

GRADED POTENTIALS AND SPIKING IN SINGLE
UNITS OF THE OVAL ORGAN, A
MECHANORECEPTOR IN THE LOBSTER
VENTILATORY SYSTEM

III. SENSORY HABITUATION TO REPETITIVE STIMULATION

BY V. M. PASZTOR

*Department of Biology, McGill University, 1205 Avenue Dr. Penfield,
Montreal, Canada H3A 1B1*

AND B. M. H. BUSH

*Department of Physiology, University of Bristol, Park Row, Bristol
BS1 5LS*

(Received 14 March 1983—Accepted 24 June 1983)

SUMMARY

1. When the oval organ is stimulated repetitively at frequencies imitating normal scaphognathite beating the responses of the three sensory afferents decline. The number of spikes per response diminishes, the firing rate declines and the latency of the first spike may increase.

2. The term 'sensory habituation' is proposed to describe the decline in response of a primary sensory unit to a repetitive train of identical stimuli.

3. The decline in spiking performance is shown to be due to some or all of these underlying changes: (a) a decline in the rate of rise of the graded potential, (b) a decline in amplitude of the graded potential, (c) a decline in the rate of growth of the active process leading to spike initiation and (d) a rise in spiking threshold.

4. The graded potential undergoes less of a reduction than impulse generation. Tetrodotoxin-treated fibres show little habituation to stretch. Normal fibres habituate to repetitive pulses of depolarizing current.

5. It is suggested that during regular quiet ventilation the analogue signal alone provides feedback. Any extraneous perturbation or change in scaphognathite rhythm restores spiking.

INTRODUCTION

In vivo the oval organ is subjected continually to a cycle of stretch and relaxation as the scaphognathite beats. This paper describes the diminution of response which occurs *in vitro* when the isolated oval organ is stimulated repetitively with pull stimuli imitating the natural situation. Both components of the response are affected, but the decline in spiking is more pronounced than that of the graded potential.

A decline in response during a train of identical stimuli has been noted in studies

on other mechanoreceptors (Catton, 1961; Hunt & Ottoson, 1976; Bush & Roberts, 1971), but has not been adequately named. A term is needed which differentiates the phenomenon both from classical adaptation which occurs during a single maintained stimulus, and from postsynaptic responses to incoming repetitive presynaptic events. 'Sensory habituation' is proposed, and defined as a diminution of the peripheral responses of primary sensory units to trains of repetitive, identical stimuli.

MATERIALS AND METHODS

Preparative procedures, mechanical stimulation and microelectrode methods were similar to those described in the first paper of this series (Pasztor & Bush, 1983). In brief, controlled stretch stimuli were delivered to the oval organ from a loudspeaker-driven puller, *via* a hook inserted into the ventral attachment of the connective tissue array. In these experiments, trains of repetitive trapezoidal or sinusoidal pulls were used, with pull frequencies imitating natural respiratory rhythms (0.5–2.5 Hz). Intracellular recordings taken with 3 M-KCl-filled glass micropipettes were either filmed or printed out on a Brush recorder on line, or taped for subsequent analysis. Recording loci were 3–6 mm central to the confluence of the sensory dendrites in afferent fibres measuring 12–15 mm from oval organ to the suboesophageal ganglion.

RESULTS

When single stretch stimuli of constant parameters were delivered to an oval organ at intervals of 1 min or more, the responses were remarkably consistent, both in graded potential magnitude and shape, and in spiking pattern. If the stimulus repetition rate was increased, however, the responses to the second and subsequent presentations declined. The example in Fig. 1 shows Y fibre's responses to a series of trapezoidal pulls delivered at 0.66 Hz. Initially there were 19 spikes attaining a

