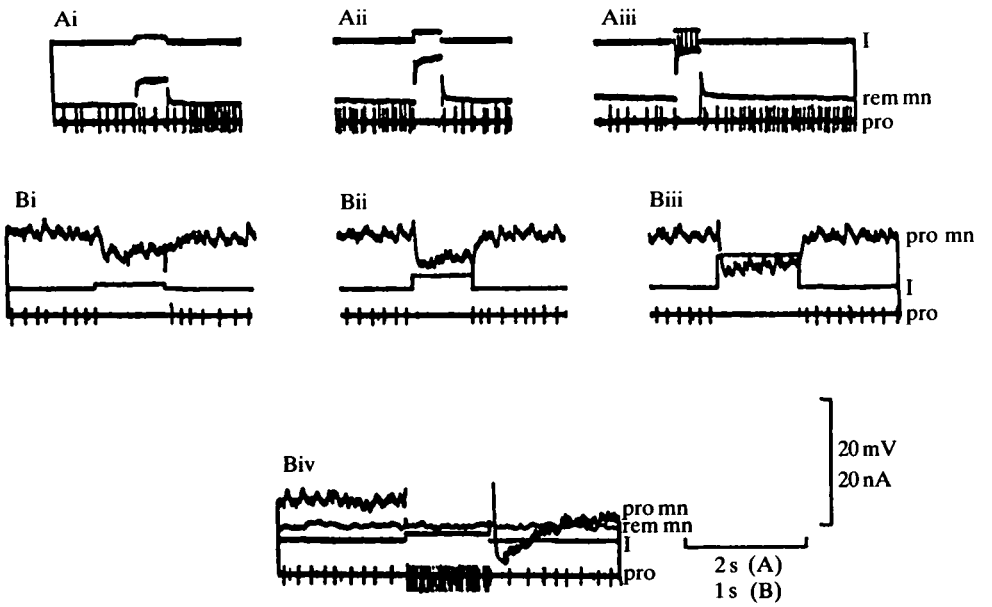

Fig. 4. (Ai,ii) Depolarization (current monitor, I) of a remotor motor neurone (rem mn) without evoking spikes reduces and then abolishes spiking activity in the promotor nerve (pro). Larger amplitude current pulses evoke spikes in the remotor motor neurone (iii). (Bi–iii) Graded depolarizations of a remotor motor neurone (not shown, current monitor middle trace) cause graded hyperpolarizations of a promotor motor neurone recorded intracellularly (pro mn) and abolish spiking in a tonically active promotor unit recorded extracellularly (pro). (iv) Depolarization of the promotor motor neurone (top trace, bridge unbalanced) does not produce any discernible change in the membrane potential of the remotor motor neurone (rem mn). Note the axon spike of the promotor motor neurone in the bottom trace.

could inhibit remotor activity (Fig. 5A). These effects could be evoked without spiking and were graded with the amount of current injected into a given motor neurone (e.g. Fig. 4A).

Paired intracellular recordings reveal that graded hyperpolarizations underlie this graded inhibition. Nine pairs of motor neurones (from over 50 paired recordings) displayed an inhibitory interaction, and in all cases this was unidirectional. In one such experiment depolarizing a remotor motor neurone hyperpolarized a promotor motor neurone that was recorded simultaneously (Fig. 4B). This hyperpolarization increased in amplitude as the presynaptic current strength was increased, up to a maximum of about 5 mV with 7 nA of presynaptic current (Fig. 4Biii). Spiking of a second, tonically active promotor motor neurone was suppressed when the remotor motor neurone was depolarized. When the *promotor* motor neurone was depolarized there was no effect on the membrane potential of the remotor motor neurone, although the firing frequency of the tonically active promotor unit was increased (Fig. 4Biv).

