*J. exp. Biol.* 113, 83–99 (1984)
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# ANALYSIS OF THE SCAPHOGNATHITE VENTILATORY PUMP IN THE SHORE CRAB *CARCINUS MAENAS*

## III. NEUROMUSCULAR MECHANISMS

BY A. JOFFRE MERCIER* AND JERREL L. WILKENS

*Department of Biology, University of Calgary, Calgary, Alberta, T2N 1N4, Canada*

*Accepted 9 April 1984*

SUMMARY

The motor output pattern to one of the ventilatory muscles of the scaphognathite (SC) in the shore crab, *Carcinus maenas*, was analysed from extracellular nerve recordings. During 'forward mode' bursting, an increase in the burst rate of L2b motor neurones is accompanied by an increase in the average intraburst firing frequency. The number of action potentials per burst, although variable, does not change consistently as a function of burst rate.

The influence of individual aspects of the motor pattern on isotonic contractions of muscle L2b was examined. Increasing the intraburst frequency leads to greater contraction and work output, and allows the muscle to lift heavier loads. This effect is correlated with an increase in the level of postsynaptic depolarization, due, at least in part, to greater summation of EPSPs. Increasing the burst rate alone also enhances muscle contraction and work, and results in greater depolarization. This latter effect appears to involve an accumulation of short-term facilitation, which becomes more acute as the time interval between the bursts is progressively shortened. In addition, contraction and depolarization are augmented by increasing the number of impulses per burst or the number of axons recruited.

These observations indicate some aspects of the motor output pattern which are appropriately modified to accommodate the changes in force and work demands which accompany a change in gill ventilation rate.

## INTRODUCTION

The rhythm and pattern of ventilatory activity in decapod crustaceans are determined by the motor programme generated in the central nervous system. The central pattern generator for the ventilatory programme includes a network of oscillatory interneurones (Mendelson, 1971; Simmers & Bush, 1980, 1983*a*) and motor neurones (DiCaprio & Fourtner, 1982), which interact to produce appropriately

* Present address: Department of Zoology, University of Iowa, Iowa City, Iowa 52242, U.S.A.
Key words: Crustacean, neuromuscular, facilitation.

phased, rhythmic bursts of motor neurone action potentials to the muscles of the bilateral scaphognathites (SCs).

The ventilatory rhythm can vary over a wide range. In a number of species, the frequency of SC beating can increase by three-fold or more over minimum values (Batterton & Cameron, 1978; Hassall, 1979; Taylor, 1976). An increase in SC beat frequency is accompanied by an increased gradient of hydrostatic pressure between the branchial chambers and the outside (Cumberlidge & Uglow, 1977*b*; Hughes, Knights & Scammell, 1969). This, in effect, increases the load on the SC, and the SC muscles must exert greater force and perform greater work at high beat frequencies (Mercier & Wilkens, 1984). Such changes in muscular force and work should be reflected by appropriate alterations in the motor programme from the central pattern generator.

The SC muscles are innervated by two to four excitatory (and no inhibitory) axons and possess highly facilitating synapses (Moody-Corbett & Pasztor, 1980; Pilkington & MacFarlane, 1978). It has been proposed that the force generated by the SC muscles is varied mainly through changes in the firing frequencies and axonal recruitment of the motor neurones (Moody-Corbett & Pasztor, 1980). To date, there has been no rigorous analysis of the motor neurone firing pattern as a function of bursting rate. Moreover, facilitation in the SC muscles has been studied largely at frequencies of 1–10 Hz (Moody-Corbett & Pasztor, 1980), which are considerably lower than recorded firing frequencies of the motor neurones (60–140 Hz, cf. Pasztor, 1968).

The present study examines some of the changes in patterned output which accompany changes in ventilatory rhythm in the shore crab, *Carcinus maenas*. The hypothesis that changes in various aspects of the motor programme can compensate for changes in load is tested. In addition, excitatory postsynaptic potentials (EPSPs) are recorded, in order to gain some insight into the neuromuscular mechanisms involved.

## MATERIALS AND METHODS

*Carcinus maenas* of various sizes were maintained in aerated sea water for up to several months prior to use. Active, semi-intact ganglia were prepared as described by Simmers (1978). The chelipeds and walking legs were autotomized, and the dorsal carapace, heart and viscera were removed. The sternal artery leading to the thoracic ganglion was rapidly cannulated and perfused with oxygenated or aerated *Carcinus* saline of the following composition (mmol $l^{-1}$): 500, NaCl; 12, KCl; 12, $CaCl_2$; 20, $MgCl_2$; 10, Tris buffer; 4·3, maleic acid; pH, 7·2 (Roberts & Bush, 1971). Motor neurone activity in the fine nerve branch innervating muscle L2b was recorded *via* conventional suction electrodes, which were connected to a Grass Model P15 preamplifier. The branch supplying muscle L2b (Young, 1975) was exposed by dissecting away the sclerite holding this muscle to the SC blade and pinning it out to one side. The remainder of the appendage was free to move during recordings but caused little or no movement artifact. Recorded motor patterns were stored on magnetic tape and, subsequently, were filmed at high speed on photographic paper for analysis.

In experiments in which nerve-evoked contractions were studied, cannulation of the sternal artery was omitted. The blade of the SC was cut away, and the sclerite

which had held the dorsal muscles to the blade were all dissected free. Contractions and EPSPs were usually recorded from the most dorsal muscle, L2b (Young, 1975), although some experiments were also performed with muscle L2a with essentially the same results. In order to record isotonic contractions, the entire preparation was orientated vertically, and the sclerite holding the appropriate muscle was hooked with a fine pin, which in turn was attached to a Harvard Apparatus heart/smooth muscle transducer. The preparation was kept on ice, and the muscle was superfused with cold, oxygenated saline (8–11 °C) at a rate of 2 ml $min^{-1}$.

Contractions were elicited by supplying electrical stimuli to the levator nerve (Young, 1975) through a suction electrode. The muscle was 'after-loaded' using a set screw, such that the weight was applied to the muscle after it began to contract. This made it possible to maintain the initial muscle length constant. Contraction recordings were amplified by conventional means and were displayed on a Grass Model M7 chart recorder (provided through the courtesy of the Grass Instrument Company, Quincy, MA). Intracellular recordings were made with 'floating' microelectrodes (Woodbury & Brady, 1956), made with fine silver wire and self-filling micropipettes containing 3 mol $l^{-1}$ KCl. This prevented the recording electrode from dislodging as a result of muscle movement and minimized movement artifacts. In many cases (e.g. Figs 5, 9B) intracellular recordings were made simultaneously with recordings of isotonic contraction of the entire muscle. The signal from the microelectrode was fed through a WPI Model M7A preamplifier to an oscilloscope.

Results were obtained from 20 preparations. Unless otherwise indicated, all experiments were performed at least four times with similar results. Regression slopes were determined by the method of least squares, and the significance of the difference between each slope and zero was determined using a two-tailed *t*-test (Mendenhall, Sheaffer & Wackerly, 1981).

