MOTOR PROGRAMME SWITCHING IN THE VENTILATORY SYSTEM OF CARCINUS MAENAS: THE NEURONAL BASIS OF BIMODAL SCAPHOGNATHITE BEATING

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SUMMARY

Intracellular recordings have been made from ventilatory neurones in semi-isolated and isolated thoracic ganglia of the crab Carcinus during spontaneous switching between the two motor programmes underlying forward and reversed beating of the scaphognathites (SGs).

Ventilatory reversals are dependent upon the central activation of two subgroups of motoneurones which are normally silent, and different from those driving the same SG muscles during the forward rhythm. Two further subgroups of motoneurones remain active throughout both rhythm modes.

The results suggest that both motor output patterns are produced mainly by periodic inhibitory synaptic input from the same oscillator network, and that neural switching between the rhythm modes occurs directly at the level of the motoneurones themselves.

It appears that both sets of ‘forward’ and ‘reversal’ motoneurones are driven continuously throughout oscillator activity, and that bursting activity in these sets is gated by the selective application or removal of additional, tonic inhibition.

INTRODUCTION

The gill chambers of decapod Crustacea are irrigated by the rhythmic beating of the bilateral pair of scaphognathites (SGs), the blade-like exopodites of the second maxillae. By its continuous biphasic sculling motion, the SG normally draws water forwards across the gill surfaces and expels it through an excurrent opening below the antenna (Pearson, 1908). Most crustacean decapods, however, are also capable of periodically reversing the direction of ventilatory current flow through the gill chambers (Bohn, 1902; Borrodaile, 1922; Arudpragasam & Naylor, 1966). In brachyurans, such as the shore crab Carcinus maenas, reversed pumping of the SG can last for several consecutive beat cycles, resulting in water being drawn in anteriorly and expelled via the normal incumbent openings at the base of the chela and walking limbs (Arudpragasam & Naylor, 1964; Hughes, Knights & Scammell, 1969).

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The functional significance of SG beat reversals is not clearly understood, but they appear to be important in cleaning detritus from the gill surfaces (op. cit., Berlind, 1977) and from branchiostegal hairs which filter incurrent water during forward beating (Wilkens & McMahon, 1972). Reversed pumping is also the predominant mode used to irrigate the gills of burrowing crabs, such as Corystes, which normally lie buried in sand (Arudpragasam & Naylor, 1966), while the intertidal habitat of Carcinus may explain the increased occurrence of ventilatory reversals in this species under hypoxic conditions and during partial exposure to air (Taylor, Butler & Sherlock, 1973).

The neuromuscular control of SG beat reversal in Carcinus has been described in some detail by Young (1975) on the basis of extracellular recordings from the peripheral SG nerves and muscles. The ten major muscles controlling the appendage are organized into two main antagonistic sets of five depressor (D) muscles and five levators (L), each set being innervated by a separate motor branch of the ventilatory nerve root arising from the thoracic ganglion. A further division related to function and timing of activity provides four muscle subgroups in all, two depressors (D1 and D2) and two levators (L1 and L2). Muscles within each subgroup are activated more or less synchronously, and during normal forward beating the recruitment sequence of the four groups is D1, D2, L1, L2, D1. During reversed beating, however, the phases of D2 and L2 within each cycle are exchanged, thereby yielding the completely inverted sequence D1, L2, L1, D2, D1. The corresponding changes in motoneurone burst activity thought to underlie SG reversals are summarized in Fig. 1. By comparing the ventilatory motor output patterns during forward and reverse beating, Young (1975) predicted that the inversion of the cyclic contractile sequence of SG muscles is dependent upon (i) activation of D1 and L1 muscles by the same sets of motoneurones throughout forward and reverse ventilation; and (ii) activation of D2 and L2 muscles by different sets of motoneurones operating during the two rhythm modes. Thus D2F and L2F motoneurones which control the D2 and L2 muscles during forward beating are silent during reversals, when they are replaced by another set of motoneurones, D2R and L2R, which fire bursts in advance of the D1 and L1 units, respectively.