More than half the recorded motor neurones that exerted inhibitory effects on

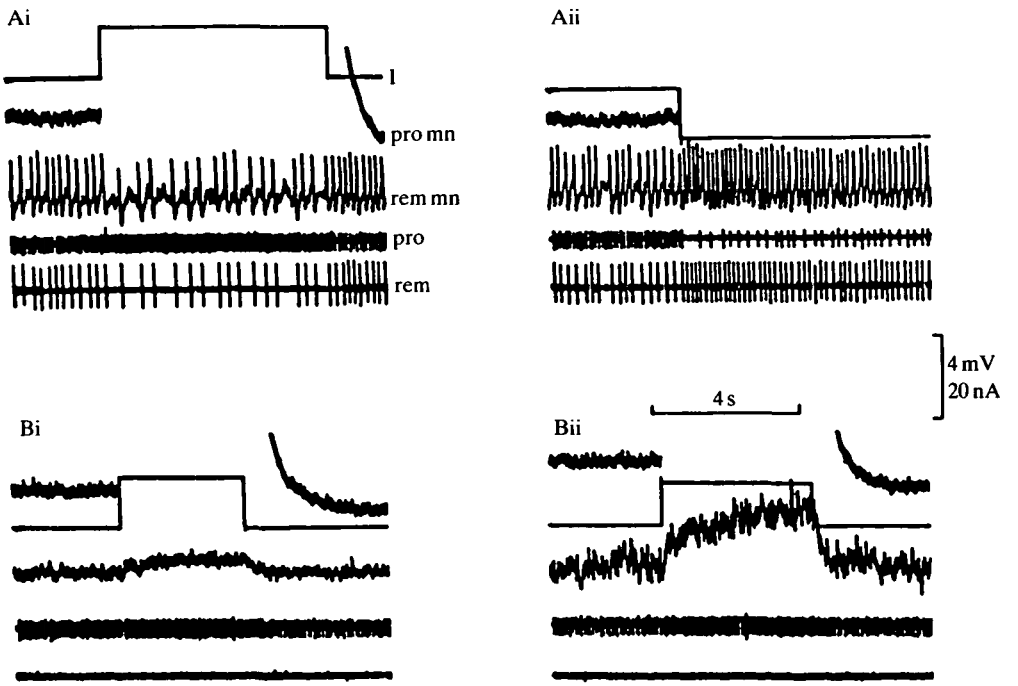

Fig. 5. Tonic inhibitory interaction between a promotor and a remotor motor neurone. Traces, top to bottom: current monitor (I), promotor motor neurone (pro mn), remotor motor neurone (rem mn), promotor nerve (pro), remotor nerve (rem). (A) The remotor motor neurone is held depolarized (trace a.c.-coupled) and spikes tonically. Depolarizing the promotor motor neurone reduces (i), and hyperpolarizing the promotor motor neurone increases (ii), the firing frequency of the remotor motor neurone. (B) Depolarizing the promotor motor neurone evokes a depolarizing IPSP when the remotor motor neurone is hyperpolarized (i), which is enhanced by further hyperpolarization of the remotor motor neurone (ii).

their antagonists produced the opposite effect on injecting current of the opposite polarity. An example of an inhibitory connection from a promotor motor neurone to a remotor motor neurone is shown in Fig. 5. The firing frequency of the remotor motor neurone was decreased when the promotor motor neurone was depolarized, but *increased* when the promotor motor neurone was hyperpolarized. The explanation for this result must be either that the promotor motor neurone was tonically releasing an inhibitory transmitter onto the remotor motor neurone, or that the promotor motor neurone made a tonic excitatory (chemical or electrical) connection with an interneurone that, in turn, tonically inhibited the remotor motor neurone.

The final stage of the inhibition appears to be chemically mediated. In the experiment illustrated in Fig. 5 the remotor motor neurone was initially held depolarized (Fig. 5Ai,ii), with the amplifier a.c.-coupled. Without an imposed polarization of the remotor motor neurone there was no discernible change in its

membrane potential in response to depolarization of the promotor motor neurone (not shown), but when held hyperpolarized the response to depolarization of the promotor motor neurone was a small depolarization (Fig. 5Bi). This depolarization could be increased in amplitude by further hyperpolarization of the postsynaptic remotor motor neurone (Fig. 5Bii). The interpretation of this result is that at its resting potential the remotor motor neurone was at or near the reversal potential of the IPSP evoked by depolarizing the promotor motor neurone, and that hyperpolarization of the remotor motor neurone was required to reveal the response as a depolarizing IPSP. More commonly the membrane potential change underlying inhibition of a given motor neurone is a graded hyperpolarizing IPSP (e.g. Figs 4B, 6), and it is not usually possible to inject sufficient current to reverse this IPSP. Nevertheless, such responses are normally increased in amplitude by postsynaptic depolarization and decreased by hyperpolarization (see Fig. 6C).

Interactions between motor neurones of different joints

The central outputs of leg motor neurones are not restricted to other motor neurones of the same joint. Fig. 6A shows the effect of injecting about 10 nA of depolarizing current into one remotor motor neurone. Spiking activity was inhibited in the promotor nerve and also in the motor nerve to the anterior levator muscle of the basipodite, while in the nerve supplying basipodite depressor muscles a single unit was activated. Therefore a motor neurone of the thoracocoxal joint can exert an interjoint effect on motor neurones of the coxobasal joint. Interjoint effects were also observed on stimulating motor neurones other than promotors and remotors. Some of these neurones were motor neurones of the distal limb, which at present remain unidentified (data not shown).

Fig. 6B shows simultaneous intracellular recordings from a remotor motor neurone and a levator motor neurone. When the remotor motor neurone was depolarized the levator was hyperpolarized for the duration of the current pulse, and several extracellularly recorded promotor units were inhibited (Fig. 6B). The hyperpolarization of the levator motor neurone increased in amplitude when it was held depolarized (Fig. 6Cii), again suggesting that the final stage of the interaction involves chemical synaptic inhibition.

Interjoint coupling of leg motor neurones always reflected the functional synergies implied by the spontaneous activity of a thoracic ganglion. Thus, during rhythmic motor output promotor and levator motor neurones are active in phase with each other and in antiphase with remotor motor neurones (Skorupski, 1985; Sillar & Skorupski, 1986; Skorupski & Sillar, 1986; Fig. 7A). Interjoint coupling of leg motor neurones reflects this premotor drive: excitatory coupling between promotor and levator motor neurones and inhibitory coupling between remotor and levator motor neurones are frequently observed. These connections would be appropriate for forward but not backward walking, which implies that such coupling must be suppressed when different coordination modes are required (see Discussion).

