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CONTROL OF 'PULMONARY' PRESSURE AND COORDINATION WITH GILL VENTILATION IN THE SHORE CRAB *CARCINUS MAENAS*

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Summary

1. In the shore crab, *Carcinus maenas* (L.), forward ventilation creates negative pulses of hydrostatic pressure while reversed ventilation causes dramatic positive pressure fluctuations in the branchial chamber. These pressures are transmitted *via* the gills to the haemolymph of the open circulatory system. The branchiostegal sinus, which is a compliant chamber, may function as a reservoir for displaced haemolymph and may operate as an accessory pump driven by the action of the dorsoventral (DV) muscles.

2. A band of dorsoventral muscles controls the volume of the branchiostegal sinuses. The muscular activity is coordinated with ventilatory activity and may assist in regulating pressure fluctuations caused by ventilatory pressure pulses. During a ventilatory reversal, the haemolymph displaced from the gills is added to the volume of haemolymph in the open circulatory system and this haemolymph may be accommodated in the branchiostegal sinus by relaxation of the DV muscles.

3. Artificially regulating the pressure either in the branchial chamber or in the branchiostegal sinus reflexively alters DV muscle activity, which suggests the occurrence of baroreceptors in this crab.

4. The branchiostegal nerve that innervates the DV muscles contains five neurones identified by cobalt backfills. Three of them are median and two are contralateral. The dendritic field of each neurone is confined to its respective hemiganglia.

5. The electrical activity of one of the motoneurones in the branchiostegal nerve corresponds to the activity of the DV muscles. *In vitro* observations of the activity of branchiostegal motoneurones in relation to ventilatory motoneurone activity indicate that both are centrally coupled and support the hypothesis that the branchiostegal motoneurones are influenced by the ventilatory central pattern generator.

Introduction

Gaseous exchange in crabs is generally thought to occur primarily across the

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gills; however, an alternative 'pulmonary' circulation and gas exchange mechanism has been described for amphibious and terrestrial crabs. This pulmonary circulation operates in parallel with the gills and consists of the movement of deoxygenated haemolymph through vascular beds in the branchiostegal walls (see Greenaway and Farrelly, 1990; Maitland, 1990). Gas exchange occurs across the cuticle-covered epithelial linings of the branchial chamber to the subepithelial branchiostegal sinuses. The course of haemolymph in the branchiostegal sinuses of amphibious crabs is not well understood. In terrestrial crabs, the lungs receive haemolymph from the dorsal sinuses and hepatic sinuses; the haemolymph passes through the branchiostegal sinuses and returns to the pericardial sinus *via* pulmonary veins (Greenaway and Farrelly, 1984, 1990). Such networks and courses of flow are observed in the crab *Cancer magister* and, in addition, anterior arteries provide input to the branchiostegal sinuses (B. R. McMahon, personal communication). This pulmonary gas exchange mechanism appears to become increasingly important in the transition from aquatic to terrestrial habitats (Diaz and Rodriguez, 1977). The cuticle facing the branchial chamber and its underlying epithelium is the branchiostegal membrane. In the crab *Cancer pagurus* the branchiostegal membrane is separated from the dorsal carapace anteriorly and is suspended from the dorsal carapace by a band of dorsoventral (DV) muscles (Pearson, 1908). In addition to gas exchange, the branchiostegal sinus may be involved in haemocoelic pressure regulation (Taylor, 1990).

How is this pulmonary circulation related to gill circulation? Crabs pump water through the branchial chambers by the rhythmic beating of scaphognathites. During forward ventilation the branchial chambers are maintained at a negative pressure, but during reversed pumping hydrostatic pressure in the gill chamber becomes positive. The variation in branchial chamber pressure in crabs may range from -0.392 to $0.588\,kPa$ (-4 to $+6\,cmH_2O$) during forward and reverse modes, respectively (Hughes *et al.* 1969; Wilkens and McMahon, 1972).

During forward ventilation the positive transmural pressure, from gill vasculature to branchial water, will facilitate haemolymph flow through the gills. During reversed ventilation the reduced transmural pressure will compress the delicate gills, causing a reduced rate of gill perfusion (L. E. Burnett, cited in Taylor, 1982) and an increase in haemocoelic pressure (Blatchford, 1971). Burggren *et al.* (1985) demonstrated that variations in branchial chamber pressure during a reversal cause an elevation of intracardiac haemolymph pressure. Any reduction in the cross-sectional area of the gill vascular channels will have a profound effect on haemolymph flow since velocity in a pipe varies in proportion to the fourth power of the radius (Poiseuille's equation). At this point an integration of gill and pulmonary circulations can be visualized. During forward ventilation the increased transmural pressure across the gills would favour circulation through the gills, but during reversed ventilation a reduction in transmural pressure might shunt haemolymph flow to the branchiostegal sinuses (Taylor, 1990). Furthermore, during reversal, branchial pressure swings, and haemolymph driven from the gills might be accommodated in the compliant space provided by the

branchiostegal sinuses, since the rest of the body is restricted by a hard exoskeleton.

In the present study we used the shore crab *Carcinus maenas* (L.) to analyze the effects of changes in branchial chamber pressure on branchiostegal sinus pressure, the activity of DV muscles that traverse this sinus and their neural control, in an effort to elucidate how haemocoelic pressure is regulated and coordinated with ventilation. A brief report of this study has appeared elsewhere (Rajashekhar and Wilkens, 1989).

Materials and methods

Carcinus maenas were obtained from commercial suppliers and maintained in filtered, recirculated artificial sea water at 12°C. They were fed on a diet of chopped fish.

Ventilatory and cardiac activity

Ventilation rate and mode (forward or reversed) was recorded by means of pressure transducers connected through polyethylene tubing to the gill chambers, as described by Wilkens and McMahon (1972). The haemocoelic pressure in the branchiostegal sinus was measured by the same technique with the polyethylene cannula connected through a hole drilled in the dorsal carapace. Heart rate was measured by impedance conversion (Biocom, Inc.) with two insulated copper wires (0.1 mm diameter) with bared tips implanted on either side of the heart. When the mode of ventilation was not relevant, the scaphognathite activity was also recorded by using impedance conversion with the electrodes placed on either side of the scaphognathites.

Neuroanatomy

The course of the branchiostegal nerve, which innervates the dorsoventral muscles, was observed and traced using 0.1 % Methylene Blue solution in sea water. The central projections of the motoneurones and their dendritic arborizations were revealed by backfilling with 5 % cobalt chloride in saline (Pitman *et al.* 1972) through the cut end of the branchiostegal nerve. The nerve was cut before it branches. Cobalt-filled ganglia were intensified with reduced silver (Bacon and Altman, 1977) following fixation in formaldehyde/acetic acid/alcohol. The ganglia were cleared in methyl benzoate and wholemounts were observed. The motoneurones and their dendritic profiles were traced using a Wild *camera lucida*.