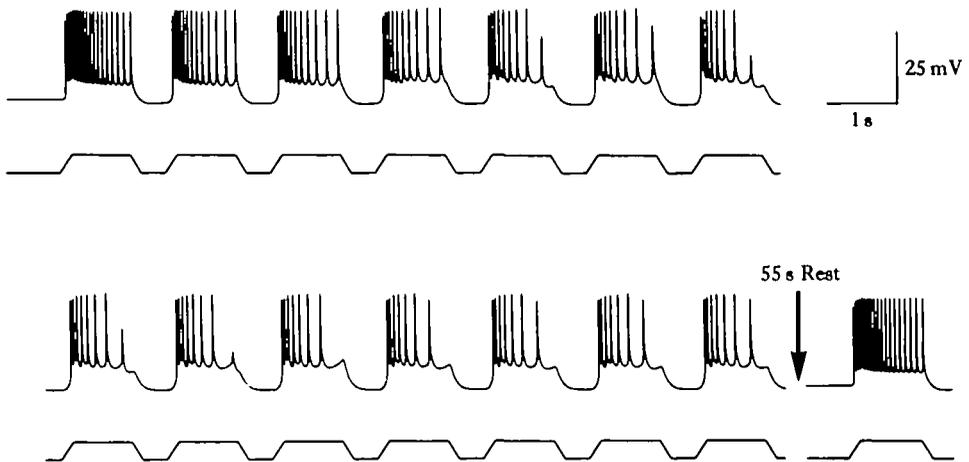


Fig. 1. Sensory habituation in fibre Y. Repetitive 0.3-mm trapezoidal pulls delivered at 0.66 Hz. Last response shows full recovery after 55 s rest.

maximum firing frequency of 38 Hz. At the second stimulus there were six spikes less, and lower firing frequencies throughout the response. Finally, after 10 identical stimuli the response stabilized at seven spikes, with a maximum frequency of 19 Hz. In all trials, rest periods of at least 55 s were required to restore the full response.

When a lower amplitude or shorter duration stimulus was used, which only elicited a brief burst in the fully rested preparation, as in the top two panels of Fig. 2A, B, spiking often ceased altogether during repetitive presentations, leaving all, or a large proportion, of the responses without impulses. Only the graded analogue component of the response was consistently retained, although this too showed some evidence of decline.

By superimposing a series of responses to repetitive stimuli, as in Fig. 3B, it can be seen that sensory habituation involves several distinct effects which are not all expressed equally in each series. (1) A decline in the rate of rise in the graded potential (also seen in Fig. 2). (2) A decline in amplitude of the graded potential (especially noticeable in X). (3) A decline in the rate of growth of the active process leading to spike initiation. (4) Finally, there may be a rise in spiking threshold. Together, these effects contribute not only to the diminution in numbers and frequency of spikes mentioned above, but in increasing delay in the appearance of the first spike and the abolition of the initial dynamic burst.

In these responses, recorded several millimetres proximal to the confluence of the

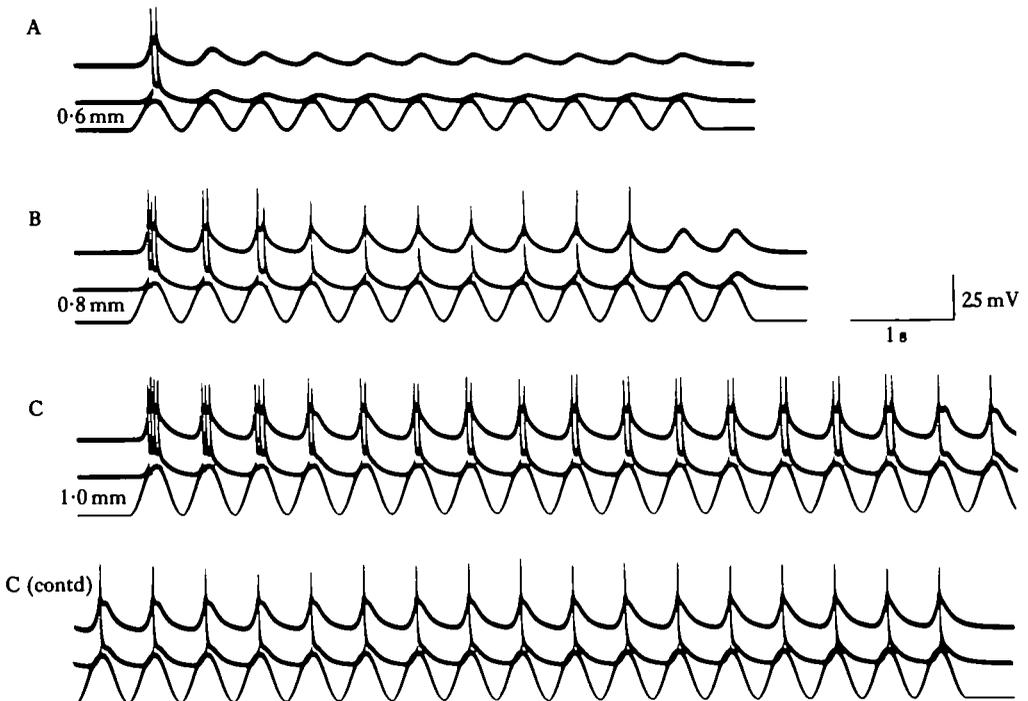


Fig. 2. Sensory habituation in fibre Y. Sinusoidal stimulation at three pull amplitudes; 2 Hz. Double impalement. Upper trace: distal electrode 3.6 mm from confluence of sensory dendrites. Middle trace: proximal electrode 5.8 mm from confluence. Bottom trace: stimulus monitor.

