J. exp. Biol. 154, 517–535 (1990)
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AN INTERNEURONE MEDIATING MOTOR PROGRAMME SWITCHING IN THE VENTILATORY SYSTEM OF THE CRAB

BY RALPH A. DICAPRIO

Department of Zoological and Biomedical Sciences and College of Osteopathic Medicine, Ohio University, Athens, OH 45701, USA

Accepted 28 June 1990

Summary

The central pattern generator controlling ventilation in the crab can generate two distinct motor programmes, which determine the direction of water flow during irrigation of the gills. An interneurone has been identified that depolarizes when the ventilatory motor output switches from forward to reverse ventilation and remains depolarized for the duration of the reverse motor progamme. Depolarization of this neurone by intracellular current injection causes a switch in the motor programme from forward to reverse ventilation, which persists for the duration of the current step. Hyperpolarization of this cell during reverse ventilation terminates the reverse motor programme. The possible role of this reversal switch interneurone is considered in the context of the observed changes in the activity of other ventilatory interneurones and motor neurones during reverse ventilation.

Introduction

Much centrally patterned motor behaviour can be reorganized to produce different motor programmes that are expressed by a common set of motor elements. Examples of this reorganization are seen in the various forms of locomotor behaviour in the cat *Felis catus* (Miller *et al.* 1975), forward and backward walking in the lobster *Homarus americanus* (Ayers and Davis, 1977; Ayers and Clarac, 1978), ingestion and egestion in *Pleurobranchaea californica* (McClelland, 1982; Croll and Davis, 1981), swimmeret beating in the crayfish *Pacifastacus leniusculus* (Heitler, 1985), swimming and withdrawal in *Tritonia diomedea* (Getting and Dekin, 1985) and in the pyloric rhythms in Crustacea (Marder, 1984; Marder and Hooper, 1985). These different motor programmes may be produced by a separate central pattern generator (CPG) network dedicated to each behaviour, as in the swimmeret system (Heitler, 1985), or by a single neuronal network that is modified to generate distinct motor patterns. Getting and Dekin (1985) have elaborated the concept of a 'polymorphic network' where the reorganization of a single ensemble of neurones results in the

Key words: motor programme, central pattern generator, interneurone, ventilation, *Carcinus maenas*.

generation of different motor programmes. The neuronal network underlying gill ventilation in the crab *Carcinus maenas* is a possible example of such a network which generates distinct motor patterns controlling the gill bailer, resulting in the pumping of water in two different directions.

Gill ventilation in decapod crustaceans is produced by the rhythmic dorsoventral movements of the scaphognathite (SG) or gill bailer of the second maxilla. The beating of the SG pumps water through the branchial chamber and over the gills. Movement of the SG is controlled by five depressor muscles, which have been classified into two functional groups, D1 and D2, and five levator muscles, also classified into two groups, L1 and L2 (Young, 1975). Movement of the SG can pump water in either of two directions, corresponding to forward and reverse ventilation. In the forward mode of ventilation, water is drawn through the branchial chamber, entering at the base of the chelae (Milne-Edwards openings) and analogous openings at the base of the walking legs. Water passes through the branchial chambers and over the gills before leaving *via* anterior exhalant channels located under the antennae. Forward pumping is the prevalent mode in *Carcinus maenas* (L.) (Wilkens, 1976) and is characterized by the muscle recruitment sequence D1–D2–L1–L2. Reverse ventilation, in which water enters anteriorly and leaves at the base of the legs, results from a change in the recruitment sequence of the SG muscles to D2–D1–L2–L1.

The transition from forward to reverse ventilation occurs during a depressor burst and is characterized by the loss of a D2 motor neurone burst (often after an extended depressor D1 burst), which is then followed by an L2 motor burst (Young, 1975). A typical motor pattern during the forward-to-reverse transition would therefore be L1–L2–D1...L2–L1–D2–D1. The return to forward ventilation is again characterized by an extended depressor D1 burst which incorporates an additional D2 motor burst followed by a levator L1 burst. In addition, the motor neurones innervating the D2 and L2 muscle groups, which are active during forward ventilation, are silent during reverse ventilation. These muscle groups are driven during reverse ventilation by a distinct set of 'reversal' motor neurones, which only spike when the reverse motor pattern is expressed (Young, 1975; Simmers and Bush, 1983*b*). Reverse ventilation is also characterized by an increased ventilatory cycle frequency for the duration of the reverse motor programme. Fig. 1 illustrates a typical extracellular recording of the ventilatory motor output pattern during forward and reverse ventilation in an isolated ganglion preparation.