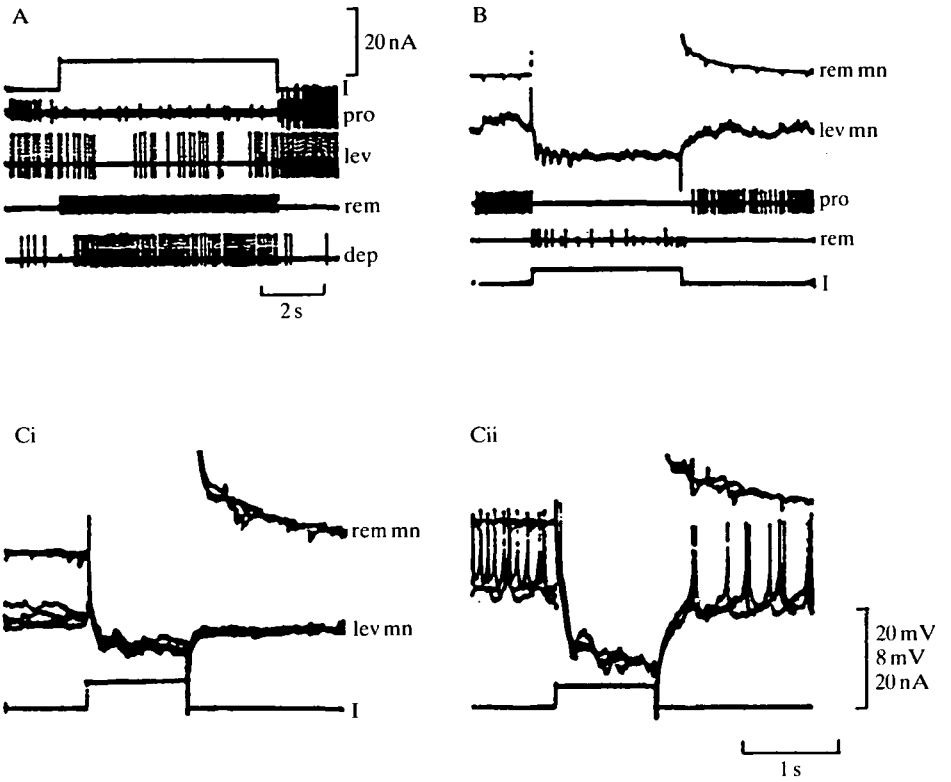

Fig. 6. Interactions between motor neurones serving different joints of the leg. (A) Depolarizing a remotor motor neurone (not displayed; current monitor, I) with 10 nA of current decreases activity in the promotor (pro) and levator (lev) nerves, and increases the activity of a unit in the depressor nerve (dep). The axon spike of the remotor motor neurone is visible in the fourth trace (rem). (B) In a different preparation, depolarization of a remotor motor neurone (rem mn) hyperpolarizes a levator motor neurone that was recorded simultaneously (lev mn), inhibits spiking activity in the promotor nerve (pro) and excites a second remotor unit (rem, smaller spike; the larger spike is the axon spike of the intracellularly recorded remotor motor neurone). (C) When the levator motor neurone is held depolarized so that it spikes tonically (ii) the hyperpolarizations evoked by the remotor motor neurone are larger than those recorded without polarization of the levator motor neurone (i).

The rhythmic motor output produced by an isolated ganglion can, in fact, override the effect of central connections between leg motor neurones. Fig. 7 shows recordings of the same pair of motor neurones displayed in Fig. 6B,C during a bout of such activity. In the absence of intense bursting activity, depolarization of the remotor motor neurone evoked a large hyperpolarization of the levator motor neurone (Fig. 6Ci,ii). Later in the same experiment the preparation began to generate sequences of rhythmic bursting. During this activity the levator motor neurone depolarized and spiked in phase with activity recorded extracellularly from the promotor nerve, while weak antiphasic oscillations

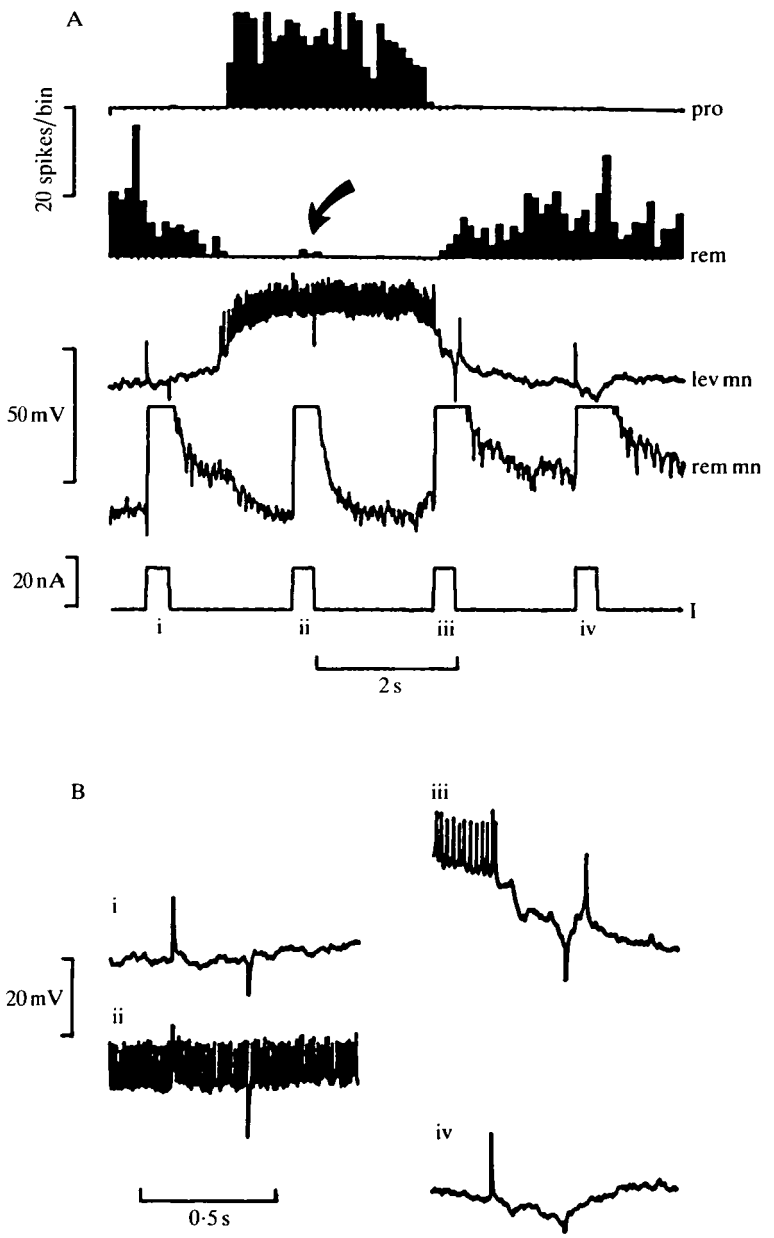

Fig. 7. Central interactions between leg motor neurones are overridden by centrally generated motor patterns (same preparation as in Fig. 6B,C). (A) Rhythmic bursting in promotor (pro) and remotor (rem) nerves is correlated with central oscillatory drive in the intracellularly recorded levator motor neurone (lev mn), which spikes in phase with the promotor. The remotor motor neurone (rem mn) is depolarized by current injection at regular intervals throughout the cycle. Current monitor (I), bottom trace. See text for explanation of arrow. (B) Expanded traces showing the response of the levator motor neurone to stimulation of the remotor motor neurone on a faster time base. i–iv correspond to the positions indicated in A.