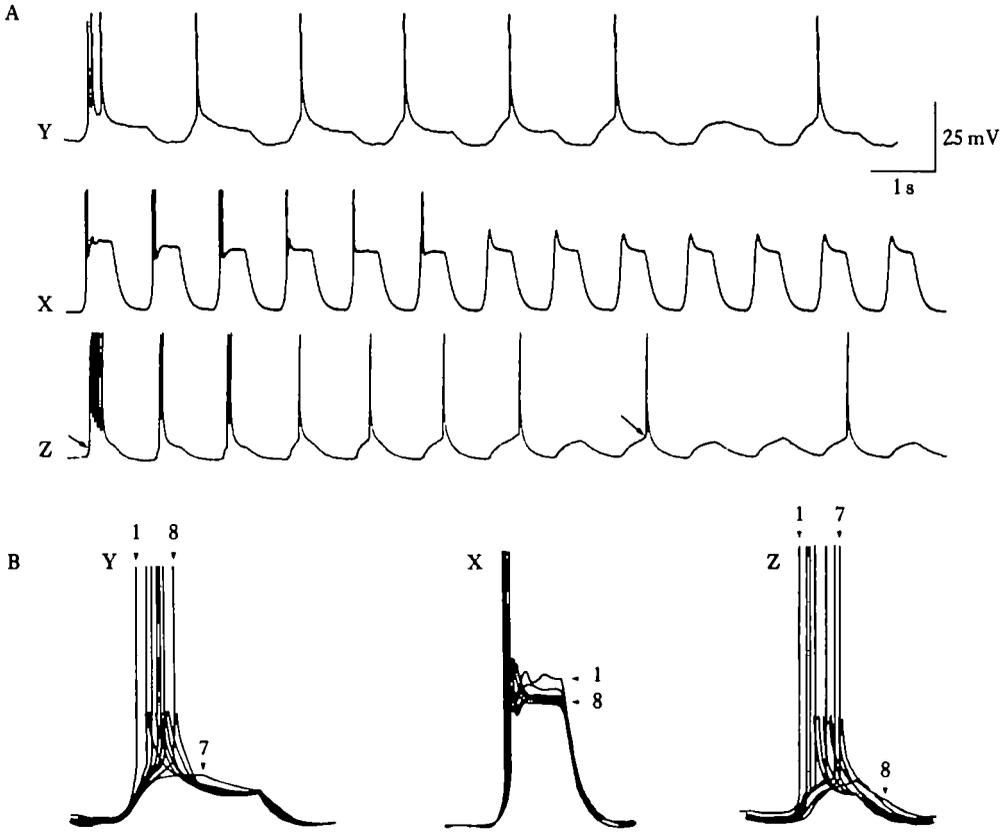


Fig. 3. Sensory habituation in X, Y and Z fibres (A) Upper trace: Y responses to 0.15-mm trapezoidal pulls at 0.66 Hz. Middle and bottom traces: X and Z responses recorded concurrently to 0.6-mm trapezoidal pulls at 1 Hz. Note the rise in the spiking threshold (arrows) in fibre Z. In the fully habituated preparation the Z spike has a phase delay of 0.5 in the X response period. (B) Same responses as in (A) redrawn and superimposed to show changes during habituation. Only the depolarizing phase of the first spike and repolarizing phase of last spike shown. Numerals indicate order of responses in the sequence.

sensory dendrites, it is difficult to ascertain whether sensory habituation is mainly a function of the transducer membrane or of the spike initiating zone. Fig. 4A shows a series of responses of a TTX-treated Y fibre to a train of identical sinusoidal stimuli at 2 Hz. The second response is 1 mV less than the first, but the decline is not nearly so marked nor progressive as in the same fibre before TTX-treatment. In this fibre therefore, it appears that sensory habituation is mainly, but not exclusively, a result of changes in the TTX-sensitive ion channels.