Electrophysiology

The activity of the DV muscles in intact crabs was observed by recording their electromyograms (EMGs) using thin insulated wire (diameter 0.1 mm) placed through holes drilled in the carapace and secured with cyanoacrylate glue and squares of dental dam. Signals were amplified using Grass P-15B a.c. amplifiers.

For recording the electrical activity of the ventilatory and branchiostegal

motoneurones, a surgically reduced preparation was used (Simmers and Bush, 1983*a*). The dorsal carapace, heart, hepatopancreas and digestive tract were removed, leaving in place the thoracic ganglion with the ventilatory and branchiostegal nerves. Ganglia were perfused at 2–3 ml min^{-1} with oxygenated saline (Wilkens *et al.* 1989) *via* the sternal artery. All recordings were carried out at room temperature (20°C). The branchiostegal nerve was separated from a branch of the cardiac nerve which is loosely attached to it. Scaphognathite and branchiostegal nerves were drawn into suction electrodes. The signals were amplified using Grass P-15B amplifiers, displayed on an oscilloscope and stored on magnetic tape. The recordings were traced using a Gould RS 3200 chart recorder.

Results

Branchiostegal sinus

The branchiostegal sinus is the vascularized space between the cuticularized epithelial lining of the branchial chamber and the dorsal carapace. In *C. maenas* this sinus consists of two distinct regions. Over the medial and posterior portions of the branchial chambers the epithelium is closely attached to the carapace by numerous short strands of striated muscle. However, over the anterior one-third of the branchial chambers the branchiostegal membrane diverges from the carapace, creating a wedge-shaped space which is mostly filled by extensions of the hepatopancreas and in females by extensions of the gonads (Fig. 1). At its anterior-most margin each sinus in a 100 g crab is about 1 cm deep.

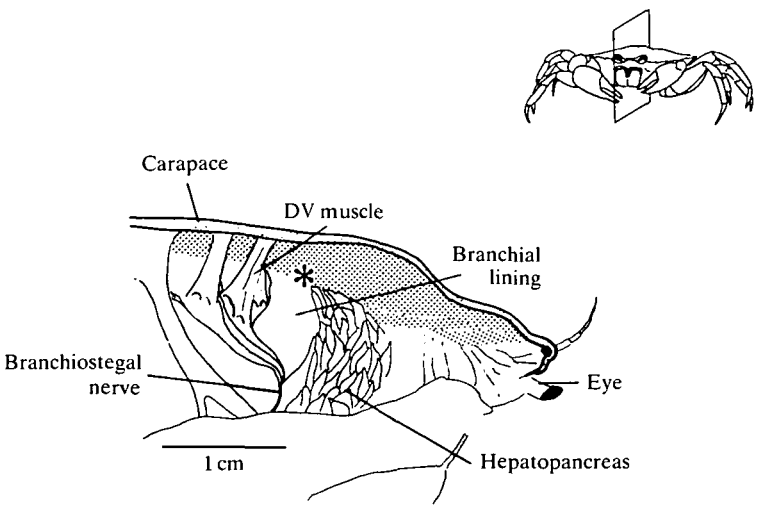


Fig. 1. Lateral sectional view of the crab showing the anterior one-third of the branchiostegal sinus (*). The stippling indicates the ventral surface of the carapace. This space also contains a portion of the hepatopancreas and gonads. Dorsoventral (DV) muscles extend from the dorsal carapace to the branchiostegal membrane. The plane of section is indicated in the inset.

At a point about midway in the anterior–posterior plane of the anterior sinus an arc-shaped band of dorsoventral (DV) muscles attaches the branchiostegal membrane to the dorsal carapace. Their points of attachment to the carapace can be visualized from the outside as an arc of slightly depressed yellow spots.

The branchiostegal nerves originate from the thoracic ganglion posterior and dorsal to the ventilatory nerves. Each runs anteriorly parallel to the ventilatory nerves, then turns dorsolaterally and curves backwards to innervate the dorsal lining of the branchial chamber. Each one divides into five branches, one of which innervates the DV muscles. The innervation of the DV muscles was confirmed by recording electromyograms while electrically stimulating the branchiostegal nerve.

Neuroanatomy

Cobalt backfilling of the branchiostegal nerve revealed five efferent neurones. Three cell bodies are placed midventrally while the other two are located in the contralateral half of the ganglion (Fig. 2). All five efferent neurones are monopolar and resemble arthropod central neurones in their geometry. Their dendritic fields are co-localized with the ventilatory motoneurones and the ventilatory central pattern generator (CPG) interneurones (Simmers and Bush, 1983*a*; DiCaprio, 1989). One of the median neurones is large, with a cell body measuring 50 μm in diameter and a dendritic field confined to the ipsilateral side (Fig. 2B). The other two ipsilateral median neurones are smaller, with 10 μm diameter somata, and have very restricted arborizations before their axons exit through the branchiostegal nerve (Fig. 2C). The two contralateral neurones are placed near the origin of the contralateral branchiostegal nerve and have their dendritic branches spanning the contralateral ventilatory neuropile region (Fig. 2D).

Dorsoventral muscle activity in situ

Electromyographic recordings of the DV muscles revealed that their activity was correlated with gill ventilation. Their activity was loosely burst-like and was phase-coupled with the depressor half of the ventilatory cycle (Fig. 3A). Simultaneous recordings of the EMG activity from two different sites on the DV muscle band showed identical EMG recording, which suggests that the DV muscles receive a common innervation (Fig. 3B). The overall spike frequency varied according to ventilatory rate (Figs 4, 11C) and during ventilatory pauses the DV muscle activity was depressed and tonic (Fig. 5). During a ventilatory reversal the activity was inhibited; this was followed by a post-inhibitory burst with the onset of forward ventilation (Fig. 6).

Hydrostatic pressure and dorsoventral muscle activity

The coordination of DV muscle activity with ventilation could arise from a reflex caused by changes in branchial pressure or as a consequence of central hard wiring, i.e. intraganglionic neural connectivity (described below). The first