occurred in the remotor motor neurone. The previous experiment was now repeated and depolarizing current pulses were injected into the remotor motor neurone throughout the sequence of rhythmic activity. When the levator motor neurone was bursting in phase with promotor firing, the inhibition previously evoked by stimulation of the remotor motor neurone was absent: the levator motor neurone continued spiking throughout the current pulse. During the interburst interval the hyperpolarization of the levator motor neurone by the remotor motor neurone was greatly reduced or absent; this may be due to the proximity of the equilibrium potential for the inhibitory interaction. However, this cannot explain the absence of inhibition during the active phase of the levator motor neurone when the latter is depolarized (compare Figs 6Cii and 7Bii). Neither can it be postulated that powerful, rhythmic postsynaptic inhibition of the remotor motor neurone shunts out the imposed depolarization, since the current pulses continued to evoke bursts of spikes in the remotor nerve during the remotor interburst (Fig. 7A, arrow). The most likely explanation is that the inhibitory effects of the remotor motor neurone were mediated by one or more interneurones, and that these interneurones were gated by the central rhythm generator.

Modulatory effects of motor neuronal coupling on TCMRO-mediated reflexes

Many, but not all, leg motor neurones are modulated by mechanical stimulation of the TCMRO. The nonspiking receptor potentials of the S and T fibres of the TCMRO are reflected in smooth changes in the membrane potentials of various motor neurones (Skorupski & Sillar, 1986). It is not yet known if any of these connections are monosynaptic; the smooth nature of the motor neurones' responses suggests, at any rate, that no *spiking* neurones are interposed (see, for example, Figs 8, 11).

Stretching the TCMRO can excite promotor motor neurones in a typical stretch reflex (Sillar & Skorupski, 1986; Skorupski & Sillar, 1986). This is a negative feedback reflex since the TCMRO *in situ* would be unloaded by promotor muscle contraction (Alexandrowicz & Whitear, 1957; Bush, 1981). Centrally generated motor patterns do not, however, sum with TCMRO-mediated reflexes in a simple linear manner. Stretching the TCMRO in some preparations results in reflexes that vary in both intensity and sign. Successive cycles of TCMRO sinusoidal stretch activate either promotor motor neurones (negative feedback) or remotor motor neurones (a positive feedback reflex). Therefore TCMRO reflexes are centrally modulated by spontaneous patterns of motor output (Skorupski & Sillar, 1986).

The central outputs of leg motor neurones are capable of enhancing or suppressing the reflex effect of TCMRO stimulation. In one experiment, for example, an intracellular recording was made from a remotor motor neurone that excited other remotor units and inhibited promotor motor neurones when depolarized. Sinusoidal stretch of the TCMRO in this preparation resulted in reflex inhibition of a promotor motor neurone recorded extracellularly (equivalent