## RESULTS

### *Motor output pattern*

Recordings of the total motor output to the SC include at least five distinguishable classes of axons to the depressors and five to the levators (Simmers & Bush, 1983*a*). In order to simplify the analysis of recorded motor output patterns, the output to a single muscle was studied. Muscle L2b, the most dorsal of the SC muscles, was chosen since it provided the best accessibility for microelectrode penetration and for recording motor neurone activity and contractions. L2b is a large, powerful muscle which raises the anterior portion of the SC during ventilation (Young, 1975). Fig. 1 illustrates the postsynaptic responses recorded intracellularly from two cells in different preparations of L2b. The nerve was stimulated at a range of intensities which covered the thresholds for stimulating all of the motor axons. L2b is innervated usually by three but occasionally by four excitatory axons, as previously reported for *Nectocarcinus antarcticus* (Pilkington & MacFarlane, 1978). Out of 26 muscle cells (in 10 preparations) two (7·7 %) were innervated by only one axon, three (11·5 %) by two axons, 19 (73·1 %) by three axons, and two (7·7 %) by four axons.

The motor neurone output to L2b was recorded from a very small nerve branch that innervates the muscle from the ventral side. Postsynaptic responses to electrical


Fig. 1. EPSPs in muscle L2b. (A) and (B) depict responses of two different muscle cells to nerve stimulation at 5 Hz. Gradual reduction of the stimulus intensity resulted in a step-wise reduction in EPSP size, indicating innervation by three excitatory motor neurones in A and by four excitatory motor neurones in B. (See also Pilkington & MacFarlane, 1978.)

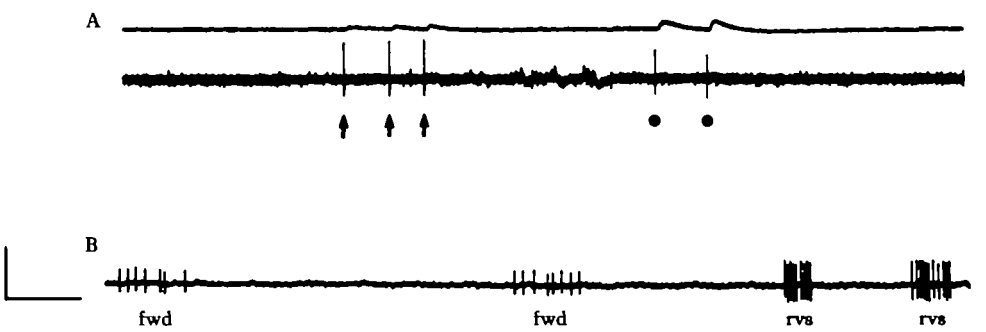


Fig. 2. Recruitment of different motor neurones to L2b. (A) During tonic activity, extracellularly recorded action potentials (lower trace) can be distinguished from two different motor neurones, as indicated by arrows and dots. Note the difference in the amplitudes of corresponding EPSPs in the muscle (upper trace). (B) Different motor neurones are recruited between forward (fwd) and reversed (rvs) ventilation. Extracellular recordings in the lower trace of A and in B were made from the fine nerve branch innervating muscle L2b. (Vertical bar, 20 mV for upper trace of A, 0·05 mV for lower trace of A, and 0·10 mV for B. Horizontal bar, 100 ms for A and 400 ms for B.)

stimulation confirmed that this branch contained all of the three to four excitatory axons to L2b. During occasional tonic activity (Fig. 2A), it was sometimes possible to distinguish extracellularly recorded action potentials from different axons, based

on action potential heights and on the amplitudes of corresponding EPSPs. In three preparations, clear differences in action potential amplitudes indicated the recruitment of different motor neurones corresponding to forward and reversed ventilatory modes (as in Fig. 2B), which agrees with previous reports (Simmers & Bush, 1983*b*; Young, 1975). Reversal motor neurones were never recruited during forward bursting. However, it was not feasible to discriminate reliably between action potentials within a burst, owing to small fluctuations in amplitude resulting from electrical interference. EPSPs were of little help in this regard, since they were facilitated and summated during bursting (Fig. 3). Thus, it was not possible to determine either the number of motor neurones activated during forward ventilation or whether the recruitment pattern of forward motor neurones changed as a function of burst rate. Intraburst characteristics (average firing frequency and the number of action potentials per burst) were determined by including all action potentials within a burst, irrespective of their size.

In some preparations, the frequency of forward bursting varied spontaneously over a wide range, while in others it was possible to alter the rhythm by stimulating the circumoesophageal connectives (Wilkens, Wilkens & McMahon, 1974). Similar

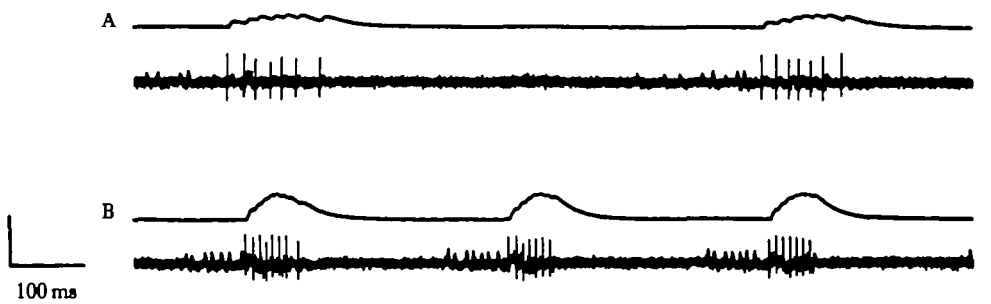


Fig. 3. Motor output pattern to L2b during forward bursting. (A) and (B) are recordings from one preparation. In each, the upper trace is an intracellular recording from muscle L2b, and the lower trace is a suction electrode recording from the nerve branch innervating L2b. (Note that in the suction electrode recordings, each burst of motor neurone action potentials is preceded by smaller, slower waves. These are electromyographic potentials from muscle L1a, which was immediately below the recording site.) An increase in burst rate is accompanied by an increase in intraburst action potential frequency and enhancement of postsynaptic depolarization. In this preparation, the number of action potentials per burst remains constant due to a decrease in the burst duration. (Vertical bar, 40 mV for intracellular recordings and 0·05 mV for suction electrode recordings.)

Table 1. *Relationships between intraburst firing frequency and burst rate for L2b motor neurones*

| Animal | $r$* | Slope | $N$* | $t$* | Significance level |
|---|---|---|---|---|---|
| 1 | 0·958 | 0·640 | 115 | 35·956 | 0·001 |
| 2 | 0·946 | 0·911 | 85 | 26·667 | 0·001 |
| 3 | 0·969 | 0·606 | 80 | 34·760 | 0·001 |
| 4 | 0·890 | 0·902 | 67 | 15·709 | 0·001 |
| 5 | 0·924 | 0·491 | 93 | 23·008 | 0·001 |

* In this and the following table, $r$ = the correlation coefficient, $N$ = the number of observations, and $t$ = the value obtained from a two-tailed $t$-test which compares the difference between the regression slope and zero.