Reverse ventilation has been suggested to have a role in cleaning or flushing the gill chamber, in aeration of water trapped in the gill chamber when the animal is exposed to air, or in irrigation of the dorsal surfaces of the posterior gills, which are not served adequately by forward ventilation (Arudpragasam and Naylor, 1964*a*,*b*).

The motor patterns controlling the left and right SGs are produced by independent CPGs (Mendelson, 1971; Wilkens and Young, 1975). The CPG responsible for the production of ventilatory pumping in Crustacea was originally

thought to consist of a single endogenously oscillating neurone (Mendelson, 1971). The ventilatory CPG was subsequently shown to consist of at least two nonspiking interneurones (Simmers and Bush, 1980; DiCaprio and Fourtner, 1981) and recently at least eight nonspiking interneurones, which appear to constitute the basic interneuronal elements of the ventilatory CPG, have been described (DiCaprio, 1989).

This study describes the physiology and morphology of a reversal switch interneurone, RSi1, which depolarizes by 15–20 mV during spontaneously occurring periods of reverse ventilation. The finding that the depolarization of this cell by intracellular current injection elicits the reverse motor pattern indicates that this cell is, at least in part, responsible for motor programme switching in the ventilatory system.

A preliminary account of this work (DiCaprio, 1985) reported that there were three interneurones with similar morphologies that could be classified as reversal switch interneurones, and that two of these cells were a dye-coupled pair.

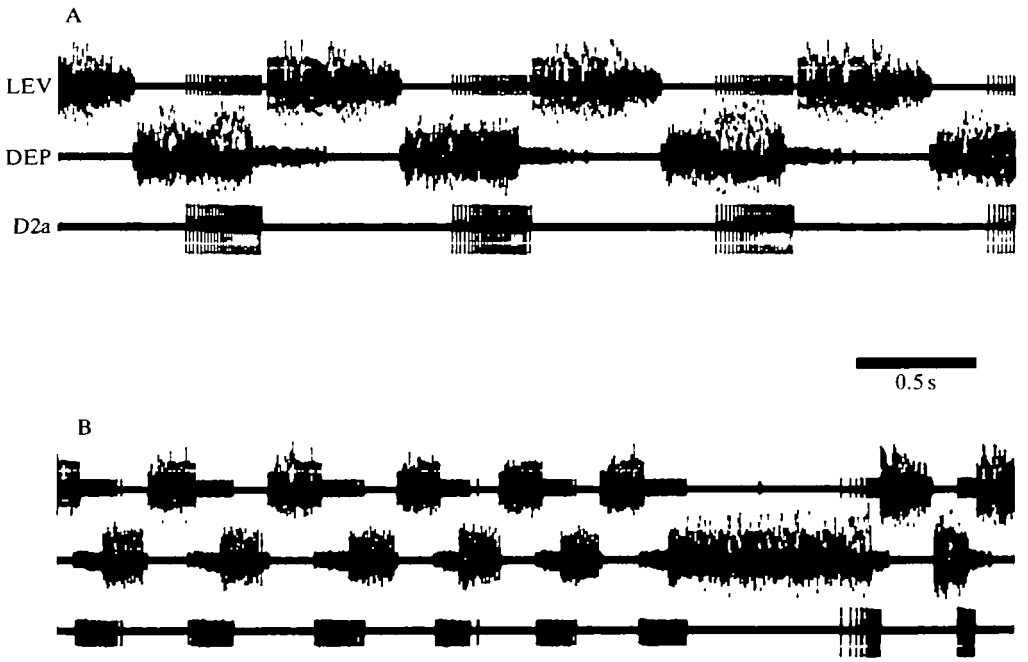

Fig. 1. Extracellular recording of the ventilatory motor output during forward (A) and reverse (B) ventilation. Note that the ventilatory motor output returns to the forward motor pattern at the end of record (B). Individual suction electrodes were placed on the levator (LEV) and depressor (DEP) motor nerves and on the branch of the levator which contains the motor axons innervating depressor muscle D2a (D2a). The levator electrode was placed close to the D2a branch point and therefore simultaneously recorded the activity of levator and D2a motor neurones. Note that the motor neurone active in D2a during forward ventilation is replaced by a different motor neurone during reverse ventilation.