Additional support is given to this notion by the responses to depolarizing current shown in Fig. 4C. Since the current was injected 6.8 mm from the confluence of the sensory dendrites, it is unlikely that the transducer membrane contributed to the response recorded. The first current injection brought the spike initiation zone rapidly to the threshold for active responses, and it responded with a sharply rising pre-potential leading to impulses. But in response to subsequent identical depolarizing pulses delivered at approximately 1.5-s intervals, the pre-potential slope became

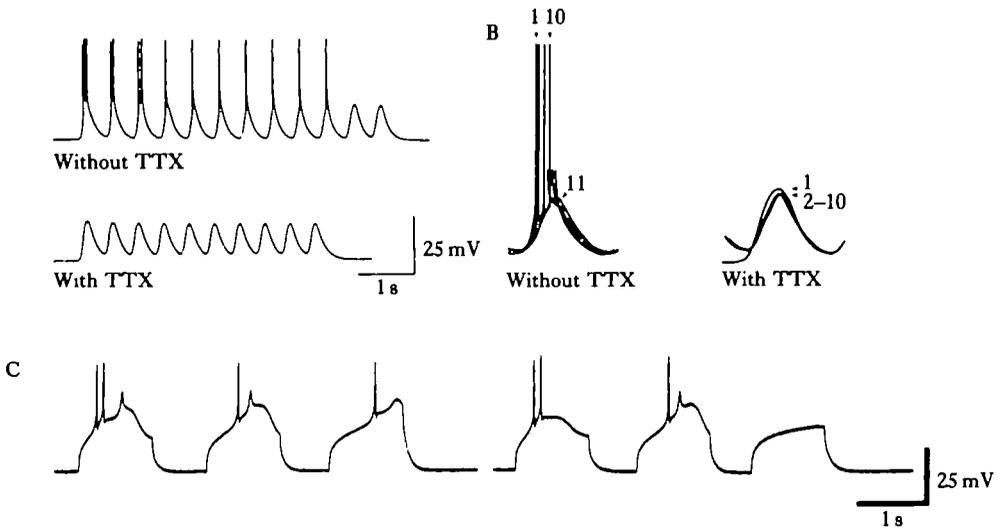


Fig. 4. Evidence for predominance of changes in spike initiation mechanism during sensory habituation. (A) Series of Y fibre responses to sinusoidal stimulation at 2 Hz before (upper) and after (lower) spike blockade with tetrodotoxin (TTX). (B) Same responses redrawn and superimposed. Note the slight decline of second and subsequent responses in the post-TTX series. (C) Two series of X fibre responses to repetitive depolarizing current injections. Current injected through second electrode 1.8 mm proximally. Note the progressive decline in the active process leading to spiking.

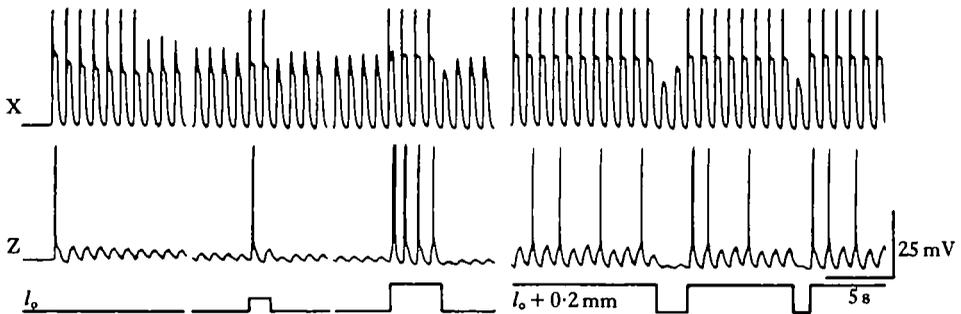


Fig. 5. Samples from long continuous series of X and Z fibre responses to repetitive trapezoidal 0.4-mm pulls at 1 Hz. Bottom line indicates the extent of stretch upon which the pulls were superimposed. l_0 , rest length. Note that two transitory increases in stretch reinstated spiking in the habituated fibres. Two transitory decreases in stretch led to reduced graded potentials and loss of spiking.

more and more gradual, and spike initiation was delayed and finally abolished.

The X and Z fibre responses shown in Fig. 3 were recorded concurrently in response to a 1-Hz trapezoidal pull. As sensory habituation developed in fibre Z, spiking was not only reduced in number and frequency, but delayed, and the peak of the receptor potential shifted to the end of the pull. Since the X response declined only in amplitude, not timing, the pair of responses underwent a significant phase delay. This can be discerned very clearly in the superimposed responses.