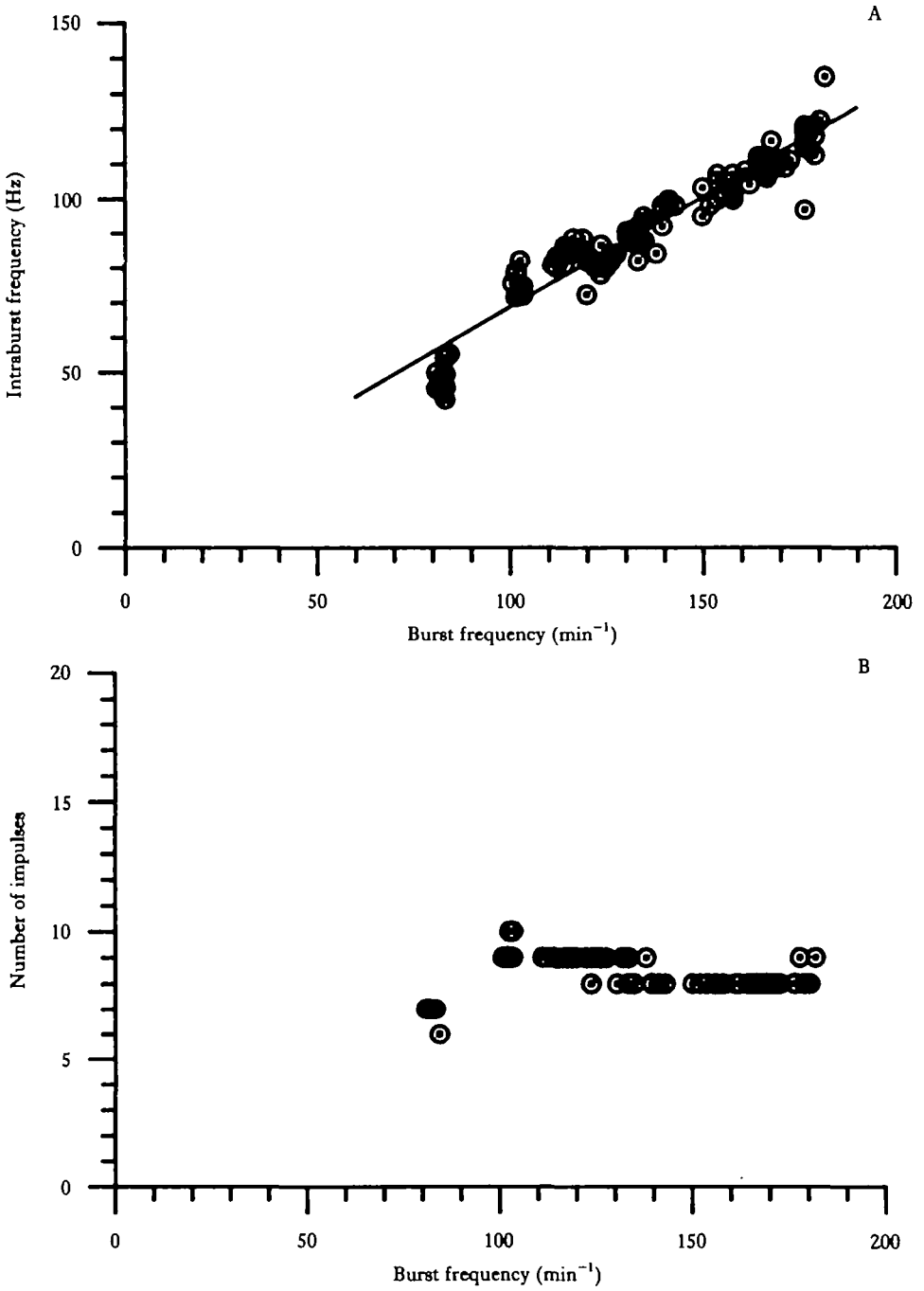


Fig. 4. Effects of burst frequency on intraburst firing rate and on the number of motor neurone action potentials per burst. Data (115 points) from the preparation of Fig. 3 were used to determine how the intraburst frequency (A) and the number of action potentials per burst (B) vary with burst rate. Intraburst frequency increased linearly with burst rate ($Y = 0{\cdot}64X + 4{\cdot}44$; $r = 0{\cdot}958$). The number of action potentials per burst did not change significantly in this preparation ($r = -0{\cdot}107$). These results correspond to the data for animal 1 in Tables 1 and 2.

Results were obtained in either case. With increasing burst rate, the average intraburst firing frequency increased and postsynaptic depolarization was enhanced (Fig. 3). The relationship between intraburst frequency and burst rate was linear (Fig. 4A). Results from five preparations (including that of Figs 3 and 4) indicate a high correlation between intraburst frequency and bursting rate (Table 1). In all cases, regression slopes were positive and statistically significant. The range of burst rates and intraburst frequencies varied from animal to animal. The largest range was that of Fig. 4, where the burst rate increased from 80 to 180 $min^{-1}$ with a corresponding increase in intraburst frequency from 40 to 135 Hz. The total range of all observed bursting rates and intraburst frequencies, respectively, was 20–180 $min^{-1}$ and 15–140 Hz.

Burst durations always became shorter with increasing burst rate, as previously reported by Young (1975). However, the relationship between burst rate and the number of action potentials per burst was variable (Table 2). In two preparations, including that of Figs 3 and 4, the number of impulses per burst was essentially constant over the entire range of burst rates. In two other preparations the number of impulses per burst decreased as a function of burst rate, and in one preparation the number per burst increased with burst rate. The number of action potentials per burst did not vary by more than 10 in any given preparation, but the total observed range was 4–29.

Thus, the only predictable change in intraburst patterning noted in the present study was an increase in average firing frequency with burst rate. The number per burst, though quite variable, did not change in a consistent manner.

### *Muscle contraction*

The influence of the output pattern on contraction was examined by applying bursts of electrical stimuli to the levator nerve (Young, 1975) and recording isotonic contractions of after-loaded L2b muscles (see Materials and Methods). The load was varied in order to determine whether changes in the output pattern could compensate for changes in load. The effects of varying the intraburst frequency as well as the number of pulses per burst were tested. Axonal recruitment was examined by adjusting the stimulus intensity in order to excite one, two or three motor neurones (as determined from intracellular postsynaptic recordings). In addition, the effect of increasing the burst rate, which necessarily shortens the time interval between bursts, was examined. In each case, only one aspect of the stimulus pattern was varied while all others were maintained constant.

Table 2. *Relationships between the number of action potentials per burst and burst rate for L2b motor neurones*

| Animal | *r* | Slope | *N* | *t* | Significance level |
|---|---|---|---|---|---|
| 1 | −0·107 | −0·0025 | 115 | −1·144 | NS** |
| 2 | −0·938 | −0·1594 | 85 | −24·650 | 0·001 |
| 3 | −0·869 | −0·0641 | 80 | −15·544 | 0·001 |
| 4 | 0·093 | 0·0308 | 67 | 0·752 | NS |
| 5 | 0·783 | 0·0638 | 93 | 12·027 | 0·001 |

** NS indicates that the slope is not significant at the 0·05 level.

Increasing the intraburst frequency enhanced contraction of the muscle (Fig. 5, top traces). Note that, as the EPSP records indicate (bottom traces), the burst duration was shortened in order to maintain a constant number of stimuli per burst. In addition, the bursts were given at 1-min intervals, so that the effects of one burst would not influence the next (see below), and supramaximal stimulus intensity was used, so that all axons to the muscle were recruited. The effect of increasing stimulus frequency on contraction correlated with an increase in the level of postsynaptic depolarization and with an increased probability for an active response to occur (Fig. 5, bottom traces). The increase in the level of depolarization is due, at least in part, to greater temporal summation of the EPSPs. Individual EPSPs near the end of the burst became smaller as the stimulus frequency was increased. It is not known whether this reflects a decrease in the amount of transmitter released or simply results from the fact that the membrane potential is closer to the reversal potential for the EPSP. In addition, the membrane conductance may increase following the action potentials at the higher stimulus frequencies, due to the activation of outward potassium currents (Mounier & Vassort, 1975*a*,*b*). An increased conductance (decreased resistance) of this type could contribute to the decrease in EPSP size, since the synaptic current would be less effective in eliciting a change in postsynaptic potential.