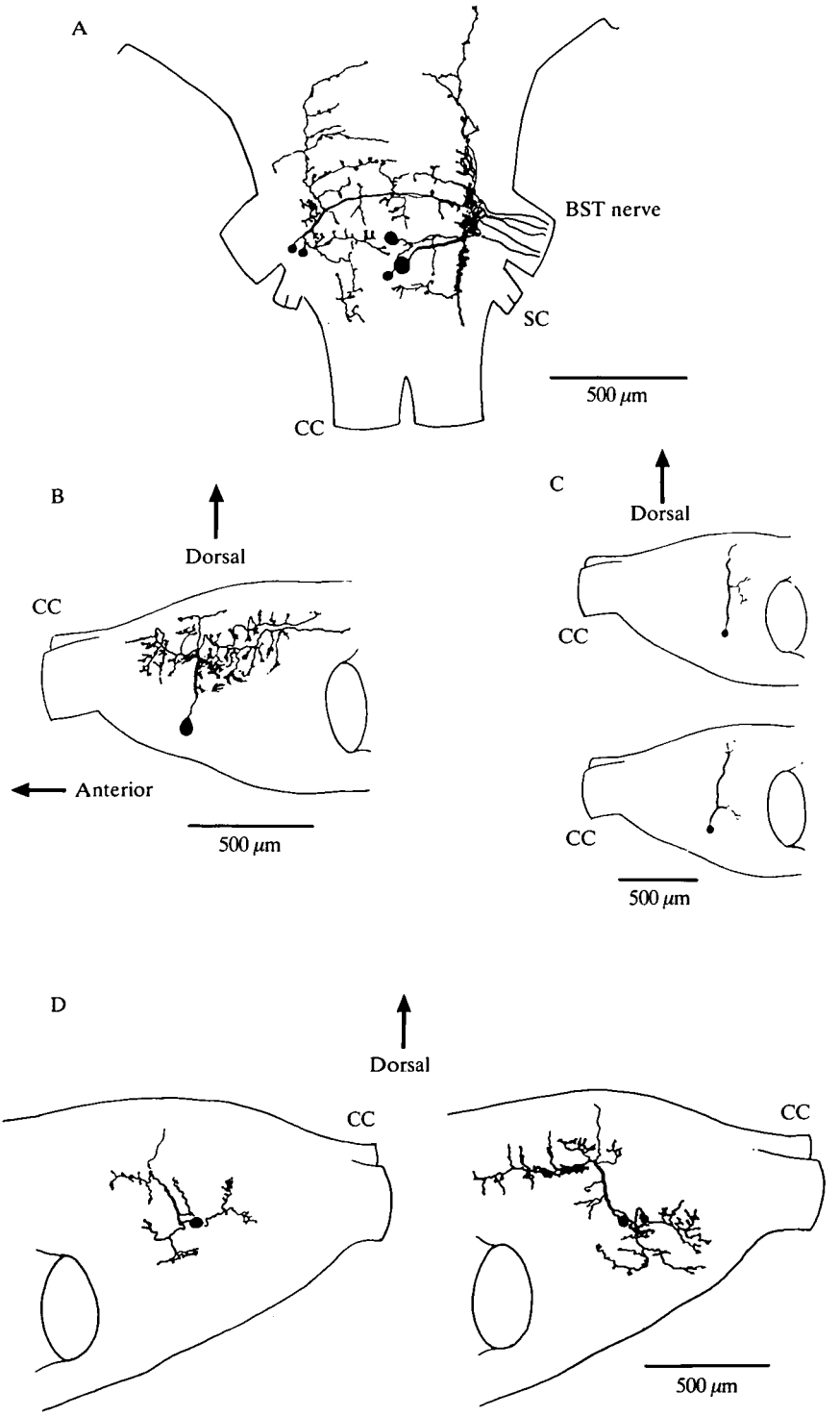

A
BST nerve
SC
CC
500 μm
B
Dorsal
CC
Anterior
500 μm
C
Dorsal
CC
CC
500 μm
D
Dorsal
CC
CC
500 μm

Fig. 2

Fig. 2. (A) Dorsal view of the thoracic ganglion showing cobalt-filled neurones of the branchiostegal nerve (BST nerve). The somata and dendritic arborizations of three of the neurones are ipsilateral, while two are contralateral. (B) Lateral view of the large ipsilateral branchiostegal efferent neurone and its dendritic tree. The neurone has extensive dendritic branches confined to the ipsilateral hemiganglion. (C) Lateral view of the two smaller ipsilateral neurones and their dendritic arborizations. The two neurones are monopolar with their cell bodies placed mid-ventrally and their dendritic field is very restricted. Their axons ascend into the branchiostegal nerve. (D) Lateral view of the two contralateral neurones. Their axons run parallel and close together (seen in dorsal view) as they cross over to the contralateral side and emerge through the branchiostegal nerve. SC, scaphognathite nerve; CC, circumoesophageal connective.

possibility was tested by plugging the scaphognathite pumping chamber and artificially manipulating the branchial and branchiostegal pressure by injecting fluid into or drawing fluid out of the appropriate chamber through a cannula attached to a syringe. The ventilatory CPG continued to generate motoneurone bursts, but at a reduced rate when the appendage was immobilized. When the branchial chamber pressure was increased, simulating reversed ventilation, DV muscle activity was depressed or inhibited (Fig. 7). Conversely, when branchial chamber pressure was reduced, DV muscle activity increased. Increasing the hydrostatic pressure in the branchiostegal sinus by injecting saline also inhibited the DV muscle activity (Fig. 8).

We next explored the effects of DV muscle activation and ventilatory pressure excursions on the hydrostatic pressure in the branchiostegal sinus. A spontaneous contraction of the DV muscles in a crab with the scaphognathite chamber plugged elevated the pressure in the sinus by about 0.196 kPa (2 cmH_2O) (Fig. 9). A ventilatory reversal in an unrestrained animal caused a surge in hydrostatic pressure of both branchial chamber and branchiostegal sinus, even though the DV muscles were inhibited at this time (Fig. 10). Cardiac bradycardia accompanies bilateral reversals, but not unilateral reversals.

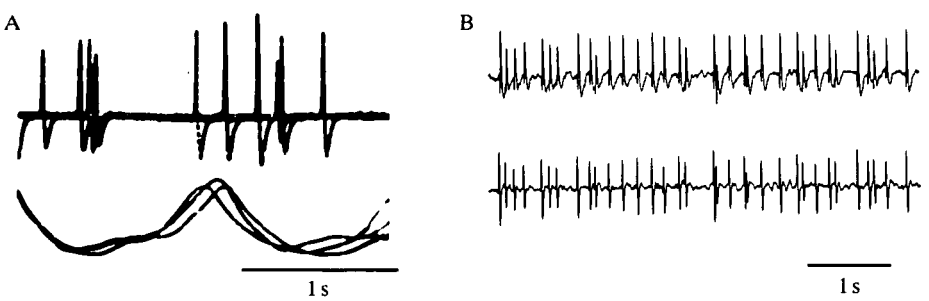


Fig. 3. (A) Three superimposed sweeps of the oscilloscope showing the phasic coupling of DV muscle activity (upper trace) to ventilatory pressure waveforms (lower trace). The oscilloscope was triggered by the lower beam at the beginning of the depressor half of the ventilatory cycle. (B) Simultaneous EMG recordings from two different sites 15 mm apart are identical, indicating common innervation.