The present study attempts to provide more direct information on the neural control of bimodal ventilation in Carcinus by recording intracellularly from ventilatory neurones within the thoracic ganglion. The basis for this approach is that, as in other arthropod motor systems, the isolated thoracic ganglion can carry on spontaneously recurring episodes of rhythmic ventilatory motor output which are similar to those driving the SG muscles in the intact animal (cf. Simmers & Bush, 1983 with Young, 1975). Furthermore, the reduced preparation can generate the motor programmes underlying both forward and reverse ventilation, and is capable of switching spontaneously between the two. Although some of the major cellular mechanisms of ventilatory rhythm generation have already been established with microelectrode investigation (Mendelson, 1971; Simmers, 1981; Simmers & Bush, 1980, 1983), the reports to date have concentrated on the neuronal basis of a single stereotyped motor programme, namely that responsible for the normal forward beating of the SG. Attention in this paper is therefore focused on the central nervous mechanisms coordinating both forward and reversed SG beating, and in particular...
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Fig. 1. Summary diagram showing the recruitment sequence of ventilatory motoneurones during forward and reverse beating of the scaphognathite. Each box represents the mean duration and position in the beat cycle of impulse bursts in motoneurones from the same functional subgroup. For clarity the D2a motoneurones whose axons are carried in the levator nerve (LN) trunk have been incorporated into the D2F subsession of the depressor nerve (DN). Arrows link those groups of units, viz D1 and L1 motoneurones, which are active during both rhythm modes. Note that the forward rhythm sequence is inverted during reversals by the activation of two different sets of motoneurones (D2R, L2R) which replace the D2F and L2F motoneurones and fire before, instead of after, the D1 and L1 bursts, respectively. The diagram is based on observations in the present study and of Young (1975).

the cellular level at which motor pattern generation is gated to produce the two different modes of behaviour. On the basis of the experiments described here, and in general accordance with the extracellular evidence of Young (1975), three distinct classes of ventilatory motoneurones can now be clearly recognized – (a) 'bimodal cells', which are rhythmically active throughout both modes of SG beating; and others which only discharge either (b) during forward beating, or (c) during the reverse rhythm. The evidence also suggests that the gating of bursting activity occurs directly at the level of the motoneurones themselves, and is dependent upon an interplay between phasic and tonic inhibitory inputs.

MATERIALS AND METHODS

Male Carcinus maenas were obtained from the Plymouth Marine Biological Laboratory and kept at 12 °C in tanks of aerated artificial sea water. Experiments were performed on semi-isolated or isolated preparations of the thoracic ganglion according to procedures described elsewhere (Simmers, 1978; Simmers & Bush, 1983). Extracellular recordings of spontaneous motor output to a scaphognathite (SG) were
obtained with polyethylene suction electrodes placed on the peripheral levator and depressor branches of the ventilatory nerve root. Simultaneously, a glass micro-electrode filled with 3 M-KCl (d.c. resistance 20–60 MΩ in crab saline) was used to record intracellularly from ventilatory neurones, mostly within the ipsilateral neuropile of the partially desheathed thoracic ganglion. Current could be injected through the recording electrode via a bridge circuit built into the electrometer amplifier. Impaled cells characterized physiologically as ventilatory motoneurones (see below) were classified according to the functional nomenclature of Young (1975).

Conventional electrophysiological equipment and techniques were used for the display, storage, and photography of all neural recordings. The data presented here are based upon intracellular recordings during spontaneous 'reversed' ventilatory motor activity in 15 preparations.

RESULTS

Intracellular recordings from the neuropilar processes of ventilatory motoneurones during spontaneous rhythmic activity reveal slow, large amplitude (8–30 mV) oscillations in membrane potential, with bursts of electrotonically decremented impulses superimposed upon the depolarized peaks (e.g. Fig. 2; see also Simmers & Bush, 1983). Recognition of a cell as a motoneurone was dependent upon a 1: 1 correspondence of intracellularly recorded action potentials with spikes recorded peripherally in either the depressor (DN) or levator (LN) motor nerve branches. It is already established that in most if not all of these cells the slow waves are produced by a periodic inhibitory synaptic drive from an oscillator network which includes non-spiking neurones (Simmers & Bush, 1980, 1983).

Motoneurones studied here were further classified, at least down to the functional subgroup level (D1, D2, L1, L2), by correlating the timing of their intra- and extracellular burst activity with the rest of the ventilatory motor output pattern. The motor command to the 10 SG muscles appears to be purely excitatory, since as yet no inhibitory SG motoneurones have been found (Young, 1975; Pilkington & MacFarlane, 1978; Moody-Corbett & Pasztor, 1980).

Bimodal motoneurones

Ventilatory motoneurones contribute to both the forward and reverse motor programmes (Fig. 2). The D1 motoneurones in the two different preparations illustrated (A, B) continued to fire bursts of impulses throughout both rhythm modes, with maintenance of a strict 1: 1 correlation between the intracellular spikes and those recorded from the corresponding axon in the peripheral nerve (DN). Furthermore, the individual bursts and their underlying slow waves were always at the same frequency as that of the prevailing ventilatory motor pattern.