Contractile responses to different stimulus frequencies were recorded at various loads and are illustrated graphically in Fig. 6. For each of the stimulus frequencies

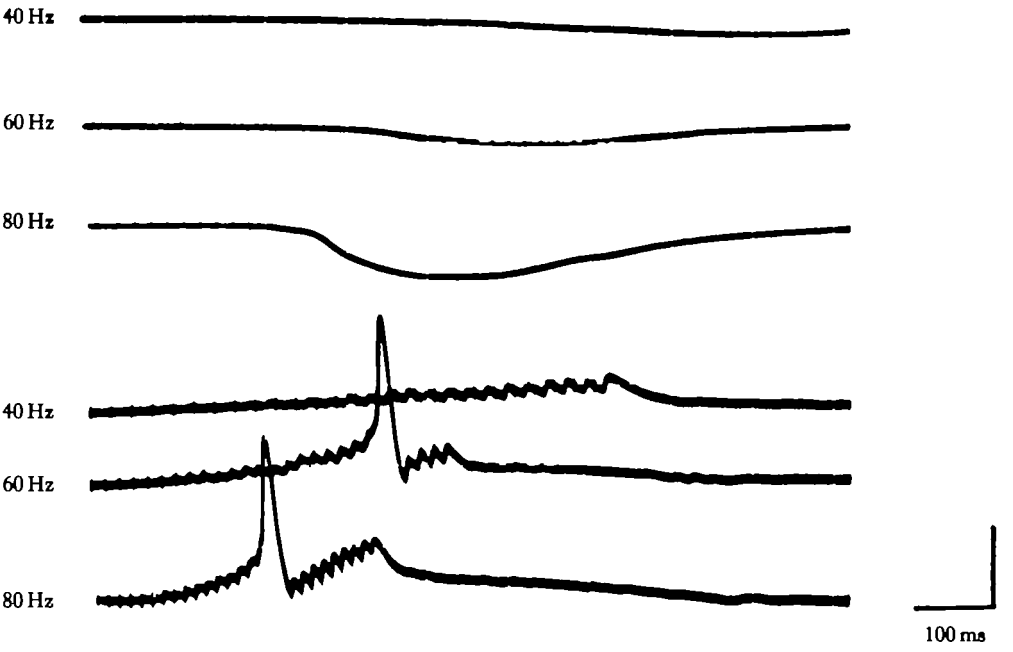


Fig. 5. Responses of L2b to nerve stimulation at various frequencies. Bursts of 25 pulses were applied to the levator nerve at the frequencies indicated. Supramaximal intensity was used, and the bursts were given at 1-min intervals. The stimulus trains began at the start of each sweep. Increasing the stimulus frequency produced greater isotonic contraction of the whole muscle (top three traces), and increased the amplitude of EPSPs recorded from a single cell (bottom three traces). Note the appearance of a spike at 60 and 80 Hz. (Vertical bar, 0·1 mm for upper traces, 20 mV for lower traces. Resting potential for the lower traces was −62 mV.)

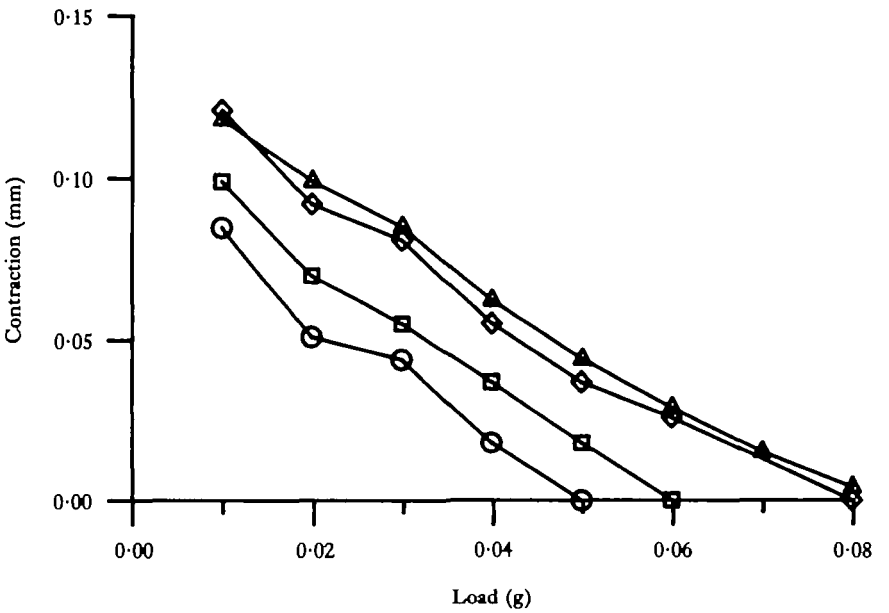


Fig. 6. Contraction (shortening) *versus* load curves for various stimulus frequencies. The levator nerve was stimulated at supramaximal intensity, and responses of L2b to bursts of 10 pulses given at 1-min intervals were recorded for various loads. (Stimulus frequencies in Hz: ○, 40; □, 60; ◇, 80; △, 100.) Increasing the stimulus frequency caused an increase in muscle contraction and in the load which could be lifted.

applied, the amount of contraction (i.e. shortening) decreased with increasing load, as would be expected. Increasing the stimulus frequency from 40 to 100 Hz increased the amount of contraction for a given load and shifted the curve to the right, such that a given contraction distance could be maintained against an increasing load. The effect of increasing intraburst frequency tended to level off above 80 Hz. This might be attributed to the fact that the burst durations were progressively shortened in order to keep a constant number of stimuli per burst, which in turn shortened the duration of the postsynaptic response (Fig. 5, bottom traces).

Increasing the number of stimuli from 5 to 25 per burst caused a substantial increase in muscle contraction and enabled the muscle to lift greater loads, as indicated by a shift to the right in the contraction *versus* load curve (Fig. 7A). This effect also correlated with an increase in postsynaptic depolarization (Fig. 7B). Similar results were obtained when the number of axons recruited was increased by varying the stimulus intensity (Fig. 8). In this case, an increased probability for a muscle spike was also apparent (Fig. 8B). In the example shown, the recruitment of two axons resulted in a smaller spike amplitude than did the recruitment of three axons. This could be due to partial inactivation of the inward current or to activation of rectifying outward currents (Mounier & Vassort, 1975*a*,*b*), since the depolarization was more gradual in the former case than in the latter.