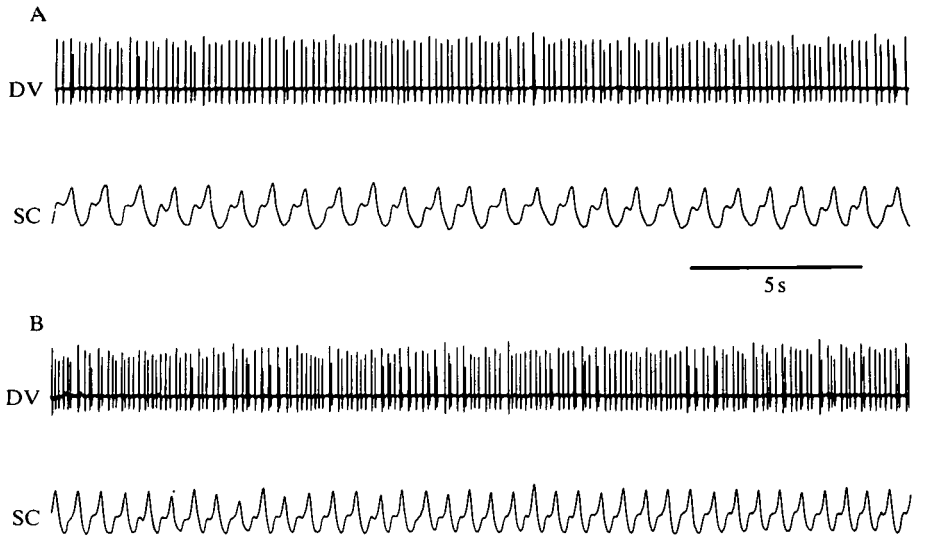


Fig. 4. The frequency of the DV muscle spikes varies proportionally with ventilatory frequency. (A) At a ventilation rate of 60 beats min^{-1} the DV muscles receive 5.02 impulses s^{-1}. (B) When ventilation rate increased to 88.5 beats min^{-1} DV spike frequency increased to 7.07 impulses s^{-1}. DV, dorsoventral muscle EMG; SC, scaphognathite-generated ventilatory rhythm recorded by impedance conversion.

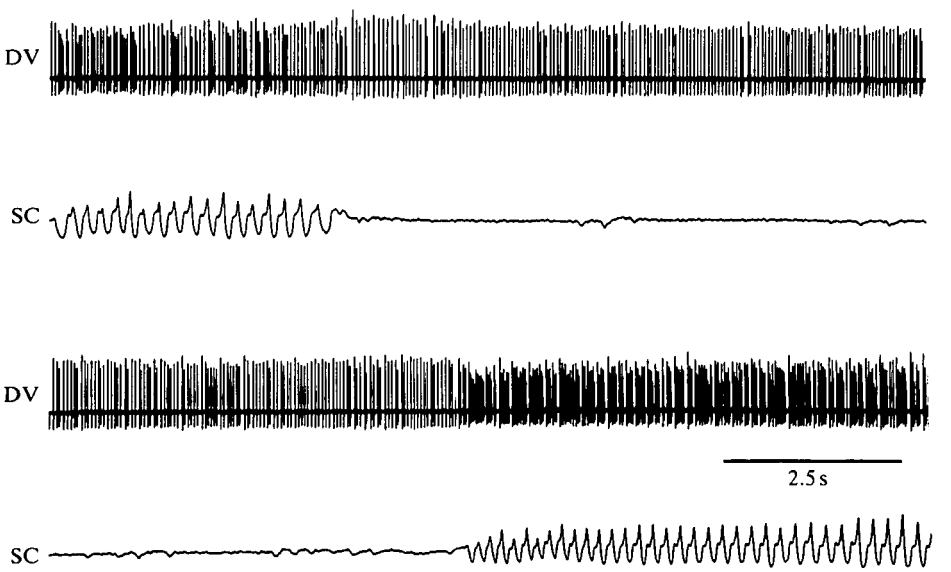


Fig. 5. During a ventilatory pause the DV muscle is tonically active at a depressed rate but, with the onset of forward ventilation, phasic firing resumes. The illustration is a continuous trace. DV, dorsoventral muscle EMG; SC, scaphognathite activity.

Electrophysiology of branchiostegal motoneurones

In surgically reduced preparations we next explored the possibility that coordination of DV muscle activity with the ventilatory rhythm could arise within the central nervous system. The deafferented ventilatory CPGs continued to produce coordinated motoneurone output typical of both forward and reversed

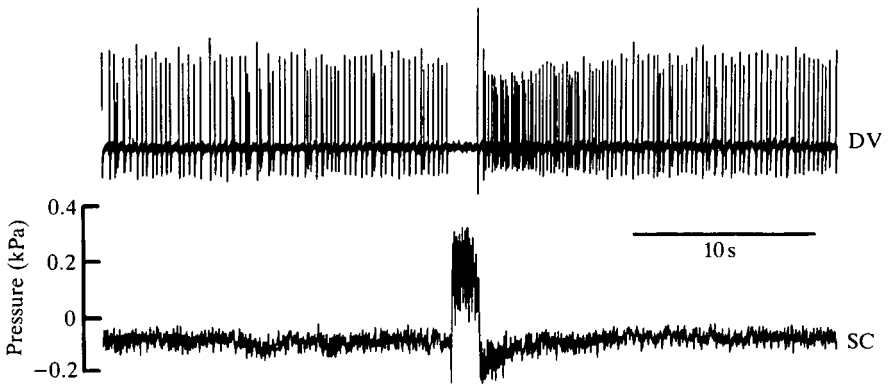


Fig. 6. During reversed ventilatory pumping, the activity of DV muscles (upper trace) is inhibited and this is followed by a post-inhibitory burst. The lower trace indicates branchial chamber pressure; reversed pumping produces positive excursions in hydrostatic pressure (0.1 kPa=1.02 cmH_2O). DV, dorsoventral muscle EMG; SC, branchial chamber pressure recording representing scaphognathite activity.

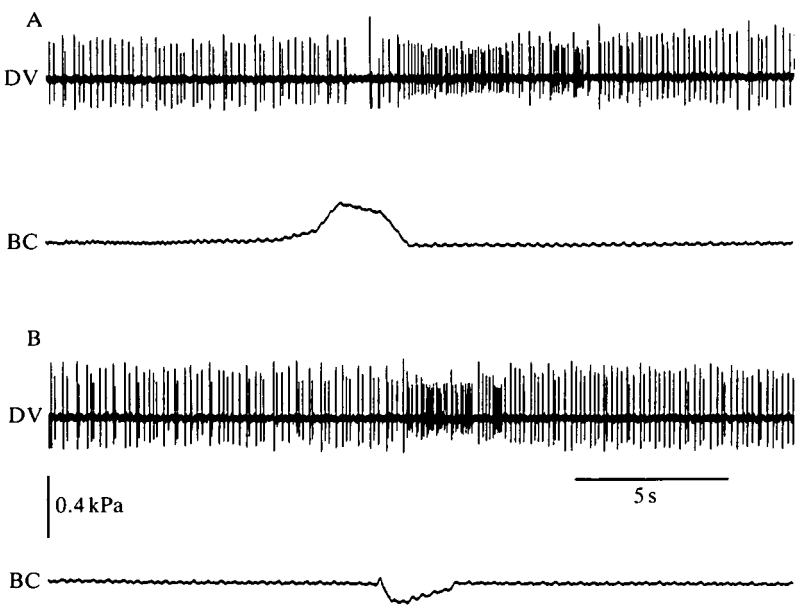


Fig. 7. (A) Artificially increasing the pressure in the branchial chamber (lower trace) reflexively depresses DV muscle activity, while reducing the pressure activates DV muscles (B). DV, muscle activity; BC, pressure in the branchial chamber (0.1 kPa=1.02 cmH_2O).