Two characteristic features of bimodal ventilation in Carcinus are also apparent in Fig. 2. Firstly, reversal periods tend to be of short duration, generally lasting for only a few consecutive cycles. The transient nature of reversals (usually less than 5 s) and their relatively infrequent occurrence (from 6 to 60 episodes per hour) appear to be typical of the intact animal under normal conditions (Arudpragasam & Naylor, 196•

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In most preparations where the thoracic ganglion was semi-
isolated or completely isolated for intracellular recording, episodes of reversal motor
output also occurred infrequently. However, in a few such preparations (e.g. Fig. 6B, C) the SG motor pattern could become locked in the reversal mode, and remain in

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Fig. 2. Neuropilar recordings from two D1 depressor motoneurones (A, B), showing that they contribute bursts of impulses to the motor programmes for both forward and reversed gill ventilation. The bar above each record in A(i), B(i), (and in subsequent Figs) indicates an episode of reversal motor output occurring spontaneously during otherwise normal forward rhythmicity. A(ii, iii), B(ii, iii). Superimposed oscilloscope sweeps on a fast timebase, triggered from spikes recorded intracellularly during the periods indicated [arrows to A(i) and B(i)], reveal phase-locked axonal impulses in the depressor nerve (DN) throughout the two motor patterns.
this state for periods lasting up to several minutes. These observations not only indicate that switching between modes can be triggered endogenously within the isolated CNS, but suggest that higher command elements in the cerebral ganglion and/or sensory inputs that are normally present in the intact animal may contribute to this switching mechanism and help maintain the dominance of the forward rhythm.

A second characteristic difference between the two motor output patterns in both intact and dissected preparations is that the cycle frequency during reversals is generally much higher than that during the preceding forward rhythm. In Fig. 2, for example, the difference between the pre-reversal and reversal cycle frequency is six-fold and three-fold in A and B, respectively. The change in frequency occurs abruptly with the onset of a reversal session. At its termination, the frequency does not always return immediately to the pre-reversal level as it does in Fig. 2B, but can decline gradually over several cycles of the resumed forward rhythm as in Fig. 2A. The implications of this in relation to the central origin of the two motor patterns will be discussed later.

Forward rhythm motoneurones

Whereas the D1 and L1 muscle groups appear to be controlled by the same sets of motoneurones throughout the two rhythm modes, the units supplying the D2 and L2 muscles during forward beating are silent during reversals and are replaced by new sets of motoneurones (Fig. 1). In accordance with this scheme, Fig. 3 shows that the two motoneurones whose cyclic bursts drive muscles D2a and D2b during the normal forward rhythm become inactive during reversals. Note that in this experiment, the D2aF motoneurone was penetrated in its peripheral axon, which is carried in the levator (LN) nerve trunk (Young, 1975). Thus, the cell's discharge that occurred during the normal forward mode comprised bursts of overshooting action potentials, with no sign of the slow membrane potential oscillations that underlay their central production. The spike bursts in the D2bF unit were part of the main extracellularly recorded depressor (DN) session, but clearly this motoneurone did not fire either during a reversal episode.