During the period immediately following a stimulus burst, muscle EPSPs are enhanced (Fig. 9A). This period of potentiation appeared to be fairly long, since it generally took 4–6 s for the EPSP to return to its pre-tetanus amplitude. Consequently,

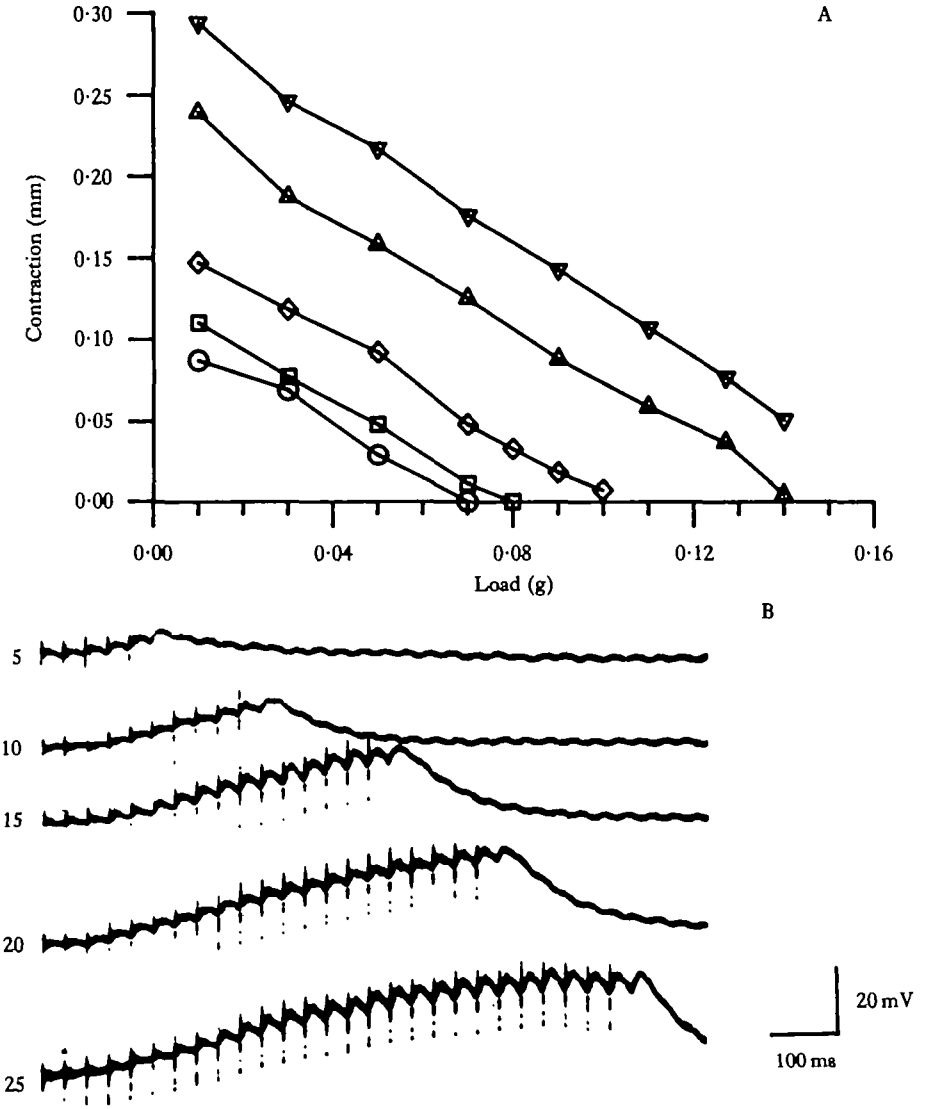


Fig. 7. Effects of varying the number of stimuli per burst. (A) Increasing the number of stimuli enhances contraction and increases the load which L2b can lift. Bursts of 80 Hz frequency were applied to the levator nerve at 1-min intervals and supramaximal intensity. (Number of stimuli per burst: ○, 5; □, 10; ◇, 15; △, 20; ▽, 25.) (B) Increasing the number of stimuli per burst enhances postsynaptic depolarization. EPSPs in response to supramaximal stimulation at 60 Hz are shown, with the number of pulses per burst indicated at the left. The small oscillations, which are prominent after cessation of the stimulus train and are also superimposed on the EPSPs, are due to 60 Hz electrical interference.

when a series of six bursts was administered at a rate of 2 bursts $s^{-1}$, the level of postsynaptic depolarization increased with each consecutive burst, and muscle contractions grew progressively as well (Fig. 9B). The increase in depolarization did not result from any temporal summation, since the membrane potential repolarized completely between bursts (lower traces). This would indicate that the increase in depolarization with successive bursts resulted from facilitation.

The range of SC beat frequencies in *Carcinus maenas* has been reported to be as high as 20–340 $min^{-1}$ (Cumberlidge & Uglow, 1977*a*). This would correspond to interburst intervals between 3 s and 170 ms. An increase in burst frequency within the physiological range, therefore, would be expected to enhance muscle contraction as a result of encroachment on the period of potentiation following each burst. Fig. 10 confirms that this is the case. Bursts consisting of a constant frequency and number of stimuli (60 Hz, 10 pulses) were applied at burst rates ranging from 0·5 $s^{-1}$ (30 $min^{-1}$) to 3·3 $s^{-1}$ (200 $min^{-1}$). The responses to every fifth burst are plotted against load.

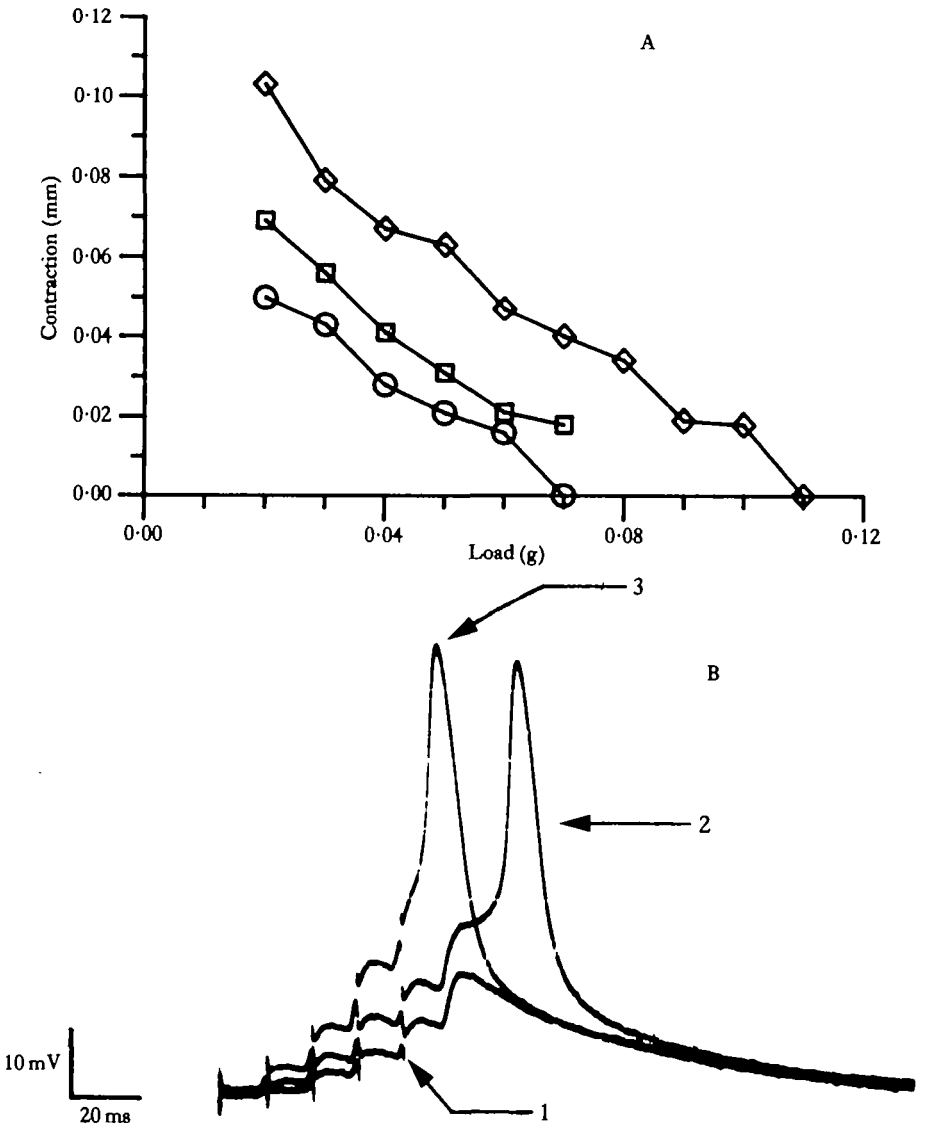


Fig. 8. Effect of increasing recruitment. (A) Contraction *versus* load curves for the recruitment of one (○), two (□) and three (◇) axons. Bursts (15 pulses at 80 Hz) were applied at 1 $min^{-1}$, and the number of axons recruited was checked before and after each series with intracellular recordings. (B) Intracellular responses to bursts of five pulses at 80 Hz with recruitment of 1, 2 or 3 axons (as indicated).