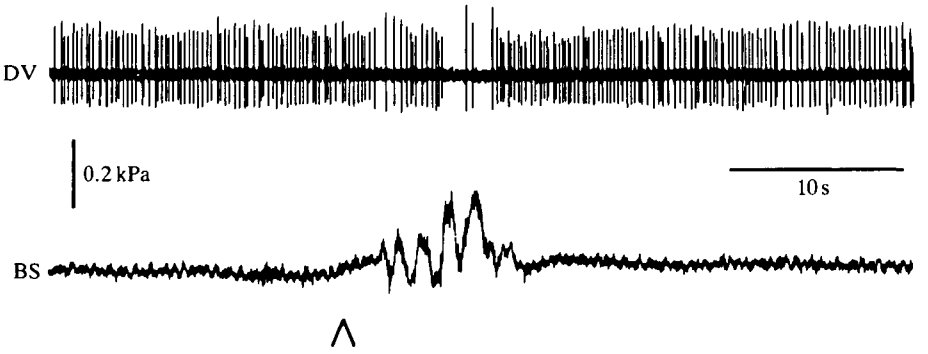


Fig. 8. Increasing the pressure in the branchiostegal sinus by injecting saline reflexively depresses DV muscle activity. The arrowhead indicates time of injection. Variations in the haemocoelic pressure, measured by a second cannula, are coupled with reduced DV muscle activity (0.1 kPa=1.02 cmH_2O).

scaphognathite pumping (Simmers and Bush, 1983*b*; DiCaprio, 1985). During forward ventilatory bursting the branchiostegal nerve typically contained two active units, one was large in amplitude (BST-mn1) and the other was smaller (BST-mn2). In the example shown in Fig. 11A the activity of BST-mn1 was phasic and coupled to levator ventilatory motoneurone bursts; BST-mn2 fired tonically. The activity of BST-mn1 corresponded to the activity of the DV muscles. The frequency of firing of BST-mn1 was proportional to the ventilatory burst rate (Fig. 11B,C). BST-mn1 frequency increased from 5.12 to 5.92 Hz (15%) as ventilatory rate increased from 81.6 to 93.6 beats min^{-1} (15%). During a pause in ventilatory motoneurone activity, BST-mn1 showed a depressed, tonic firing; it resumed phasic activity with the onset of a ventilatory motor burst (Fig. 12).

During a scaphognathite motoneurone switch to the reversed ventilation pattern BST-mn1 was depressed (we assume inhibited), as were DV muscle activity and bursts in another unit (BST-mn3) (Fig. 13A). The inhibition of BST-mn1 preceded the scaphognathite motoneurone switch, sometimes by 2–3 ventilatory motoneurone bursts. Intact crabs did not exhibit reversals during

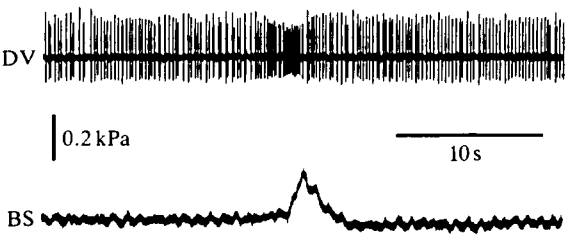


Fig. 9. A spontaneous contraction of DV muscles in a crab with its scaphognathite pumping chamber plugged causes elevation of haemocoelic pressure in the branchiostegal sinus. DV, dorsoventral muscle EMG; BS, pressure in the branchiostegal sinus (0.1 kPa=1.02 cmH_2O).