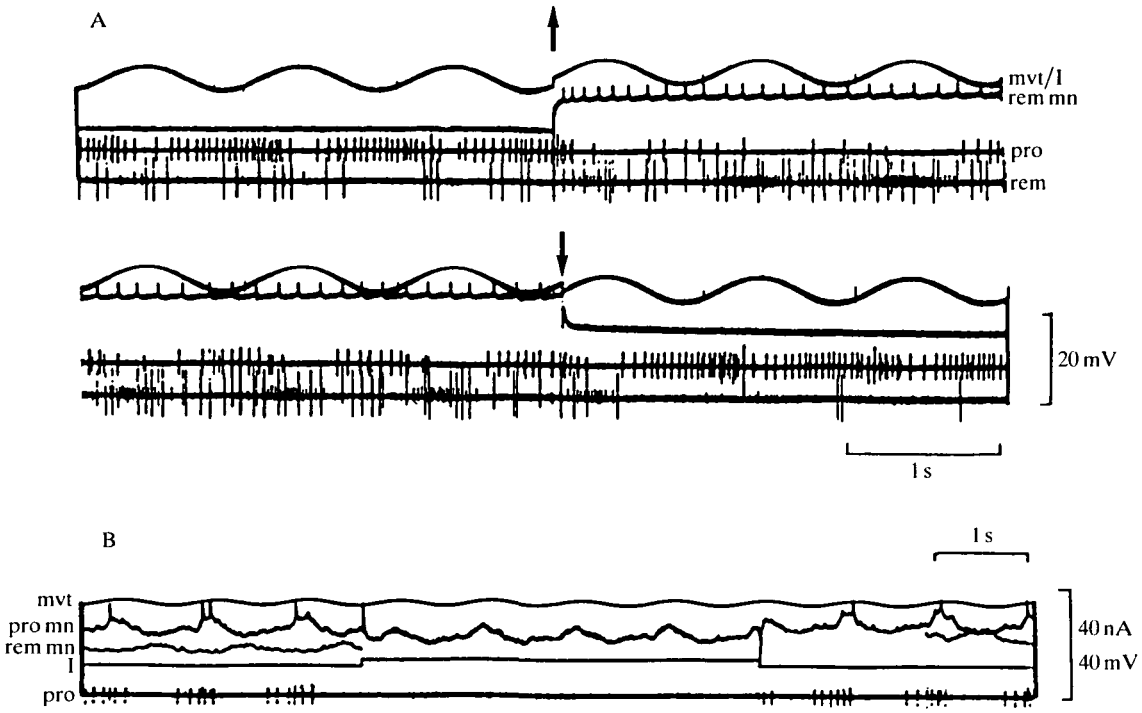

Fig. 8. Interaction of central outputs of leg motor neurones with TCMRO input. (A) Sinusoidal stretch (top trace, mvt) of the TCMRO elicits a promotor reflex (pro) but no activation of remotor motor neurones (rem). An intracellularly recorded remotor motor neurone (rem mn) receives no apparent input from the TCMRO. When this neurone is depolarized suprathreshold it is still not modulated by TCMRO stretch, but the promotor activation is abolished and a small remotor unit is now activated on each cycle of stretch. On release from depolarization the reflex returns to its former pattern. The upper and lower records are continuous. (B) Simultaneous intracellular recordings from a promotor (pro mn) and a remotor (rem mn) motor neurone while sinusoidally stretching the TCMRO (mvt). When the remotor motor neurone is depolarized (current monitor, I) the promotor motor neurone hyperpolarizes and the reflex responses are reduced in amplitude so that they no longer evoke spikes. Spiking is also abolished in a second promotor motor neurone recorded extracellularly in the promotor nerve (pro).

to a positive feedback reflex). There was no discernible modulation of the intracellularly recorded remotor motor neurone. Nevertheless, a maintained depolarization of this neurone superimposed on the mechanical stimulation resulted in powerful excitation of a small remotor unit on each cycle of stretch, and a marked decrease in promotor firing (Fig. 8A). The reflex modulation of the remaining promotor activity was such that further inhibition occurred on TCMRO stretch, in correlation with the positive feedback reflex drive of the small remotor unit.

In a similar experiment, simultaneous intracellular recordings were made from a

promotor and a remotor motor neurone while the TCMRO was continually stretched and released at 1 Hz (Fig. 8B). When the remotor motor neurone was depolarized with a 10 nA pulse of current two things occurred. First, the promotor motor neurone hyperpolarized by about 6 mV for the duration of the presynaptic current pulse. Second, the reflex depolarizations in the promotor motor neurone were reduced in amplitude from about 6 mV to 4 mV, and spiking was abolished (Fig. 8B). The reduction in amplitude of the responses could be accounted for by a maintained conductance increase associated with postsynaptic inhibition of the promotor motor neurone.

Any motor neurone that affects the activity of its synergists and antagonists is potentially capable of modulating the *gain* of reflex output; some motor neurones are apparently capable of changing the *sign* of a reflex. Figs 9 and 10 provide support for this hypothesis. A promotor motor neurone was penetrated in a preparation where stretch of the TCMRO excited either promotor or remotor motor neurones, depending on the relative levels of central excitation in these two classes of neurone (Sillar & Skorupski, 1986). The promotor motor neurone penetrated was extensively coupled centrally, exciting other promotor units and inhibiting at least one remotor motor neurone when depolarized. These effects were graded and tonic since they were reversed on hyperpolarization (Fig. 9A).

The insets in Fig. 9B show the effect of polarizing this neurone on the reflex activation of other promotor and remotor motor neurones. Each set of histograms shows the occurrence of promotor and remotor spikes in response to eight cycles of TCMRO stretch during the periods indicated in Fig. 9A. Depolarizing the promotor motor neurone with about 3 nA of current during repetitive stretch of the TCMRO increased the firing intensity of extracellularly recorded promotor motor neurones on stretch (although the neurone did not spike itself at this level of depolarization), and abolished any remotor firing (Fig. 9A,Bii). When the promotor motor neurone was *hyperpolarized*, a remotor motor neurone was powerfully activated by TCMRO stretch, and promotor activity was dramatically reduced (Fig. 9A,Biv). A bias in the membrane potential of a single motor neurone is apparently capable of effecting a reversal in reflex sign from a *negative feedback reflex* activation of promotor to a *positive feedback reflex* activation of remotor motor neurones. This result suggests that the switch between alternative reflex modes could be influenced by the relative level of excitation in one or the other of two strictly antagonistic motor pools.

In the experiment illustrated in Fig. 9 spontaneous transitions between promotor and remotor reflex activation were recorded in the absence of current injection into the promotor motor neurone (data not shown, but see, for example, the later part of the hyperpolarizing pulse in Fig. 9A: a brief reversal from remotor to promotor activation occurs). The form of the reflex input in the impaled promotor motor neurone was similarly changed according to the prevailing central drive: during promotor activation the input was predominantly depolarizing, during episodes of remotor activation it was predominantly hyperpolarizing (Fig. 10A). The depolarizing input from TCMRO stretch was sufficient to elicit spikes when

the motor neurone was depolarized by about 5 nA (Fig. 10B). Fig. 10C shows the averaged waveform of the motor neurone's response to TCMRO stretch compared (i) when the motor neurone was depolarized with about 3 nA, (ii) without current injection and (iii) when it was hyperpolarized with about 3 nA of current. This result shows that a motor neurone may not only influence the reflex activity of other motor neurones, but also the form of its own reflex input. When the motor neurone was depolarized (Fig. 10Ci) excitatory reflex input to itself and other promotor motor neurones was facilitated, but when the motor neurone was hyperpolarized excitatory input to promotor motor neurones was disfacilitated and inhibitory input became prevalent (Fig. 10Ciii). In correlation with this, the