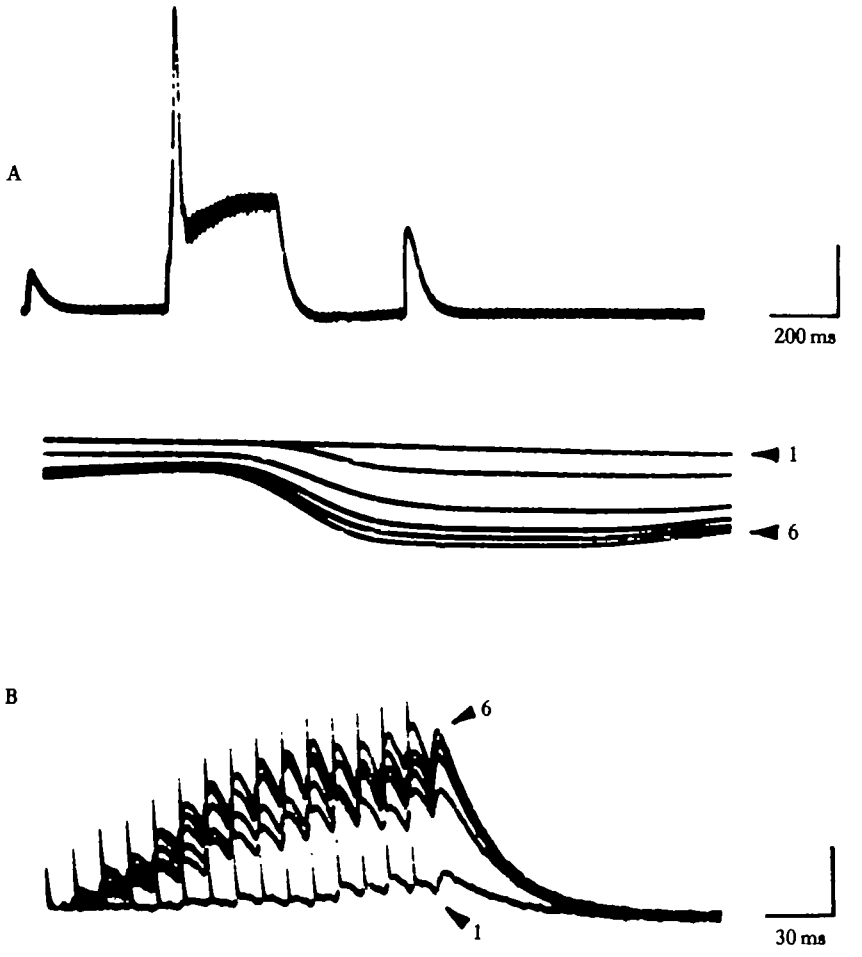


Fig. 9. Facilitation following high frequency bursts. (A) Single stimuli were applied before and after a burst of 25 pulses at 80 Hz to the levator nerve. The post-tetanic EPSP was facilitated compared to the EPSP amplitude preceding the burst. In this case, two excitatory axons were recruited, although similar results were obtained by recruiting one and three axons. (Vertical bar, 5 mV.) (B) Six consecutive bursts (sequence indicated by arrows) were applied at a rate of $2\,s^{-1}$, resulting in a gradual rise in depolarization of a single cell (lower traces) and an increase in contraction of the whole muscle (upper traces). Each burst contained 15 pulses at 80 Hz. (Vertical bar, 0·2 mm for upper traces and 10 mV for lower traces.)

Increasing the burst rate enhanced contraction and shifted the curve to the right, indicating that the muscle could lift greater loads.

## DISCUSSION

The present investigation demonstrates that an increase in forward ventilation rate is accompanied by two significant changes in motor output pattern: (a) a decrease in the time interval between bursts and (b) an increase in the average intraburst firing frequency. These results, obtained by directly recording the motor neurone output to muscle L2b, corroborate similar results obtained for a depressor muscle (D2a) wit

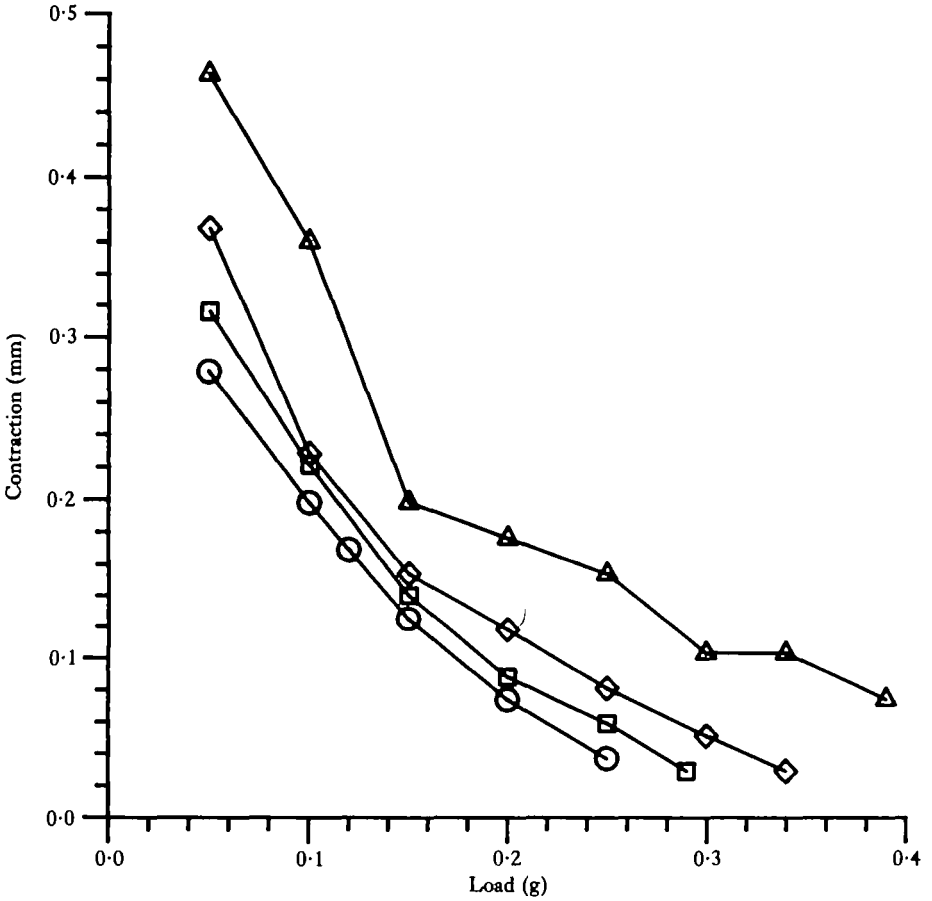


Fig. 10. Contraction *versus* load curves for various burst rates. Repetitive bursts (10 pulses at 60 Hz) were applied to the levator nerve at various burst rates and supramaximal intensity. Responses to the fifth burst in each series were plotted against load. Each series was given at 2-min intervals. (Burst rates: ○, $0{\cdot}5\,s^{-1}$; □, $1\,s^{-1}$; ◇, $2\,s^{-1}$; △, $3{\cdot}3\,s^{-1}$.) Increasing burst rate enhanced contraction and increased the load which could be lifted.