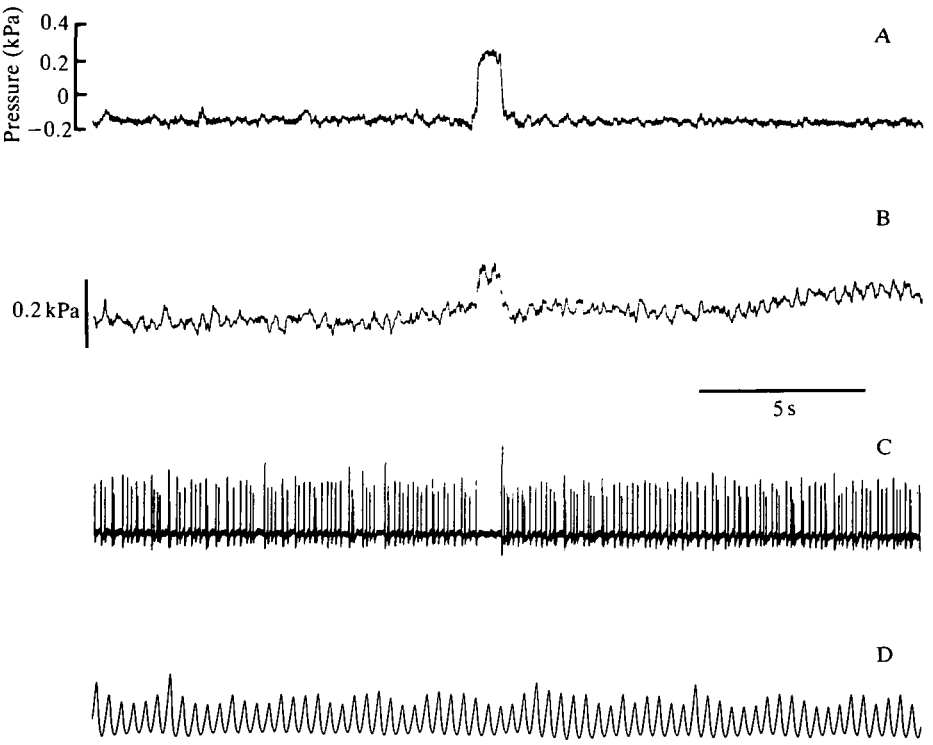


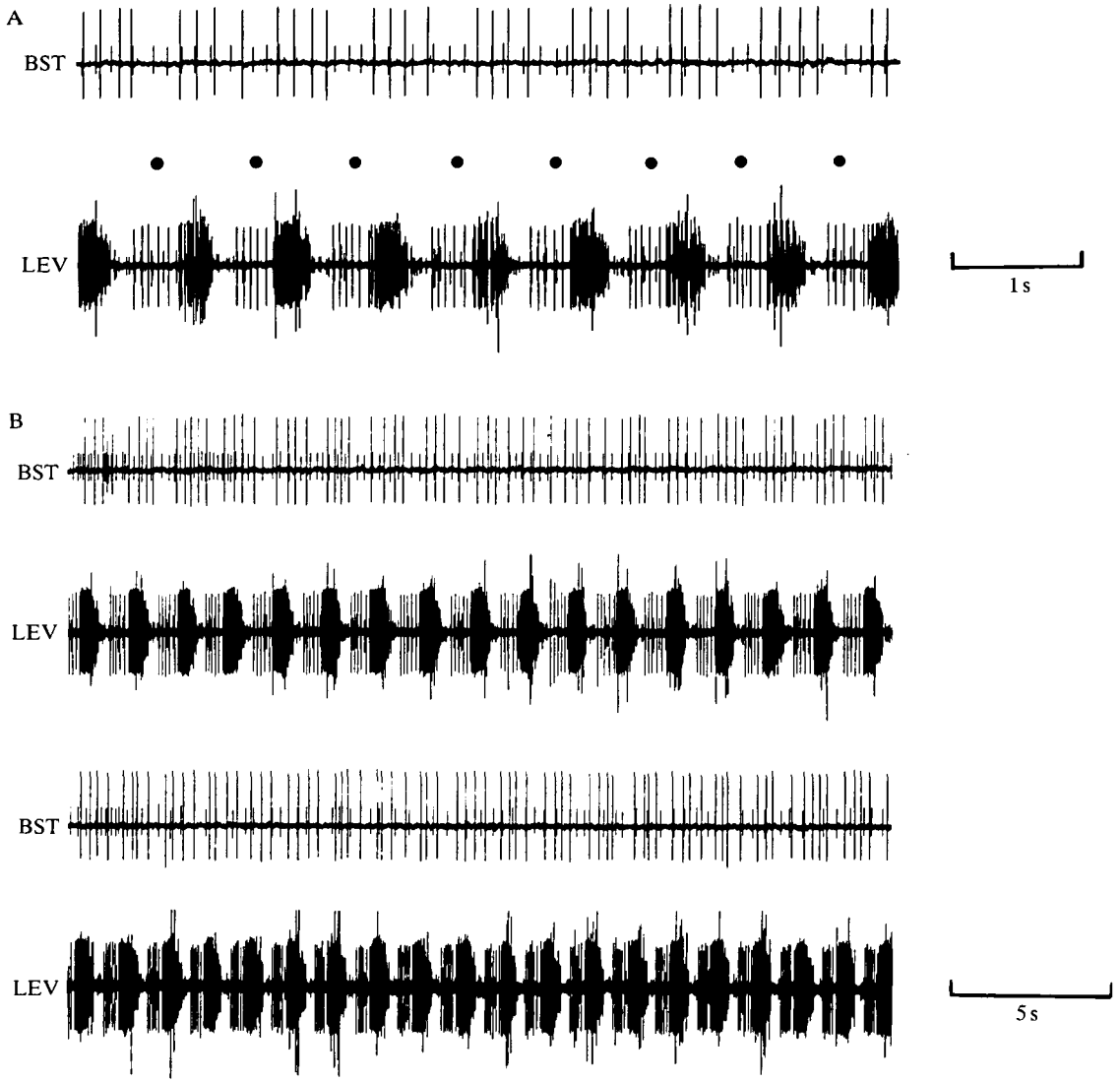
Fig. 10. (A) Branchial pressure during a period of reversal-induced positive pressure. (B) Haemocoelic pressure also increases during reversal. (C) DV muscle activity is inhibited and (D) cardiac responses (recorded by impedance conversion) accompany bilateral, but not unilateral, reversal (0.1 kPa=1.02 cmH_2O).

ventilatory pauses, but thoracic ganglion preparations did. During such reversals BST-mn1 was inhibited and BST-mn3 was activated (Fig. 13B). It was interesting that brushing or pinching the gills produced reversed bursting patterns in ventilatory motoneurones. This reflex resembles the 'cough' reflex in fish (Satchell, 1959). Such 'induced' reversals also elicited reversal-associated responses in the branchiostegal nerve that looked identical to those shown here (data not shown).

Discussion

Role of branchiostegal sinuses in the regulation of haemolymph pressure

Fluctuations in haemolymph pressure in crabs are primarily produced by cardiac pumping; however, ventilatory pressure changes in the branchial chambers are transmitted to the haemolymph *via* the fragile and voluminous gills (Blatchford, 1971; L. E. Burnett, cited in Taylor, 1982). Any haemolymph displaced from the gills during reversed ventilation must be accommodated elsewhere and the exoskeleton severely constrains this for most of the body. In addition, the gills receive a low-pressure venous haemolymph supply and any increase in branchial


A
BST
LEV
1 s
B
BST
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BST
LEV
5 s

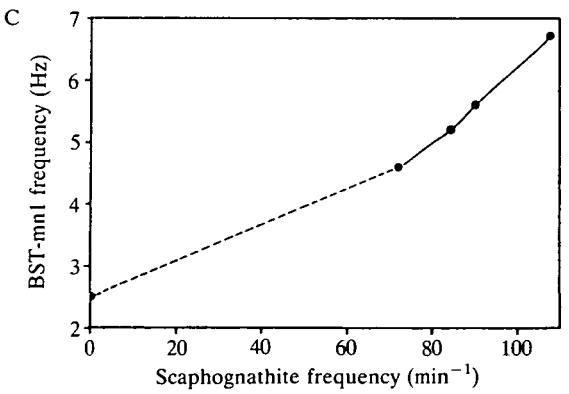
C
BST-mnl frequency (Hz)
7
6
5
4
3
2
0
20
40
60
80
100
Scaphognathite frequency (min^{-1})

Fig. 11

Fig. 11. (A) In a semi-isolated thoracic ganglion preparation the activity of a large-amplitude motoneurone (BST-mn1) of the branchiostegal nerve corresponds to DV muscle activity in intact animals. The activity of BST-mn1 is coupled to the ventilatory rhythm and ● indicates the silent period that occurs during transition from depression to levation. The small-amplitude neurone (BST-mn2) fires tonically. (B) The frequency of BST-mn1 in a thoracic ganglion preparation increases with increasing rates of ventilatory motoneurone bursting, but continues to maintain a phase-coupled pattern. (C) BST-mn1 frequency at different frequencies of ventilatory bursts in a thoracic ganglion preparation. BST, branchiostegal nerve; LEV, levator branch of scaphognathite nerve.