Additional experiments have failed to confirm the existence of three different reversal switch interneurones. Based on morphological and physiological criteria, there is only one distinct, identifiable interneurone that can be classified as RSi. The previous conclusion that there were two dye-coupled neurones in this class of cells was based on the artefactual filling of a second neurone due to movement of the microelectrode during Lucifer Yellow ionophoresis into interneurone RSi1.

Materials and methods

Preparation

Male and female green shore crabs, *Carcinus maenas*, were used in all experiments. The animals were maintained for 2–10 weeks in artificial seawater aquaria at 10°C until use.

The isolated ganglion preparation used in this study has been described in detail elsewhere (Simmers and Bush, 1983*a*; DiCaprio and Fourtner, 1984). The walking legs and chelae were autotomized and the dorsal carapace, viscera and brain were removed. The thorax was pinned down in a Sylgard-lined dish containing oxygenated crab saline (Ripley *et al.* 1968). The sternal artery was immediately cannulated, allowing the perfusion of the thoracic ganglion with oxygenated saline at a rate of 2–3 ml min^{-1}. The nerves to both SGs were dissected from the SG musculature and all remaining nerves from the thoracic ganglion were severed. The thoracic ganglion was removed from the thorax and pinned dorsal surface upwards on the Sylgard base. The ganglionic sheath above the ventilatory neuropile was removed with fine forceps to facilitate intracellular recording. All experiments were performed at room temperature (21–22°C).

The isolated ganglion preparation used in this study spontaneously expresses the motor pattern corresponding to forward ventilation for periods of 6–8 h, over a typical frequency range of 45–100 cycles min^{-1}. Pauses in the ventilatory motor output and spontaneous bouts of reverse ventilation occurred infrequently in all experiments.

Recording procedures

Extracellular recordings of the ventilatory motor pattern from both left and right SG levator (LEV) and/or depressor (DEP) motor nerves were made with polyethylene suction electrodes (Fig. 1). In most experiments, only the levator nerve was monitored on the side contralateral to the intended site of intracellular recording. Recordings from the levator nerve were made proximal to or adjacent to the branch of this nerve that innervates depressor muscle D2a; this usually allowed recording of activity in axons innervating muscle D2a along with the levator motor neurone activity. A suction electrode was placed on the intact circumoesophageal connective ipsilateral to the intended site of intracellular recording, to permit stimulation of descending fibres that affect the ventilatory rhythm (Wilkens *et al.* 1974). No attempt was made to dissect the connective in order to stimulate discrete descending fibres.

Intracellular recordings from ventilatory interneurones were made in the ventilatory neuropile with microelectrodes pulled from 1.2 mm o.d. fibre-filled glass capillary tubing. The tips of all electrodes used in this study were filled with a 4% solution of the dye Lucifer Yellow (Stewart, 1978), and the shafts were filled with 1 mol l^{-1} lithium chloride. The Lucifer-Yellow-filled electrodes had resistances in the range 120–160 MΩ. Although these electrodes could usually pass currents in the range of ±5 nA, their nonlinear behaviour usually resulted in bridge imbalance while passing current and the subsequent inability to observe intracellular events while passing current. Intracellular potentials were amplified with a capacity-compensated bridge electrometer (WPI 707A or 767) and intracellular currents were measured with a virtual earth circuit (WPI IVA). All signals were recorded on an eight-channel instrumentation tape recorder (HP model 3968A) for later filming and analysis. Some of the figures were produced using digitized data (CED 1401 interface, 33 kHz $channel^{-1}$ sample rate) and printed on a laser printer (HP LaserJet II).