The neuropilar recordings in Fig. 4 demonstrate that there are also L2 motoneurones which become inactive during rhythm reversals. Throughout the forward
Fig. 4. Neuropilar recordings from a levator L2 motoneurone which fires only during forward rhythm motor output. During a spontaneous reversal episode (A), the membrane potential oscillations of the cell are considerably reduced in amplitude and it remains silent. The dotted line on the intracellular trace in A(i) indicates the resting membrane potential level of the motoneurone during pauses in rhythmic activity. A(ii) shows the indicated portion of A(i) with a faster time scale and greater amplification. B, C. The cell's oscillations result from periodic synaptic inhibition, since the waveform during the normal forward rhythm could be diminished (B) and eventually reversed in polarity (C) by maintained hyperpolarization with injected current. Note the similarity between the amplitude changes induced by current in B and those occurring spontaneously during a reversal as in A.
rhythm, the penetrated cell discharged bursts of spikes superimposed upon approximately 20 mV oscillations in membrane potential. As for SG motoneurones in general, these slow waves result mainly from a periodic inhibitory synaptic input to the cell (Simmers & Bush, 1983). Thus with increasing levels of injected hyperpolarizing current the membrane oscillations decreased in amplitude (Fig. 4B), and eventually became phase inverted so that they were now depolarizing (Fig. 4C). During a spontaneous reversal (Fig. 4A) the motoneurone continued to display slow wave activity phase-locked to the reverse rhythm frequency, but the oscillations were much reduced in amplitude and the cell never reached threshold for spiking. The weakened oscillatory activity expressed in this motoneurone during reversals could reflect a selective decrease in the strength of periodic input from some premotor level. Alternatively it could be due to decoupling influences exerted directly at the level of the motoneurone itself. This latter possibility was initially suggested (see later) by the observation that the attenuated waveform during a reversal session was similar in amplitude to that artificially induced during the forward rhythm when the cell was injected with small levels (approx. 2 nA) of negative current (cf. Fig. 4A with 4B). It could be, therefore, that although the motoneurone continues to receive a strong oscillatory drive throughout reversals, it is prevented from firing by an additional, tonic input which keeps the cell's membrane relatively hyperpolarized, near equilibrium potential for the periodic input. The resting position (−49 mV) of this motoneurone during quiescent periods lay close to the level of the depolarized peak of each forward rhythm cycle, at which time the membrane potential oscillated between −46 and −66 mV. This indicates, therefore, that any tonic decoupling influence impinging upon the cell during reversals must be inhibitory in order to hold the cell's membrane potential down towards the more negative end of this range, as seen in Fig. 4A(i). At the end of the reversal period, then, the tonic inhibition is removed and the motoneurone depolarizes immediately, thereby re-augmenting its response to the oscillator's inhibitory drive, and threshold for spiking is once again reached during the disinhibitory phase of each forward rhythm cycle.

Reverse rhythm motoneurones

If the motoneurones which activate D2 and L2 muscles throughout the forward mode become silent during reversals, then these cells must be replaced by activity in other motor units, themselves silent during forward SG beating (see Fig. 1). Examples of two different L2 reversal (R) motoneurones are shown in Figs 5 and 6. Throughout the forward motor pattern, the membrane potential of these cells appeared to remain at a steady state level with a complete absence of any spiking. During reversals, however, these cells displayed strong membrane oscillations, with bursts of action potentials riding on the positive peaks (Figs 5A, 6A). Each spike burst occurred at the correct phase in the cycle for the reverse mode recruitment of the L2 muscle group, preceding rather than following the motor bursts to the L1 muscle groups (cf. Figs 5A and 6A with 4A).

The entire range of membrane potential fluctuation during reversals in these cells was depolarized relative to the membrane potential level during the forward rhythm (Figs 5A, 6A). Nevertheless, these underlying slow waves are still due largely to synaptic inhibition between spike bursts, rather than periodic excitation during each
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Fig. 5. A levator (L2) motoneurone which normally fires only during rhythm reversals. A. Throughout forward rhythmicity the cell's membrane potential oscillates weakly along with the motor output pattern. During a spontaneous reversal episode, the oscillations increase in amplitude and a burst of spikes occurs on each depolarized peak. The first reversal burst in A(i) is shown at a faster sweep-speed and higher gain in A(ii). B–E. The injection of constant current during the forward motor pattern reveals a continuous cyclic inhibitory drive to the motoneurone. B, C. Depolarizing current increases the amplitude of membrane oscillation and eventually causes the cell to fire rhythmic bursts of impulses [e.g. C(ii)] whose intensity is directly related to the level of applied current. D, E. Conversely, imposed hyperpolarization results in an inversion of the waveform's polarity and an increase in its amplitude in the opposite direction.
Fig. 6. Neuropilar recordings from a levator (L2) reversal motoneurone. A. As for the motoneurone of Fig. 5, this cell fires bursts of spikes astride large membrane potential oscillations during reversals, but otherwise slow wave activity is barely discernible and it remains silent. A(ii) shows the first two reverse rhythm bursts in A(i) at faster speed and greater amplification. B. During an extended reversal episode, an inhibitory synaptic input to the motoneurone is revealed by the injection of hyperpolarizing current pulses (the bridge circuit is unbalanced). Note the suppression of impulse activity in the injected cell, the inversion in polarity of the membrane oscillations and the correlation between the amplitude of the inverted waveform and the two different strengths of negative current. There is also a slight reduction in the frequency of the entire motor output pattern during current injection, suggesting a reciprocal interaction between the penetrated cell and the elements driving it. C. Transition from reverse to forward rhythm motor output (B and C are continuous). The marked decline in amplitude of oscillation and the cessation of spiking is associated with a gradual hyperpolarization of the motoneurone.
This is indicated by the complete phase-inversion of the membrane wave when an L2R motoneurone was held sufficiently hyperpolarized with injected current during a sustained reversal episode (Fig. 6B). Once inverted, the waveform continued to increase in amplitude with increasing negative current.