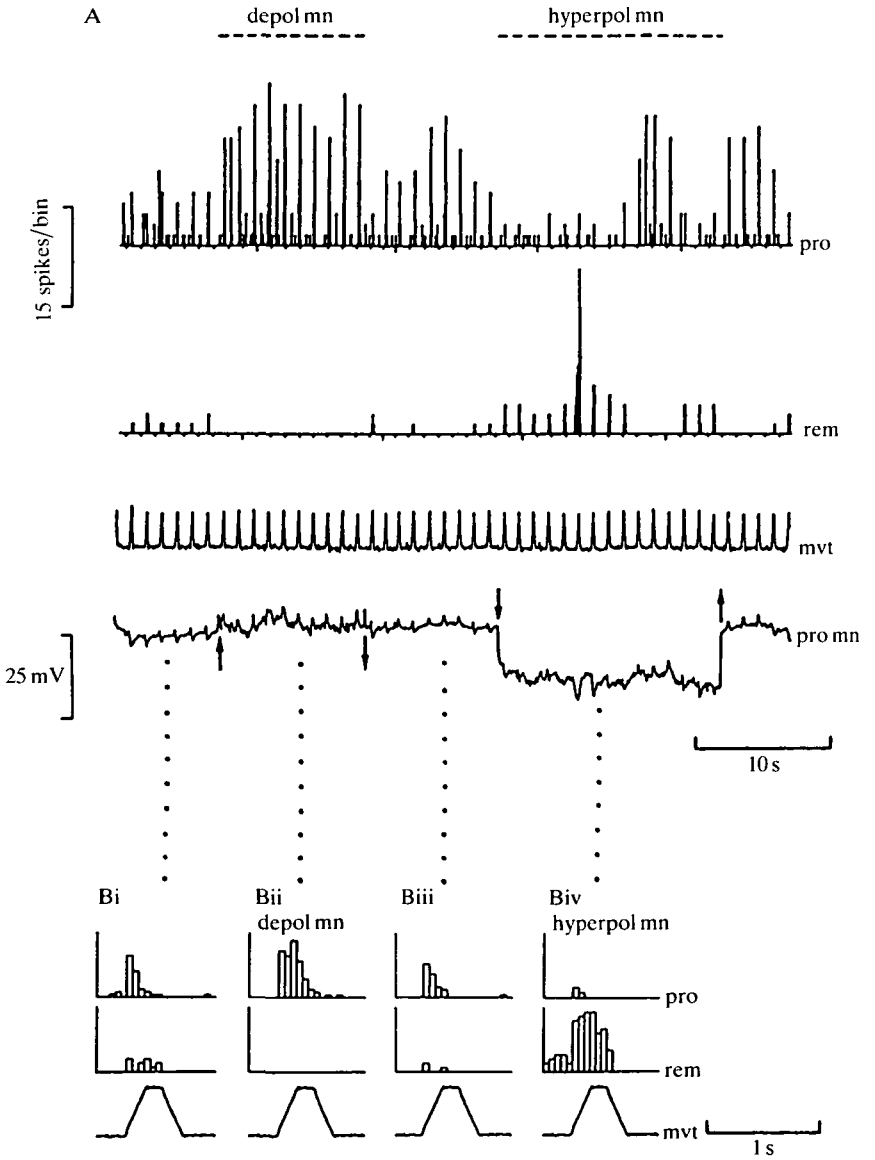

Fig. 9

reflex excitation of remotor motor neurones was most intense when the motor neurone was hyperpolarized (Fig. 9Biv). This result suggests that some motor neurones have access to premotor circuits involved in regulating the flow of reflex excitation in antagonistic motor pools.

Discussion

It is emerging as an organizational principle in crustacean neurobiology that motor neurones are not passive output elements interposed between a central rhythm generator and the effector muscles, but may themselves participate in the generation of behaviour. It has long been known that motor neurones are intimately involved in pattern generation in the stomatogastric ganglion of the lobster (Selverston *et al.* 1976; Miller & Selverston, 1985). The phenomenon, however, is not restricted to a specialized, 'autonomic' ganglion; it also applies in the motor control of the segmental appendages. For example, the timing of the neural rhythms underlying swimmeret beating in the crayfish and ventilatory beating of the scaphognathites in the crab can be altered by injecting current into single motor neurones (Heitler, 1978; Simmers & Bush, 1983). Such motor neurones must be integral parts of the neural oscillator circuit that generates the rhythmic behaviour. Central synaptic coupling has also been observed among uropod motor neurones in the crayfish (Nagayama *et al.* 1983). To this list must now be added the motor neurones of crayfish walking legs.

Nature of central coupling among leg motor neurones

A detailed assessment of the nature and extent of the synaptic interactions among walking leg motor neurones will be the object of future study. Three observations are relevant at present. (1) The central outputs of a given motor neurone may effect both excitatory and inhibitory changes in other motor

Fig. 9. Graded control of promotor and remotor reflexes by a promotor motor neurone. (A) Reflexly evoked spikes in the promotor (pro) and remotor (rem) nerves triggered window discriminators and were plotted as continuous time histograms (100 ms bin width). Maintained depolarization (depol mn) and hyperpolarization (hyperpol mn) of a promotor motor neurone (pro mn) was superimposed on a background of regularly repeated ramp stretches (mvt). The intensity of promotor reflex activation is increased when the promotor motor neurone is depolarized and decreased when the motor neurone is hyperpolarized, whereas the intensity of remotor reflex firing is increased during hyperpolarization. See text for further details. (B). Peristimulus-time histograms to show the modulation of extracellularly recorded reflexes in promotor and remotor motor neurones by current injection into the intracellularly recorded promotor motor neurone. Each pair of histograms shows the occurrence of promotor spikes (upper histograms) and remotor spikes (lower histograms) in response to eight ramp stretches of the TCMRO, prior to current injection (i), during passage of 3 nA positive current through the recording electrode (ii), with zero current (iii), and during passage of 3 nA negative current (iv). A single cycle of the movement monitor is aligned beneath each pair of histograms.