electromyograms (Mercier & Wilkens, 1984). Both of these changes in output pattern appear to contribute to the increase in postsynaptic depolarization (Fig. 3), and both are appropriate for satisfying increased force and work requirements at high ventilation rates. Contraction *versus* load curves demonstrate that increasing either the intraburst frequency or the burst rate within the physiologically observed range results in: (a) enhancement of the nerve-evoked contraction, (b) the ability of the muscle to lift greater loads, and (c) an increase in the work output of the muscle.

The experimental paradigm used in the present study can be compared to the function of the SC muscles during ventilation. When the stroke volume is constant, the excursion of the SC and, hence, the contraction distance of the SC muscles must be maintained constant (Cumberlidge & Uglow, 1977*b*; Pilkington & Simmers, 1973). An increase in ventilation rate effectively increases the load against which the SC muscles must contract, as indicated by changes in branchial pressure (Mercier & Wilkens, 1984). Figs 6 and 10 illustrate that a fixed contraction distance can be

maintained against an increasing load *via* either of the observed changes in the output pattern. When the stroke volume is variable (Burggren & McMahon, 1983; Wilkens, 1981), it might be expected that contraction distance would be variable. Changes in intraburst frequency or interburst interval would also allow for variations in contraction distance (Figs 6 and 10). This could provide some neurophysiological basis for reports of lack of correlation between SC beat frequency and ventilation volume (e.g. Burggren & McMahon, 1983; Wilkens, Wilkes & Evans, 1984). Neural control of the first maxilliped may also be an important factor in regulating stroke volume (see Wilkens, 1981).

The enhancement of contraction and work correlates with an increase in the level of muscle depolarization and with an increased probability for muscle spikes. The appearance of muscle spikes, which were common in the present study, is consistent with other phasic properties previously reported for the SC muscles (Moody-Corbett & Pasztor, 1980). The high frequency bursts which activate the SC muscles result in a considerable degree of summation and facilitation of the EPSPs. It was not possible in the present study to discern the relative contribution of each. The observed decrease in EPSP size with increasing stimulus frequency (Fig. 5) probably results, at least in part, from the fact that the membrane potential more closely approaches the reversal potential. It would be difficult to determine the relationship between transmitter release and stimulus frequency over the physiological range without a direct measurement of quantal content. In any event, it is apparent that temporal summation increases as a function of stimulus frequency and contributes significantly to the enhancement of depolarization.

The enhancement of EPSPs during the period following each burst (post-tetanic potentiation) probably results from an accumulation of short-term facilitation (Atwood, 1976), since the burst durations in the present study were relatively brief (range of 50–500 ms). This build-up of facilitation has significant effects on membrane potential and contraction at physiological bursting rates. As a result, the reduction of interburst intervals concomitant with an increase in burst rate leads to the augmentation of contraction and work. It would be interesting to know whether the SC muscles also display long-term facilitation, which typically develops in crustacean preparations over several minutes of stimulation and can last for minutes to hours afterwards (Sherman & Atwood, 1971; Atwood, 1976).

Changes in axonal recruitment or in the number of impulses per burst can have very substantial effects on postsynaptic potentials and contraction, as indicated by experiments involving stimulation of the levator nerve. Such changes in the output pattern would be appropriate for compensating for changes in load. However, no evidence for changes in axonal recruitment as a function of ventilation rate has been provided, and no consistent relationship between burst rate and the number of impulses per burst was observed.

In *Carcinus*, changes in recruitment of the D2 and L2 motor neurones appear to play a significant role with respect to the production of forward and reversed ventilatory patterns (Simmers & Bush, 1983*b*; Young, 1975). In the present study, it was not possible to discern the number of L2b motor neurones activated during forward or reversed bursting, although the axons recruited between these two burst modes were clearly different. The possibility that axonal recruitment varies as a function of bur

rate requires a more careful analysis utilizing action potential heights, shapes and rise times. In addition, the motor output to the D1 and L1 muscles should be analysed, since these muscles are activated by the same set of motor neurones in the forward and reversed modes (Simmers & Bush, 1983*b*).

Sensory feedback can affect the motor pattern to the SCs (Young & Coyer, 1979), but its importance is not yet clear. Although the SC was free to move during recordings of motor neurone output in the present study, the movements were not normal since L2b had been partially dissected away (see Materials and Methods). Sensory feedback from the SC comes mainly from the oval organ, which is thought to respond to deformations arising from movement and water pressures, and from hook sensillae, which are orientated toward the normal water current (Bush & Pasztor, 1983; Pasztor, 1969; Pasztor & Bush, 1983*a*,*b*). Disruption of the integrity of the branchial chambers and pumping channels, which is necessary to record the output pattern, probably has disastrous effects on feedback since the normal pressures and currents are no longer present.

Clearly, more work is needed in this area. It would be particularly interesting to know whether sensory information can shape the output from the central pattern generator in order to accommodate changes in load, as in crustacean walking (Evoy & Ayers, 1982). However, it is not known whether the oval organ or any other sensory structures associated with the SC function in a manner analogous to load-sensitive receptors of the walking legs. Young (1975) reported that restriction of the SC may increase the burst duration, which presumably would affect the number of impulses per burst. The lack of a consistent relationship between the number of spikes per burst and burst rate could result from abnormal feedback, but this remains speculative.

One aspect of the motor pattern which was not examined is the timing of impulses within each burst. Variations in the pattern were tested using trains of evenly spaced stimuli reflecting the average intraburst frequencies of recorded motor patterns. However, the normal intraburst pattern is parabolic (Young, 1975; unpublished observations). Changes in the timing of impulses within a burst could conceivably have a significant effect on contraction in crustacean muscle (Gillary & Kennedy, 1969) as in mammalian muscle (Stein & Parmiggiani, 1979). The effects of parabolic bursting on contraction of SC muscles and possible changes in the parabolic pattern with burst rate are subjects for future study.

Most of these experiments were performed at the Marine Biological Laboratory, Woods Hole, MA, throughout the summer of 1982. The authors are very grateful to the Grass Foundation, who supported AJM as a Grass Fellow during that period. This work was also supported in part by a studentship from the Alberta Heritage Foundation for Medical Research. The authors also thank Dr Ralph DiCaprio for helpful advice regarding the dissection.

## REFERENCES

Atwood, H. L. (1976). Organization and synaptic physiology of crustacean neuromuscular systems. *Prog. Neurobiol.* **7**, 291–391.

Batterton, C. V. & Cameron, J. N. (1978). Characteristics of resting ventilation and response to hypoxia, hypercapnia, and emersion in the blue crab *Callinectes sapidus* (Rathbun). *J. exp. Zool.* **203**, 398–418.