Fig. 5 gives several excerpts (recorded at a slower paper speed) from a long recording of the same preparation as in Fig. 3. At the beginning of the series the resting

length (l_0) of the oval organ strands was adjusted to ensure an immediate onset of depolarization at the start of a stretch stimulus. In this preparation habituation led to the cessation of spiking in both X and Z in response to 0.5-mm pulls presented at 1 Hz. The last sample was taken from a later portion of the same stimulus train after l_0 had been increased by 0.2 mm. Now the habituated responses of X retained one spike per response and a larger graded potential. Z responses were also larger and retained a higher ratio of spiking to non-spiking responses. Thus, even though habituation leads to diminished responses during repetitive stimulation, the fibres are still capable of encoding stimulus parameters such as extent of stretch.

In the habituated state, small transient perturbations in stretch, introduced manually, produced highly significant modulation of the responses. Small increments in background stretch temporarily restored spiking and amplitude to the responses, while small decrements abolished spiking and reduced the graded potentials markedly (Fig. 5).

DISCUSSION

During gill ventilation in Crustacea, long term recordings from muscles and nerves of the spontaneously active scaphognathite have shown that beating maintains constant parameters over many cycles (Pasztor, 1968; Pilkington & Simmers, 1973; Young, 1975; Best, 1982; Simmers & Bush, 1983a). When these conditions were mimicked experimentally, by presenting repetitive pulls to the isolated oval organ, the responses of its three afferent fibres habituated. Using moderate amplitude, sinusoidal stretch stimuli, spiking diminished and often disappeared, leaving the afferent fibres responding solely in the non-impulsive mode. This implies that under normal conditions of undisturbed ventilation, feedback information can be provided by small oscillations in membrane potential without the intervention of spikes.

Fibre X has the least labile response during habituation, and though spiking may disappear, the graded response retains a steep dynamic component so that the amplitude maximum undergoes the least phase shift of the three fibres. X is strongly implicated in the role of beat marker and is able to continue signalling beat frequency throughout long periods of regular ventilation.

Water flow through the gills however is not invariant, and several authors have demonstrated changes in stroke volume, scaphognathite reversals, sudden frequency changes and sporadic apnoeic periods (Hughes, Knights & Scammel, 1969; Wilkens & Young, 1975; Best, 1982; Simmers & Bush, 1983b). Sometimes these are reflex responses to noxious elements in the respiratory current, at other times these variations in rhythm appear to be spontaneous. Any such perturbations in the regular cycle of stretch and relaxation of the oval organ would disrupt habituation and restore spiking. Temporary obstructions, which might be caused by detritus lodging in the pumping chamber, forcing the scaphognathite into an abnormal configuration, would cause the transient appearance of spikes for a few cycles, while the commonly observed respiratory pauses are of sufficient duration to permit the restoration of the fully non-habituated response. Hyperventilation involving a faster, more powerful stroke, could be expected to elicit trains of bursts, with prolific spiking at any change in rhythm.

Thus the graded potentials would predominate during bouts of regular beating, while spiking would provide an enhanced release of transmitter at any perturbation in rhythm. This interplay between the two methods of signalling would seem to be an effective means of discriminating between a regular and changing ventilatory activity. Such immediacy could hardly be attained by either the analogue or the impulsive mode alone.

Sensory habituation is likely to occur in any receptor subject to rhythmical stimulation at moderate or fast repetition rates. Insect song patterns, for example, frequently provide such a stimulus situation, and Esch, Huber & Wohlers (1980), recording from single primary auditory units of *Gryllus campestris*, noted declining responses during groups of sound syllables. There was a significant decrease in the number of spikes per response with a corresponding increase in latency. The 325 ms silent period between groups was sufficient for complete recovery of the receptors before the arrival of the first syllable of the next group. Sensory habituation has also been reported in Pacinian corpuscles (Lowenstein, 1961), frog tactile receptors (Catton, 1961), crab T-C MRO (Bush & Roberts, 1971; Cannone & Bush, 1980) and cat muscle spindles (Hunt & Ottoson, 1976). All these receptors would receive repetitive stimulation during rhythmical locomotion.