The motoneurone in Fig. 6 must also have had access to the rhythm generating circuit controlling it, since imposed hyperpolarization increased both the period of oscillation in the penetrated cell, as well as the cycle period of the reversal motor output pattern as a whole. For example, 1.4 nA hyperpolarizing current caused a 15% increase in the rhythm period, while 2.0 nA slowed it by 30% (Fig. 6B), with these effects lasting only for the duration of applied current. Further evidence for feedback connections and their presynaptic driver elements has been considered elsewhere (Simmers & Bush, 1983).

Central origin of reversal motor programme

Given that patterned motor output for ventilation is determined largely at the premotor level, then the generation of rhythmic bursting in reversal motoneurones could be derived from two possible sources. One possibility is that D2R and L2R motoneurones are coupled to an oscillator system which becomes active only during rhythm reversals. This would imply that there are two separate ventilatory oscillators, one of which drives the forward rhythm (including D2F and L2F motoneurones) and the other the reverse rhythm (including D2R and L2R motoneurones). However a dual arrangement of this sort would require that the D1 and L1 motoneurones have equal access to the output of both oscillators since these cells remain active throughout the two rhythm modes (see Fig. 2). Given the infrequent and transitory nature of reversals in crabs, it is difficult to see the advantages of duplicating rhythm-generating circuitry which for most of the time remains redundant. Purely on the basis of neural economy, therefore, it would seem more likely that the two ventilatory motor rhythms are driven by a single central oscillator, with the appropriate changes in coupling occurring at a lower level in the hierarchy of the system.

A number of physiological observations suggest that this is the case. For example, in recordings from the L2R motoneurones of Figs 5-7, it was possible to demonstrate a continuous cyclic input to both these cells throughout the forward rhythm mode when they normally remain silent. Close inspection of their membrane potentials during forward rhythmicity reveals weak oscillatory activity phase-locked to the rest of the motor programme (Figs 5A, 7A). Furthermore, the injection of increasing levels of tonic depolarizing current caused an increase in the amplitude of these oscillations, until at sufficiently high levels of current (less than 1 nA), the individual cells began to fire bursts of spikes on the depolarized peaks of every cycle (Figs 5B, C, 7B-D). The number of spikes occurring within each burst was directly proportional to the level of imposed depolarization. It is important to note that these L2R bursts induced during the forward rhythm immediately followed burst activity in the D1 motoneurones. This corresponds exactly to the relative discharge positions of these two motoneurone subgroups during a complete rhythm reversal (cf. Figs 5C and 7D with 5A and 6A, respectively). The continuous oscillatory activity in these cells could also be unmasked by artificial hyperpolarization. Again there was a marked increase in the amplitude of the membrane waves with increasing level of injected negative current.
The effects of injecting current into the neuropilar process of a reversal (L2R) motoneurone during forward rhythm motor output. Records are from the same cell as in Fig. 6. A–D. Increasing strengths of injected depolarizing current cause a progressive increase in the amplitude of membrane oscillation and at sufficient levels (B–D) evoke spike bursts of increasing intensity in the otherwise silent motoneurone. D(ii) shows a higher gain record during imposed depolarization similar to that in D(i). E, F. Hyperpolarizing currents invert the polarity of oscillation then increase its amplitude. These effects indicate that, as during reversals in this cell (see Fig. 6B), the weak forward-mode oscillations also result from continuous periodic synaptic inhibition.

The effects of injecting current into these two reversal motoneurones during forward rhythmicity are summarized by the graph shown in Fig. 8. In both cases the amplitude of oscillation varied linearly with currents of either polarity. The current-induced responses of an L2F motoneurone are also plotted in order to compare parameters of forward-mode oscillation in the two functional types of motoneurone. The complete inversion of the membrane waves with hyperpolarizing currents indicates that oscillation in both cell types results from periodic inhibition at chemical synapses.

Thus reversal motoneurones are driven throughout the forward rhythm in the same way as during reversals. Furthermore, the observations that this drive is always locked
the frequency of the prevailing motor pattern, and that it is capable of producing correctly-timed bursts of spikes in an otherwise quiescent cell, suggest that it originates from the same premotor source during both rhythm modes. A similar explanation would account for the subthreshold oscillatory activity seen to persist in L2F motoneurones during rhythm reversals (Fig. 4A).