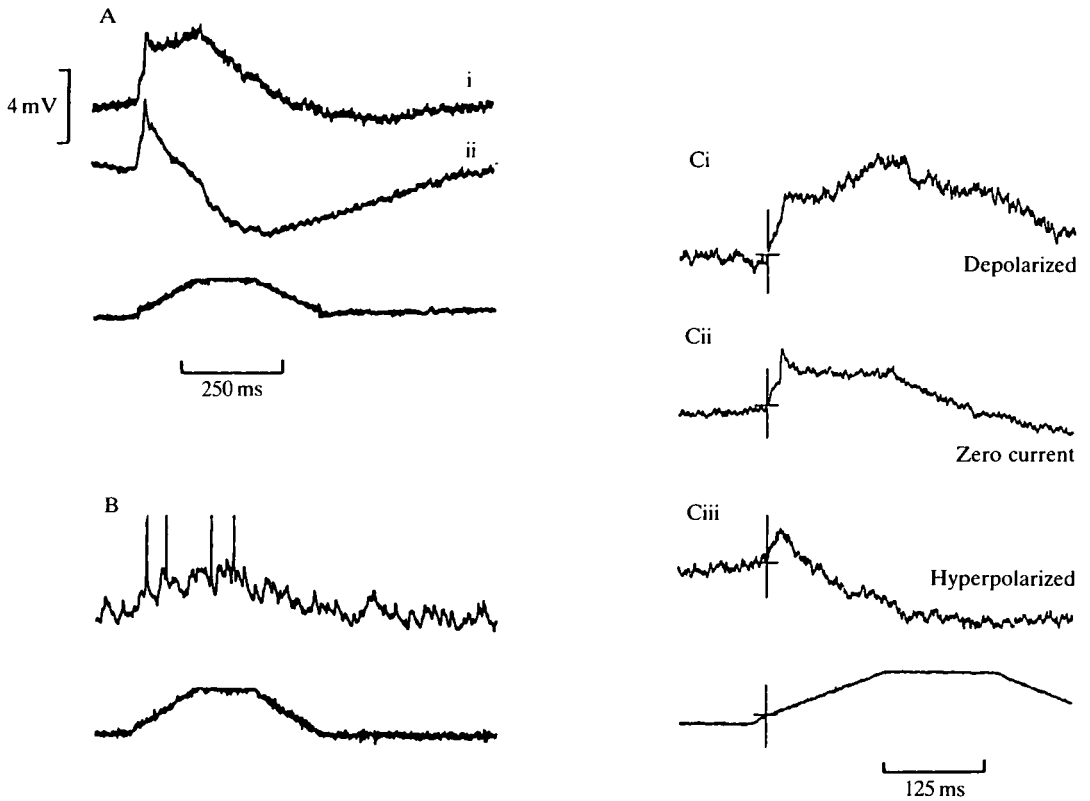

Fig. 10. (From the same preparation as Fig. 9.) (A) During expression of the promotor reflex the promotor motor neurone is depolarized on TCMRO stretch (i), but when the reflex changes to remotor activation the input to the motor neurone becomes biphasic, with a prominent hyperpolarizing component (ii). Averaged records, 15 sweeps each. (B) When the motor neurone is sufficiently depolarized with current injection the excitatory reflex input gives rise to spikes. (C) When the motor neurone is held depolarized (below threshold for spiking) the depolarizing input (i) becomes more prominent than at rest (zero current) (ii). When the motor neurone is held hyperpolarized (iii) its input becomes predominantly hyperpolarizing. See text for further details. Averaged records, 10 sweeps each.

neurones. Furthermore, these interactions are not restricted to within classes of *anatomical* synergists and antagonists; coupling is extended to motor neurones of other joints that may, in the appropriate behavioural circumstances, be *functional* synergists and antagonists (Ayers & Davis, 1977). (2) These effects do not depend on spike-mediated synaptic transmission; depolarizing a motor neurone without generating spikes may affect the activity of other motor neurones. (3) Many of these interactions are tonic, that is, they occur continuously at a motor neurone's resting potential. This is demonstrated by the fact that hyperpolarizing a motor neurone often elicits the opposite effect to that seen on depolarization.

Two classes of tonic, nonspiking synaptic transmission are recognized: electrotonic and graded chemical transmission. The excitatory interactions among

synergistic motor neurones may be mediated by direct electrical synapses. The occasional occurrence of dye-coupling among motor neurones provides some circumstantial evidence for this (Stewart, 1978). The coupling potentials that are sometimes recorded in a motor neurone on antidromic stimulation of its motor nerve may be due to electrical synapses between synergistic motor neurones (Fig. 2B).

Coupling among synergistic motor neurones does not appear to be uniform. Coupling potentials in one neurone are only occasionally correlated with spiking activity in others (Figs 2E, 3D). This suggests that coupling within a pool may be quite specific, and some synapses between synergistic motor neurones may be strongly rectifying while others are moderately or nonrectifying.

To test this hypothesis directly, however, it would be necessary to record simultaneously from pairs of interacting, synergistic motor neurones. In the present series of experiments this was achieved only once (Fig. 3A,B). It remains possible that some of the excitatory interactions between motor neurones utilize tonic, chemically mediated synaptic transmission.

The inhibitory interactions between motor neurones involve the intervention of graded chemical synapses. Underlying the inhibition in the postsynaptic motor neurone is a graded, usually hyperpolarizing, IPSP (Fig. 4B), which can be increased in amplitude on depolarization (Fig. 6C) and occasionally reversed on hyperpolarization (Fig. 5B). The inhibition involves an increase in postsynaptic conductance, as it appears to shunt depolarizations resulting from TCMRO afferent input (Fig. 8B).