Gill withdrawal in *Aplysia* is perhaps the best known example of behavioural habituation to have been analysed at the cellular level (reviewed by Kandel & Schwartz, 1982). Byrne, Castellucci, Carew & Kandel (1978) have shown conclusively that the mechanoreceptors mediating the withdrawal reflex show no decrement in afferent discharge at stimulation rates producing habituation. Thus, in contrast to the peripheral mechanism described for the oval organ and the other examples listed above, in *Aplysia* the progressive decline in motor output to the gills during repeated skin stimulation is a function of central synapses between sensory neurones and interneurones. A similar situation obtains in the crayfish escape reflex (Zucker, 1972).

Our study has established an example of habituation occurring at the receptor level which is amenable to intracellular investigation. The significance of sensory habituation and the consequent reduction in feedback to the respiratory pattern generator is currently under investigation.

This work was supported by research grants to BMHB from the Royal Society and the Science Research Council (U.K.) and to VMP from the Natural Sciences and Engineering Research Council of Canada. We thank Alison Walford for technical assistance.

REFERENCES

- BEST, S. R. (1982). Central and peripheral control of ventilation in the shore crab, *Carcinus maenas*. Ph.D. thesis, University of Bristol.
- BUSH, B. M. H. & ROBERTS, A. (1971). Coxal muscle receptors in the crab: the receptor potential of S and T fibres in response to ramp stretches. *J. exp. Biol.* **55**, 813–832.
- BYRNE, J. H., CASTELLUCCI, V. F., CAREW, T. J. & KANDEL, E. R. (1978). Stimulus response relations and stability of mechanoreceptor and motorneurons mediating defensive gill-withdrawal reflex in *Aplysia*. *J. Neurophysiol.* **41**, 402–417.
- CANNONE, A. J. & BUSH, B. M. H. (1980). Reflexes mediated by non-impulsive afferent neurones of thoracic-coxal muscle receptors in the crab, *Carcinus maenas*. I. Receptor potentials and promotor motoneurone responses. *J. exp. Biol.* **86**, 275–303.

- CATTON, W. T. (1961). Threshold, recovery and fatigue of tactile receptors in frog skin. *J. Physiol., Lond.* **158**, 333-365.
- ESCH, H., HUBER, F. & WOHLERS, D. W. (1980). Primary auditory neurons in crickets: physiology and central projections. *J. comp. Physiol.* **137**, 27-38.
- 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 in the shore crab *Carcinus maenas*. *J. exp. Biol.* **51**, 203-220.
- HUNT, C. C. & OTTOSON, D. (1976). Initial burst of primary endings of isolated mammalian muscle spindles. *J. Neurophysiol.* **39**, 324-330.
- KANDEL, E. R. & SCHWARTZ, J. H. (1982). Molecular biology of learning: Modulation of transmitter release. *Science, N.Y.* **218**, 433-443.
- LOWENSTEIN, W. R. (1961). Excitation and inactivation in a receptor membrane. *Ann. N.Y. Acad. Sci.* **94**, 510-534.
- PASZTOR, V. M. (1968). The neurophysiology of respiration in decapod crustacea. I. The motor system. *Can. J. Zool.* **46**, 585-596.
- PASZTOR, V. M. & BUSH, B. M. H. (1983). Graded potentials and spiking in single units of the oval organ, a mechanoreceptor in the lobster ventilatory system. I. The characteristics of dual afferent signalling. *J. exp. Biol.* **107**, 431-449.
- 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.
- SIMMERS, A. J. & BUSH, B. M. H. (1983a). Central nervous mechanisms controlling rhythmic burst generation in the ventilatory motoneurons of *Carcinus maenas*. *J. comp. Physiol.* **150**, 1-21.
- SIMMERS, A. J. & BUSH, B. M. H. (1983b). Motor programme switching in the ventilatory system of *Carcinus maenas*: the neuronal basis of bimodal scaphognathite beating. *J. exp. Biol.* **104**, 163-181.
- WILKENS, J. L. & YOUNG, R. E. (1975). Patterns and bilateral coordination of scaphognathite rhythms in the lobster *Homarus americanus*. *J. exp. Biol.* **63**, 219-235.
- YOUNG, R. E. (1975). Neuromuscular control of ventilation in the crab *Carcinus maenas*. *J. comp. Physiol.* **101**, 1-37.
- ZUCKER, R. S. (1972). Crayfish escape behaviour and central synapses. II. Physiological mechanisms underlying behavioural habituation. *J. Neurophysiol.* **35**, 621-637.