Further evidence to support the idea of a common ventilatory oscillator for both forward and reverse motor patterns comes from penetrations of elements within the rhythm generating system itself. Fig. 9 shows recordings from two non-spiking neurones which have already been established as intrinsic components of the oscillator network producing the normal forward motor programme (Simmers & Bush, 1980). The membrane potential of these cells continued to oscillate, with little change in amplitude, throughout both rhythm modes. Furthermore, this slow wave activity remained phase-locked to the motor output pattern, whether it was that of the forward mode or the higher-frequency reverse mode. If separate oscillator systems are generating the two ventilatory rhythms, then it could be expected that each system would reduce (or cease) its cyclic drive, or be decoupled from the other, during the rhythm

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Fig. 8. Relationship between the amplitude of membrane potential oscillation during forward rhythm motor output and the amount of current injected into a forward rhythm motoneurone and two reverse rhythm motoneurones. Measurements were taken from recordings of the L2F cell (△) in Fig. 4 and the L2R cells (○, ●) in Figs 5 and 7, respectively. Note the relatively small amplitude of oscillation occurring in the L2R motoneurones in the absence of current and the correspondingly low levels of negative current required to invert their waveforms. This suggests that, in contrast to the L2F motoneurone, the membrane potential of these cells normally lies close to the equilibrium potential for their inhibitory drive.
Fig. 9. Spontaneous rhythm reversals during intracellular recordings from two non-spiking neurones (Vn) which are part of the ventilatory central pattern generator. The membrane potential of these neurones continues to oscillate throughout, and in phase with, both motor programmes. Note that the single unit bursts recorded extracellularly in the depressor nerve (DN) in A are from a D1 bimodal motoneurone.

in which it does not participate. That this does not appear to be the case, at least for the two non-spiking cells in Fig. 9, suggests that these elements are involved in the production of both motor patterns.

**Gating of reversal motoneurones**

That the membrane oscillations expressed by L2R cells are markedly reduced in amplitude during the forward rhythm mode (as are L2F motoneurones during reversals) does not appear to be due to a corresponding decrease in the strength of their periodic synaptic inputs. Rather it seems more likely that the forward-rhythm deactivation of these cells is due directly to tonic changes in responsiveness of the postsynaptic motoneurones themselves. The arguments for this mechanism are an extension of those already suggested above for the reciprocal deactivation of forward rhythm motoneurones during reversals, and are as follows.

Given the continuous and inhibitory nature of the premotor drive to these cells, then a convenient way effectively to decouple the two (during the forward rhythm, in this case) would be for the postsynaptic membrane potential to remain close to the equilibrium potential for this inhibitory input (Fig. 8). The amplitude of the postsynaptic response would be diminished accordingly, with the cell membrane failing to reach threshold for spiking during the disinhibitory phase of each forward rhythm cycle. Either a depolarization (whether imposed as in Fig. 7B–D or during a spontaneous reversal as in Fig. 6A) or a hyperpolarization (Fig. 7E, F) away from this negative steady state level would cause an increase in the driving force of the ionic currents which underlie the inhibitory postsynaptic response. This is reflected by a corresponding increase in the amplitude of membrane oscillation (Fig. 8) until, with sufficient overall depolarization, the cell is able to reach spike threshold during the positive-going phase of each cycle.
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Fig. 6C shows the changes in activity of an L2R motoneurone over the period of spontaneous transition from the reverse to forward rhythm mode. Throughout the reversal episode, the large amplitude (15 mV) slow waves arose from a hyperpolarized maximum of 64 mV. On resumption of the forward motor programme, the cell's membrane gradually repolarized to a negative limit of approx. 70 mV, with a concomitant reduction in the amplitude of the membrane wave and a cessation of any spiking activity. Moreover, it is significant that, for this motoneurone, the negative injected current required to produce an inverted waveform of 10 mV amplitude during a reversal was 1.4 nA (Fig. 6B), whereas only 0.9 nA was needed to produce an inverted wave of similar amplitude during the forward rhythm (Fig. 7E). This could be explained by the observed bimodal difference in the mean membrane potential of the cell relative to the equilibrium potential for its rhythmic inhibitory input.