Lucifer Yellow was injected into neurones using a constant hyperpolarizing current of 3–5 nA or with current pulses of similar magnitude with a duration of 0.5 s at a frequency of 1 Hz for 5–10 min. Ganglia were fixed in 10% formalin, dehydrated in a graded ethanol series and cleared in methyl salicylate. Whole mounts of the ganglia were viewed and photographed with a fluorescence microscope. After first drawing and photographing a dye-filled cell from a dorsal aspect, the ganglion was sectioned to obtain transverse views of the cell. The section was then remounted, and the cell photographed and drawn. All drawings presented here were made from fixed material, and the cells were drawn as completely as possible. A faithful rendering of cellular morphology was often complicated by the background autofluorescence of the tissue and scattering of light due to the thickness of the tissue.

Results

All the intracellular recordings from RSi1 were made after penetrations of this neurone in the ventilatory neuropile. This cell was encountered in the course of experiments where over 400 penetrations of cells in the ventilatory system were made. RSi1 was identifiable in different preparations based on physiological and morphological criteria, and has been encountered seven times in separate experiments. In one instance, attenuated action potentials were observed in RSi1, and data from this experiment are presented here. All other penetrations of this cell indicate that other portions of this cell are functionally nonspiking, and an example from such an experiment is also presented.

Intracellular recordings from RSi1 during forward ventilation demonstrate a modulation of the membrane potential of this neurone in-phase with the ventilatory rhythm (Figs 2A,C, 3A). The amplitude of this oscillation was small, 3–4 mV peak-to-peak, and was hyperpolarizing with respect to the membrane potential observed during a ventilatory pause (Fig. 3A). Hyperpolarization of this