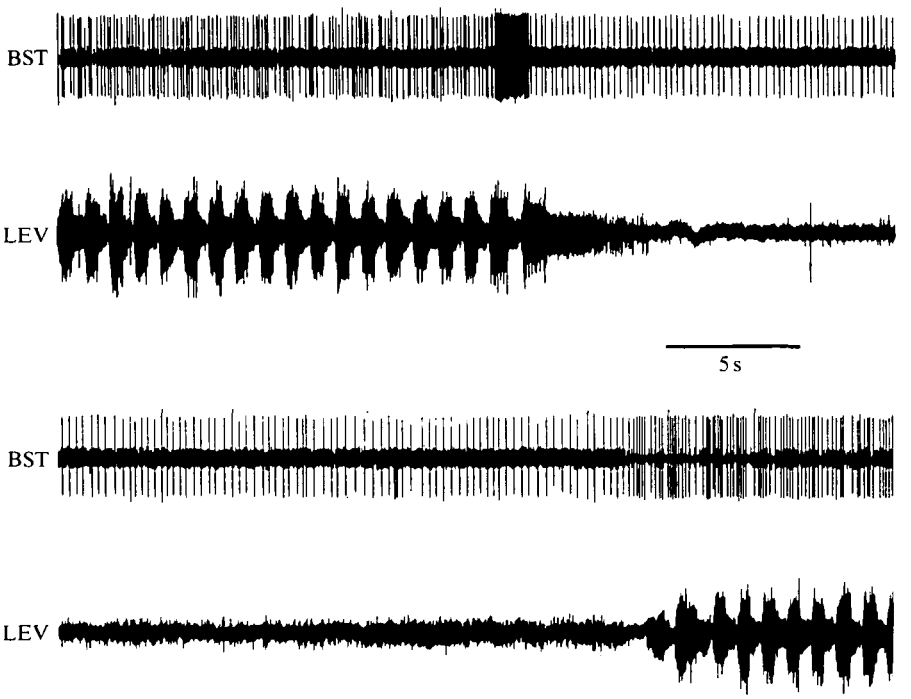

Fig. 12. During a ventilatory pause, BST-mn1 fires tonically at low frequency (upper trace); it resumes phasic firing with the onset of ventilation. The illustration is a continuous trace. BST, branchiostegal nerve; LEV, levator branch of the ventilatory nerve.

resistance may reduce gill perfusion (see Introduction). If gill perfusion is reduced and shunted during ventilatory reversals an alternative venous return and gas exchange pathway can be predicted. The anterior branchiostegal sinuses of *Carcinus maenas* are compliant venous spaces and would therefore be suited for these gill bypass functions. Indeed, the venous return from the various sinuses can be facultatively switched from the gills to the branchiostegal sinuses in *Holthuisana transversa* as it moves from water to air (Taylor and Greenaway, 1984).

During forward ventilation, when negative branchial pressure would tend to

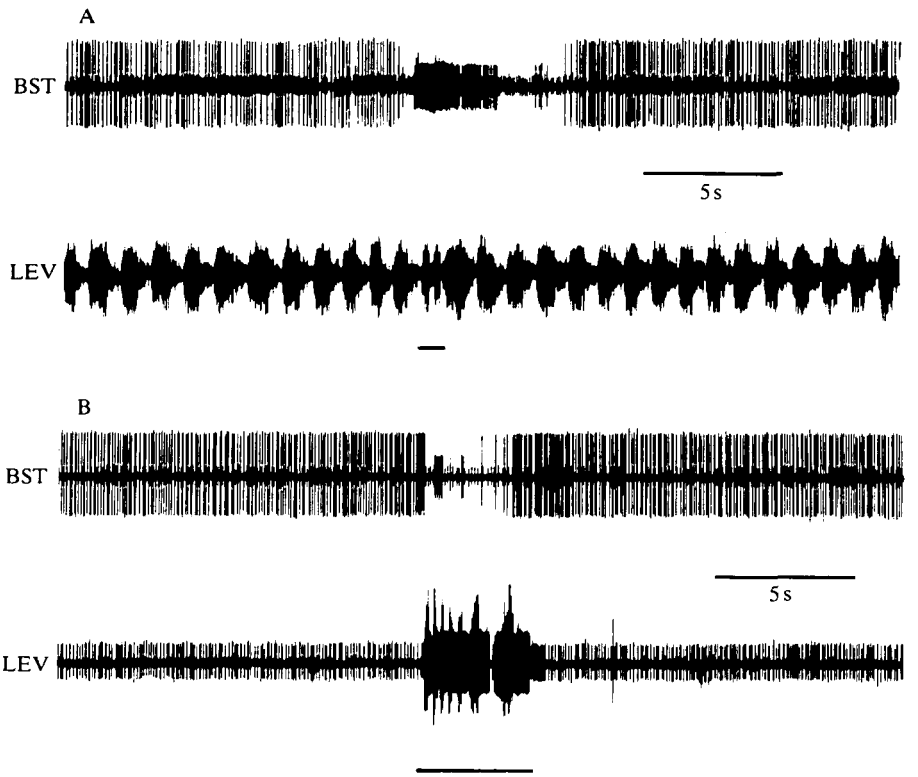

Fig. 13. (A) During a switch of scaphognathite motor neurone activity from the forward to the reversed pattern (bar) BST-mn1 is depressed and another motor unit (BST-mn3) bursts. (B) A reversal bout (bar) occurring during a pause is also accompanied by a similar depression of BST-mn1 and activation of BST-mn3. BST, branchiostegal nerve; LEV, levator branch of the ventilatory nerve.

cause the gills and branchiostegal sinus to expand, we observed phasic, sometimes tonic, activation of the DV muscles. Contraction of these muscles would prevent the expansion of this sinus. Phasic contractions might also function as an auxiliary pumping mechanism to aid venous return through this pulmonary pathway to the pericardial sinus.

During short bouts of reversed ventilation the pressure in the branchiostegal sinus increased. This could arise either from the positive pressure pulse in the branchial chamber or from the displaced haemolymph from the gills or from both. Relaxation of the DV muscles at this time would allow the branchiostegal sinus to expand to accommodate the additional haemolymph volume expressed from the gills. It can be argued that the branchial sinus is subjected to the same positive pressure as are the gill lamellae. The ratio of sinus volume expansion resulting from DV muscle relaxation to compression from the elevated branchial pressure is not known; however, the sinus is not an empty space but contains the hepatopancreas, and the gonads in females, and would therefore resist compression by