In the pyloric motor system of the lobster similar graded inhibitory transmission occurs between spiking neurones, and here the interactions are thought to be monosynaptic (Graubard *et al.* 1983). This question has not been directly addressed in the present investigation, although it must inevitably arise. At least three possibilities must be considered. (1) A motor neurone directly inhibits its antagonist by tonically releasing an inhibitory neurotransmitter so that the rate of release is increased by depolarization of the motor neurone and decreased by hyperpolarization. (2) A motor neurone synapses electrically onto a nonspiking neurone that tonically inhibits an antagonistic motor neurone. (3) A motor neurone tonically excites an interneurone by the graded release of a chemical transmitter, and this interneurone in turn tonically inhibits an antagonistic motor neurone. At present we cannot distinguish between these three possibilities. The usual physiological test for monosynapticity is the latency to the onset of a response, but this is difficult to apply to interactions that are not spike-mediated. It would in any case be difficult or impossible to distinguish between hypotheses 1 and 2 above by purely physiological criteria. Hypothesis 2 has the attraction of explaining all the results while only requiring that motor neurones bear one type of central output synapse: namely, electrical. Indeed, in a recent study of the ultrastructure of crayfish uropod motor neurones with known central outputs, no morphological evidence was found for chemical output synapses (Kondoh *et al.* 1987).

Functional implications of synaptic coupling among motor neurones

The reciprocal inhibitory interactions between promotor and remotor motor neurones must reinforce the reciprocal reflex drive to these two classes of motor neurone. For example, TCMRO stretch may excite either promotor or remotor motor neurones, depending on the relative levels of excitation in these two motor pools (Sillar & Skorupski, 1986; Skorupski & Sillar, 1986). Promotor resistance and remotor assistance reflexes do not occur simultaneously: the two patterns are strictly reciprocal. This could be maintained, at least in part, by extensive inhibitory cross-coupling between the two classes of motor neurone.

Central connections between leg motor neurones are not only capable of reinforcing the prevailing pattern of rhythmic activity generated by a ganglion, but may also modulate the *sign* of reflex output (Figs 9, 10). There are both excitatory and inhibitory pathways from the TCMRO to promotor motor neurones (Skorupski & Sillar, 1986), and during rhythmic motor output transmission in these pathways alternates in a phase-dependent manner. We now present evidence that the central outputs of an individual motor neurone exert control over whether excitatory or inhibitory transmission occurs in the TCMRO reflex pathway. It seems likely that the transition between excitatory and inhibitory input from the TCMRO is accomplished at the level of as yet unidentified interneurones. If this is so then the results of Figs 9 and 10 imply that certain motor neurones have access to such putative interneurones.

The ganglionic rhythm generator itself has previously been shown to modulate the sign of TCMRO-mediated reflexes (Skorupski & Sillar, 1986); we now demonstrate that the effector motor neurones by themselves can achieve a similar result. If the motor neurones are capable of reversing the sign of reflex effects, a task previously ascribed to the central rhythm generator, then this suggests that they have access to, or comprise part of, the rhythm-generating circuitry.

Comparison with other motor systems

The walking legs and the swimmerets are segmentally homologous appendages and both swimmeret motor neurones and walking leg motor neurones make widespread central outputs (Heitler, 1978, 1983; this paper). The swimmeret rhythm, however, is rather stereotyped and invariant, whereas walking is a complex and flexible behaviour. Crayfish can walk forwards, backwards or sideways, and the legs also have the function of maintaining posture, as well as participating in other activities such as feeding. Furthermore, muscles of a leg may be multifunctional: the timing of activity in one muscle relative to others is a function of the behaviour in question (Ayers & Clarac, 1978). In view of this requirement for flexibility of motor control in the walking legs it may seem surprising that central coupling among leg motor neurones is apparently so extensive. Nevertheless, central inhibitory connections between motor neurones can be gated by the CNS (Fig. 7), which may be important in allowing motor output patterns with alternative coordination modes (e.g. backward walking).

The active involvement of motor neurones in sensorimotor integration and motor patterning has not hitherto been shown for the arthropod walking legs. In the cockroach, for example, the deafferented thoracic ganglia can produce a rhythmic motor pattern resembling that underlying walking or struggling (Pearson & Iles, 1970; Zill, 1985), but recordings from the motor neurones involved have failed to reveal any central outputs that may contribute to patterning in this system (Fourtner & Pearson, 1977). In the locust, central outputs of motor neurones are rare (Watson & Burrows, 1982). Instead, many of the interactions we observe between crayfish leg motor neurones are paralleled in the locust by one-way inhibitory interactions between nonspiking local interneurones (Burrows, 1979), which exert excitatory and inhibitory control over sets of leg motor neurones (Burrows, 1980). Such interneurones are also capable of effecting changes in proprioceptive reflexes in a leg (Siegler, 1981).

Our results strengthen the assertion that neural circuitry reflects evolution as well as functional principles of neural organization (Dumont & Robertson, 1986). The presence of central output synapses on motor neurones is rare in insects, but appears to be the rule in decapod crustaceans. This seems to suggest that many details of the components of neural circuitry involved in the patterning of motor behaviour are phylogenetic characteristics rather than functions of the behaviour in question. The relatively stereotyped rhythm of respiration, for example, appears to be generated exclusively at the interneuronal level in locusts (Burrows, 1978), yet crayfish leg motor neurones, subserving an appendage with a highly varied behavioural repertoire, make extensive central connections (this paper). Investigations into the neural basis of a variety of 'simple' behaviours may reveal as much about an organism's phylogenetic history as they do about principles of neural organization.

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