What natural tonic influences could be responsible for changing the responsiveness of reversal motoneurones to the ventilatory oscillator? One possibility is that, throughout the forward rhythm, a separate tonic inhibitory input is superimposed upon that of the oscillator, thereby holding the cell relatively hyperpolarized and inactive. During a reversal episode, the cell membrane is released from this tonic influence and is able once again to reach threshold for spiking in response to the oscillator's continuous drive. Alternatively, it is just as likely that reverse-mode activation could be due to a tonic excitatory input which is absent during the forward rhythm.

Although further experiments are needed to resolve these two possibilities in the crab ventilatory system, there is some evidence to suggest that deactivation of L2R motoneurones during the forward rhythm results, at least in part, from tonic inhibition. For example, the forward-mode oscillations in the L2R motoneurone of Figs 6 and 7 occurred between membrane potential values of -67 and -70 mV. In the absence of any rhythmic ventilatory activity, however, the membrane potential of this cell settled at a 'resting level' of -56 mV. Assuming the absence of synaptic input to the neurone during such quiescent periods, then an inhibitory influence must be responsible for the relatively hyperpolarized membrane level attained during the forward rhythm. If the intracellular ionic concentrations are assumed to be comparable to those of known neurones in other marine invertebrates (e.g. squid giant fibres), the equilibrium potentials for K⁺ and Cl⁻ ions would be around 70–80 mV. The observation that the membrane potential of L2R motoneurones lies close to this region throughout the forward rhythm is therefore consistent with the hypothesis that it is being 'clamped' at this level by a tonic synaptic inhibition mediating a relatively high K⁺ or Cl⁻ conductance.

DISCUSSION

The results of the present study permit several conclusions to be drawn on the neuronal basis of bimodal ventilation. The first concerns the cellular level at which the two different motor programmes originate from within the ventilatory rhythm-generating system. The possibility that the two rhythms are driven by separate central oscillators is perhaps suggested by the sudden increase in cycle frequency which invariably accompanies the transition from forward to reverse SG beating. In a given
preparation, the mean cycle frequency during periods of reversal activity is consistently higher than at any time during the normal forward rhythm. Such bimodal differences could thus be derived from a switch between two independent oscillators operating at different intrinsic rhythm frequencies.

However, several lines of evidence indicate that both motor output patterns are in fact produced by the same oscillator network. First, the slow re-entrainment of the forward rhythm frequency which commonly follows a reversal episode (Fig. 2A) does not readily comply with independent control at the oscillator level. Secondly, the observation that some ventilatory motoneurones, namely the L1 and D1 units, participate during both rhythm modes is more simply explained in terms of a single oscillator rather than a dual system. Thirdly, motoneurones which are active only during forward (i.e. D2F, L2F units) or reversed (D2R, L2R units) SG beating continue to receive an underlying oscillatory input throughout the rhythm mode in which they normally remain silent (Figs 4—7). This input is always phase-locked to the current motor pattern and, furthermore, it can be unmasked by artificial membrane polarization at any time, regardless of rhythm mode, to produce correctly-phased burst activity in the penetrated cell. Fourthly, non-spiking interneurones that have been identified as intrinsic components of the rhythm generating system for ventilation (Mendelson, 1971; Simmers & Bush, 1980), continue to show strong oscillations in membrane potential during both modes of rhythmic behaviour (Fig. 9). Again this oscillatory activity remains synchronized to the prevailing motor programme.

The production of the motor output patterns underlying forward and reverse ventilation appears, therefore, to be dependent on: (i) a premotor rhythm generator which is common to both forward and reverse modes, (ii) some ventilatory motoneurones (viz D1, L1 subgroup units) being continuously driven at suprathreshold levels by the oscillator throughout both rhythm modes, (iii) the gating of other pairs of motoneurone subgroups (viz D2F and L2F, or D2R and L2R) to the oscillator’s drive according to the mode in which they are required to participate.