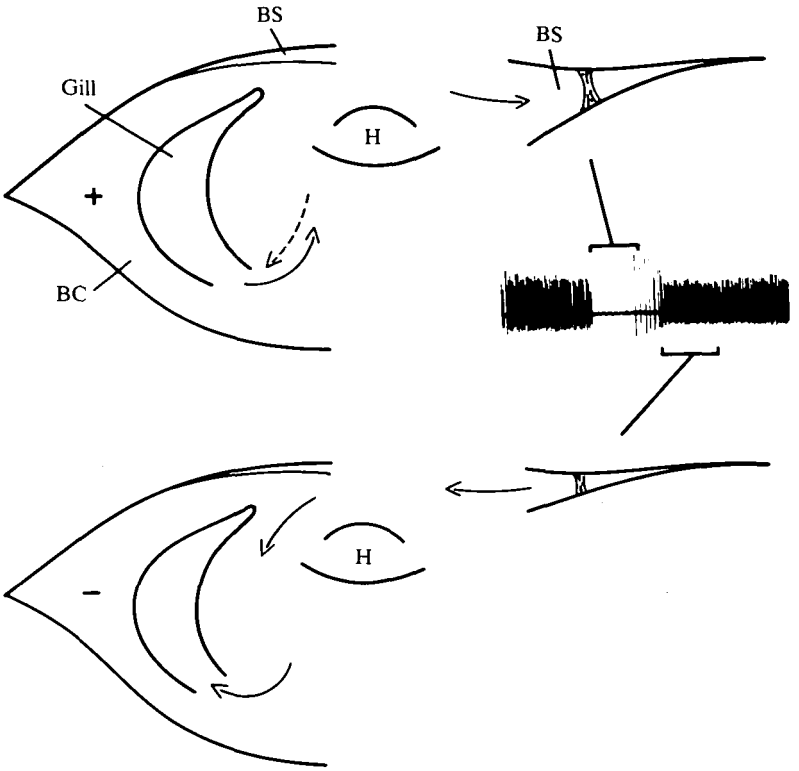

Fig. 14. Schematic diagram of haemolymph flow during reversal (+ indicates positive pressure in the branchial chamber) and during forward ventilation (−), when branchial chamber pressure becomes negative. Solid arrows indicate the direction of haemolymph flow and the broken arrow indicates possible resistance to the flow of the haemolymph. EMG recordings from DV muscle and the proposed changes in the branchiostegal sinus are shown on the right. BC, branchial chamber; BS, branchiostegal sinus; H, heart.

branchial pressure. The weight of the gonads and hepatopancreas would also force the membrane down when the DV muscles relax (Pearson, 1908).

Immediately upon return to forward ventilation the post-inhibitory burst of DV muscle activity and consequent contraction should pump the accumulated haemolymph from the sinuses, reduce sinus perfusion and favour the rapid return to predominantly gill perfusion. A schematic illustration of this hypothesis is provided in Fig. 14. It would be interesting to measure DV muscle activity when reversed ventilation is the dominant mode, e.g. in *Corystes cassivelaunus* when buried in mud (Arudpragasm and Naylor, 1966).

Coordination of ventilation and dorsoventral muscle activity

Our data indicate that DV muscle activity is coordinated with the ventilatory

rhythm both by reflexive responses to branchiostegal sinus pressure and by synaptic connections existing between the two systems in the thoracic ganglion.

Reflex coordination

Artificial manipulation of the pressure levels in the branchial chamber produces reflex responses in the activity of the DV muscles. These reflex responses of the DV muscles and of BST-mn1 during spontaneous or induced increases of branchial chamber pressure or branchiostegal sinus expansion lead us to propose the existence of baroreceptors. These receptors may feed back onto BST-mn1, regulating its activity. The likely site for these receptors is the branchiostegal membrane, which would be stretched during both manipulations. By analogy with the vertebrate baroreceptor reflex (Guyton, 1986), this reflex would serve to dampen or reduce blood pressure fluctuations following stimuli that would otherwise increase it. Similar compensatory mechanisms involving variation in heart rate in response to artificial increases in haemolymph pressure in the land crab *Cardisoma guanhumi* have been reported by Burggren *et al.* (1990). The burst of DV muscle impulses following a naturally occurring reversal may represent post-inhibitory rebound, since it is also observed in deafferented preparations (Fig. 13A,B).

Central coordination

The activity of DV muscles is phase-coupled with ventilatory beats and the rate is proportional to ventilatory frequency. This activity becomes tonic during ventilatory pauses and is inhibited during a ventilatory reversal. Each of these attributes could arise as reflexive responses to scaphognathite-induced branchial pressure waveforms or each could result from central coordination between the two systems.

In semi-isolated deafferented preparations, the phasic coupling of BST-mn1 to ventilatory bursts during forward bursting, the covariance in rates (Fig. 11A, B), the tonic firing during pauses in ventilatory bursts (Fig. 12) and the inhibition of BST-mn1 and the activation of BST-mn3 only during reversals (Fig. 13) demonstrate central, or hard-wired, coordination. An anatomical basis for this coupling is revealed by visualizing the central projections of the BST motoneurones. The dendritic fields of branchiostegal motoneurones occur in the region occupied by the dendritic arborizations of the ventilatory motoneurones (Simmers and Bush, 1983*a*) and the ventilatory non-spiking interneurones (DiCaprio, 1989). This is suggestive of interaction of the branchiostegal motoneurones with the ventilatory neuropile.

Electrophysiological recordings of the branchiostegal nerve account for only three of the five neurones filled by cobalt backfilling. The spikes from BST-mn1 are larger than the others and may arise from the largest neurone seen in cobalt backfills. The occurrence of contralateral efferent neurones indicates possible bilateral interaction. The possibility exists that the two cobalt-filled neurones not electrically active in reduced preparations may be neurosecretory or sensory in

nature, as some sensory neurones in crustaceans are known to have large-diameter axons and central somata (Laverack, 1987).

The ventilatory motoneurones are classified into two categories, the 'forward only' and 'reversal only' ventilatory motoneurones (Simmers and Bush, 1983*b*), because they are active exclusively during forward or reversed modes of ventilatory pumping. BST-mn1 corresponds to the forward only and BST-mn3 to the reversal only category of motoneurones, as they are active during forward and reversed modes of ventilation, respectively. The frequency of ventilatory motor bursts is known to be regulated by the frequency-modulating interneurones of the ventilatory CPG (DiCaprio and Fourtner, 1988). It appears that these frequency-modulating interneurones of the ventilatory CPG drive BST-mn1 during forward ventilation, since the frequency of BST-mn1 co-varies with ventilatory burst frequency. Similarly, the interneurone inducing motor pattern switch during reversal (DiCaprio, 1985; and personal communication) may participate in the BST-mn1 and BST-mn3 switch. The inhibition of BST-mn1 sometimes precedes ventilatory motoneurone switch by 2–3 ventilatory motor bursts and the inhibition may last longer than the reversal bout. This parallel switch is also seen when bouts of reversed ventilation occur during a ventilatory pause *in vitro* (Fig. 13B). These observations suggest that the interneurones of the ventilatory CPG, rather than the ventilatory motoneurones, influence the branchiostegal motoneurones.

The role of BST-mn3 is unclear. We speculate that it is an inhibitory neurone facilitating relaxation of the DV muscles during the inhibition of BST-mn1 associated with reversed ventilation. Attempts to confirm this by intracellular recording from DV muscle fibres while stimulating the branchiostegal nerve have been unsuccessful.

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