BURGGREN, W. W. & MCMAHON, B. R. (1983). An analysis of scaphognathite pumping performance in the crayfish *Orconectes virilis*: compensatory changes to acute and chronic hypoxic exposure. *Physiol. Zoöl.* **56**, 309–318.

BUSH, B. M. H. & PASZTOR, U. M. (1983). Graded potentials and spiking in single units of the oval organ, a mechanoreceptor in the lobster ventilatory system. II. Individuality of the three afferent fibres. *J. exp. Biol.* **107**, 451–464.

CUMBERLIDGE, N. & UGLOW, R. F. (1977*a*). Heart and scaphognathite activity in the shore crab *Carcinus maenas* (L.). *J. exp. mar. Biol. Ecol.* **28**, 87–107.

CUMBERLIDGE, N. & UGLOW, R. F. (1977*b*). Size, temperature, and scaphognathite frequency-dependent variations of ventilation volumes in *Carcinus maenas* (L.). *J. exp. mar. Biol. Ecol.* **30**, 85–93.

DICAPRIO, R. A. & FOURTNER, C. R. (1982). Motor neurons as functional elements of the ventilatory oscillator in the crab. *Soc. Neurosci. Abstr.* **8**, 161.

EVOY, W. H. & AYERS, J. (1982). Locomotion and control of limb movements. In *The Biology of Crustacea*, (editor-in-chief, D. E. Bliss; eds D. C. Sandeman & H. L. Atwood), pp. 61–105. New York: Academic Press.

GILLARY, H. C. & KENNEDY, D. (1969). Neuromuscular effects of impulse pattern in a crustacean motoneuron. *J. Neurophysiol.* **32**, 607–612.

HASSALL, C. D. (1979). Respiratory physiology of the crayfish *Procambarus clarki*. M.Sc. thesis, University of Calgary, Calgary, Alberta.

HUGHES, G. M., KNIGHTS, B. & SCAMMELL, C. A. (1969). The distribution of $P_{O_2}$ and hydrostatic pressure changes within the branchial chambers in relation to gill ventilation of the shore crab *Carcinus maenas* L. *J. exp. Biol.* **51**, 203–220.

MENDELSON, M. (1971). Oscillator neurons in crustacean ganglia. *Science, N.Y.* **171**, 1170–1173.

MENDENHALL, W., SHEAFFER, R. L. & WACKERLY, D. D. (1981). Linear models and estimation by least squares. In *Mathematical Statistics with Applications*, 2nd Edition, pp. 421–479. Boston: Duxbury Press.

MERCIER, A. J. & WILKENS, J. L. (1984). Analysis of the scaphognathite ventilatory pump in the shore crab *Carcinus maenas*. I. Work and power. *J. exp. Biol.* **113**, 55–68.

MOODY-CORBETT, F. & PASZTOR, V. M. (1980). Innervation, synaptic physiology, and ultrastructure of three muscles of the second maxilla in crayfish. *J. Neurobiol.* **11**, 21–30.

MOUNIER, Y. & VASSORT, G. (1975*a*). Initial and delayed membrane currents in crab muscle fibre under voltage clamp conditions. *J. Physiol., Lond.* **251**, 589–608.

MOUNIER, Y. & VASSORT, G. (1975*b*). Evidence for a transient potassium membrane current dependent on calcium influx in crab muscle fibre. *J. Physiol., Lond.* **251**, 609–625.

PASZTOR, V. M. (1968). The neurophysiology of respiration in decapod crustacea. I. The motor system. *Can. J. Zool.* **46**, 585–596.

PASZTOR, V. M. (1969). The neurophysiology of respiration in decapod crustacea. II. The sensory system. *Can. J. Zool.* **47**, 435–441.

PASZTOR, V. M. & BUSH, B. M. H. (1983*a*). Graded potentials and spiking in single units of the oval organ, a mechanoreceptor in the lobster ventilatory system. I. Characteristics of dual afferent signalling. *J. exp. Biol.* **107**, 431–449.

PASZTOR, V. M. & BUSH, B. M. H. (1983*b*). Graded potentials and spiking in single units of the oval organ, a mechanoreceptor in the lobster ventilatory system. III. Sensory habituation to repetitive stimulation. *J. exp. Biol.* **107**, 465–472.

PILKINGTON, J. B. & MACFARLANE, D. W. (1978). Numbers and central projections of crab second maxilla motor neurones. *J. mar. biol. Ass. U.K.* **58**, 571–584.

PILKINGTON, J. B. & SIMMERS, A. J. (1973). An analysis of bailer movements responsible for gill ventilation in the crab *Cancer novae-zelandiae*. *Mar. Behav. Physiol.* **2**, 73–95.

ROBERTS, A. & BUSH, B. M. H. (1971). Coxal muscle receptors in the crab: the receptor current and some properties of the receptor nerve fibres. *J. exp. Biol.* **54**, 516–524.

SHERMAN, R. G. & ATWOOD, H. L. (1971). Synaptic facilitation: long-term neuromuscular facilitation in crustaceans. *Science, N.Y.* **171**, 1248–1250.

SIMMERS, A. J. (1978). A preparation for analysis of the neural control of ventilation in crabs. *J. Physiol., Lond.* **277**, 15P–16P.

SIMMERS, A. J. & BUSH, B. M. H. (1980). Non-spiking neurones controlling ventilation in crabs. *Brain Res.* **197**, 247–252.

SIMMERS, A. J. & BUSH, B. M. H. (1983*a*). Central nervous mechanisms controlling rhythmic burst generation in the ventilatory motoneurones of *Carcinus maenas*. *J. comp. Physiol.* **150**A, 1–22.

SIMMERS, A. J. & BUSH, B. M. H. (1983*b*). Motor programme switching in the ventilatory system of *Carcinus maenas*: the neuronal basis of bimodal scaphognathite beating. *J. exp. Biol.* **104**, 163–181.

STEIN, R. B. & PARMIGGIANI, F. (1979). Optimal motor patterns for activating mammalian muscle. *Brain Res.* **175**, 372–376.

TAYLOR, A. C. (1976). The respiratory responses of *Carcinus maenas* to declining oxygen tension. *J. exp. Biol.* **65**, 309–322.

WILKENS, J. L. (1981). Respiratory and circulatory coordination in decapod crustaceans. In *Locomotion and Energetics in Arthropods* (eds C. F. Herreid & C. R. Fourtner), pp. 277–298. London: Plenum Press.

WILKENS, J. L., WILKES, P. R. H. & EVANS, J. (1984). Analysis of the scaphognathite ventilatory pump in the shore crab *Carcinus maenas*. II. Pumping efficiency and metabolic cost. *J. exp. Biol.* **113**, 69–81.

WILKENS, J. L., WILKENS, L. A. & MCMAHON, B. R. (1974). Central control of cardiac and scaphognathite pacemakers in the crab *Cancer magister*. *J. comp. Physiol.* **90**, 89–104.

WOODBURY, J. W. & BRADY, A. J. (1956). Intracellular recording from moving tissues with a flexibly mounted ultramicroelectrode. *Science, N.Y.* **123**, 100–101.

YOUNG, R. E. (1975). Neuromuscular control of ventilation in the shore crab *Carcinus maenas*. *J. comp. Physiol.* **101**, 1–37.

YOUNG, R. E. & COYER, P. E. (1979). Phase co-ordination in the cardiac and ventilatory rhythms of the lobster *Homarus americanus*. *J. exp. Biol.* **82**, 53–74.