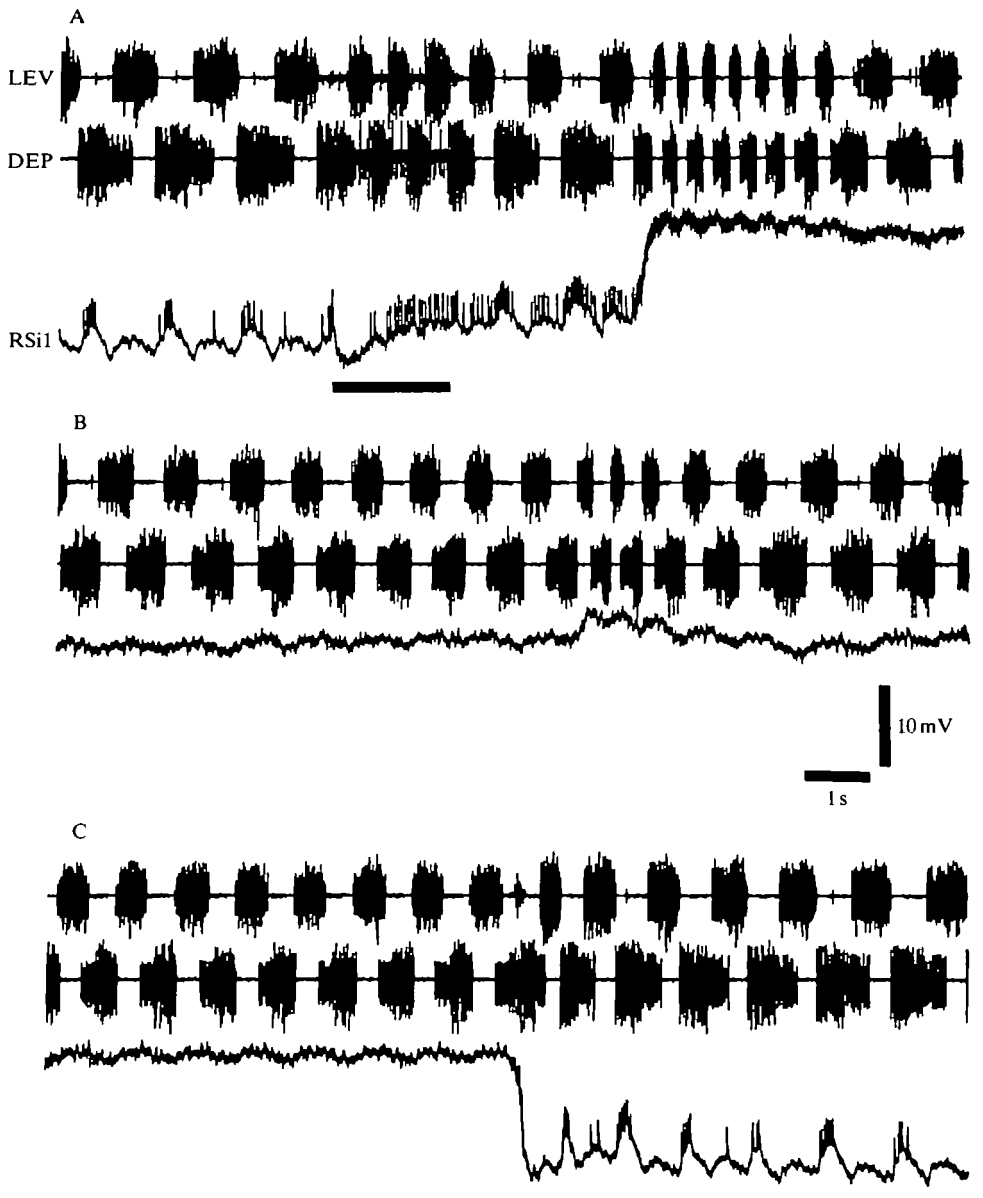

Fig. 2. An intracellular recording from interneurone RSi1 during forward and reverse ventilation. The spontaneous switch to reverse ventilation was apparently triggered by a brief period of stimulation of the ipsilateral connective (bar: 2 s duration, 150 μs pulses at 80 Hz). The membrane potential of RSi1 starts to depolarize during the depressor burst bridging the forward and reverse motor programmes (A). Note that there is an additional increase in the depolarization of RSi1 when the rate of reverse ventilation increases transiently for two ventilatory cycles (B). When the ventilatory rhythm returns spontaneously to forward ventilation, the membrane potential of RSi1 repolarizes (C) to the level observed in A. Note that D2a motor neurone activity is not present in the LEV trace owing to the placement of the recording electrode with respect to the D2a branch in this experiment. This is also the case in Figs 3 and 4.