Biasing different motoneurones into or out of a common oscillator system could occur at one of two cellular levels. The first possibility is that changes in coupling could take place at the level of interneurones interpolated between the oscillator and motoneurones, as suggested by Young (1975). In his model of the crab ventilatory system, Young proposed that the four functional muscle subgroups of an SG are controlled by six ‘group level’ interneurones, three depressors and three levators. Two of these interneurones (D2* and L2*) are activated by the oscillator only during reversed beating, when they replace the equivalent forward mode premotor units (D2 and L2), and drive the different sets of motoneurones to the muscles in these groups. A similar scheme has been suggested by Miller (1973) to account for alterations in the synchronization of spiracular activity with tracheal ventilatory movements in the cockroach. In this system it is thought that the change in coupling of the spiracle opener motoneurones to the respiratory oscillator is mediated by two different sets of coordinating interneurones with the appropriate set being selected by command input biases. An important difference, however, is that whereas the bimodal phase changes in the activity of crab SG muscles are dependent in part upon the coupling of different subsets of motoneurones, in cockroach the spiracle opener muscles are driven by the same set of motoneurones during both rhythm modes.
The possibility that crab ventilatory motoneurones are also gated at the premotor level (Young, 1975) does not, however, agree with the intracellular evidence of the present study. This inference derives mainly from the observation that the cyclic output of the ventilatory oscillator can be shown to reach all its follower motor elements throughout production of both rhythms. If changes in coupling were taking place at the level of interneurones presynaptic to the motoneurones, then it is difficult to see how the oscillator's drive could remain discernible at the lower level of the motoneurones themselves, even during periods when they normally remain silent. This observation would be compatible, however, with the interpretation that neural switching takes place directly at the level of the motoneurones rather than at the premotor level. A possible scheme is then as follows.

Both sets of 'forward' and 'reversal' motoneurones are driven continuously in the appropriate phase by a single ventilatory oscillator with multiphasic output. During production of the forward motor pattern, the reversal motoneurones, D2R and L2R, are held relatively hyperpolarized by an independent inhibitory input which effectively overrides that of the oscillator and suppresses burst activity in these cells. When switching to the reverse mode occurs, this tonic inhibition is removed and the reversal motoneurones are then free to fire bursts of spikes in response to the oscillator's ongoing drive. At the same time the appropriate forward motoneurones, D2F and L2F, are inhibited and they in turn remain silent during the reversal period. Other motoneurones, viz D1 and L1, do not receive these biasing inputs and consequently continue to fire throughout both rhythm modes.

Although this explanation is compatible with the available data, the real situation is no doubt more complex. For example, there is nothing in the data to exclude the possibility that the gating mechanism involves the application/removal of tonic excitation, as well as inhibition, from the command biasing elements. Furthermore, the above scheme takes no account of the probable existence of synaptic interactions between components of the same cellular level (Simmers & Bush, 1982). Lateral couplings, whether at the motoneurone, premotor or command level, may serve not only to coordinate motor output within each rhythm mode, but also to ensure strict reciprocity between the two modes.

Some provision must be made for the marked variation in cycle frequency between the forward and reverse rhythm. The simplest explanation is that the command inputs for forward/reverse beating (Wilkens, Wilkens & McMahon, 1974; Best, 1983), in addition to exerting their biasing effects at loci distal to the oscillator, also supply collaterals directly onto the oscillator itself. Switching from the forward to reverse mode could be associated with an excitatory or disinhibitory input from the reverse command to the oscillator. At the end of the reversal episode, there is a gradual decline in this influence which may last for several cycles of the resumed forward rhythm. Further, because of the infrequency of rhythm reversals, the whole system must be inherently biased so as normally to suppress reversal activity and promote coupling in the forward mode. The occurrence of more prolonged episodes of reversal motor output after isolating the thoracic ganglion suggests that appropriate descending and afferent inputs help maintain this asymmetry.

The switching of motor programmes in the ventilatory system of crabs is in several ways analogous to the multifunctional activity of other rhythmic motor systems. For
example, single pattern generating networks are thought to produce the motor programmes appropriate for a variety of behaviour patterns involving the same muscles in the legs and wings of insects (e.g. Wilson, 1962; Kammer, 1968; Elsner, 1974; Pflüger & Burrows, 1978), buccal movements of ingestion and egestion in Limulus (Wyse & Dwyer, 1973) and molluscs (Croll & Davis, 1981), and different stepping patterns of limbs in lobsters (Ayers & Davis, 1977; Ayers & Clarac, 1978) and cats (Miller, van der Burg & van der Meché, 1975). To date the only example of motor programme switching studied at the cellular level is the swimmeret system of crayfish (Heitler, 1981). In this system, spontaneous or induced changes in the membrane potential of a single identifiable interneurone can lead to a switch between two different patterns of swimmeret motor output in the isolated preparation, although the mechanism of the switch and its functional significance remain unknown. Thus, the control of different forms of behaviour by the same basic motor system appears to be a widespread phenomenon in the organization of central nervous systems, but clearly there is a need for considerably more information, particularly that derived from direct intracellular recording, before the neuronal basis of such behavioural flexibility can emerge.

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REFERENCES

Motor programme switching in crabs