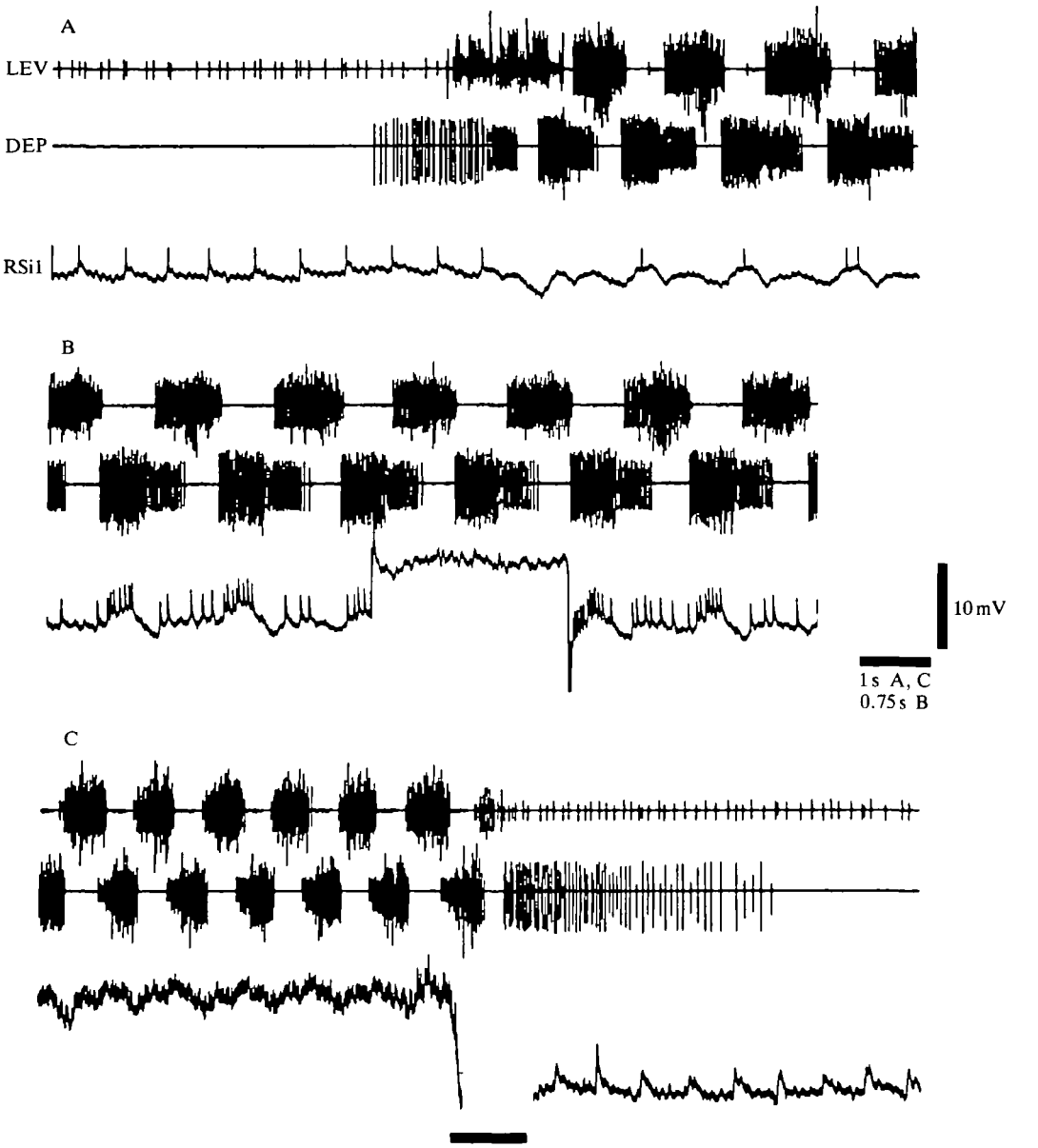

Fig. 3. (A) Low-frequency action potentials are generated in RSi1 during pauses in ventilation while no modulation of its membrane potential is present. When forward ventilation starts spontaneously, a cyclic hyperpolarizing drive to RSi1 is evident. The initial irregular 'activity' in the levator trace is due to the break-in voice channel which is shared with the data recording. (B) Injection of a hyperpolarizing current step (0.5 nA, bridge is unbalanced) into RSi1 inhibits the generation of action potentials and reduces the amplitude of the rhythmic modulation of the membrane potential. (C) A bout of reverse ventilation is correlated with a depolarization of RSi1 and is terminated by the injection of hyperpolarizing current (bar: 1.5 nA) into RSi1.

neurone by intracellular current injection decreased the amplitude of this oscillation (Fig. 3B), indicating that RSi1 is cyclically inhibited during forward ventilation. Attenuated action potentials (4–5 mV amplitude) occurred in short bursts on the relatively depolarized phase that occurred during each D1 motor neurone burst.

A depolarizing shift in the membrane potential of RSi1 was correlated with a switch to reverse ventilation (Fig. 2A). In this case, a short period of stimulation of the circumoesophageal connective preceded the switch from forward to reverse ventilation. The mean membrane potential of RSi1 depolarized by 16 mV during reverse ventilation, to an absolute level of −33 mV. The depolarization of RSi1 started at the beginning of the transitional depressor motor neurone burst and reached a maximum value at the end of this burst. The membrane potential remained at this depolarized level for the entire period of reverse ventilation. There was also a slight increase (2–3 mV) in the level of this depolarization during a transient increase in the rate of reverse ventilation (Fig. 2B). The membrane potential of RSi1 repolarized to the membrane potential characteristic of forward ventilation when the ventilatory motor programme returned to forward ventilation (Fig. 2C). The repolarization started at the mid-point of the depressor motor neurone burst which occurs between the reverse and forward ventilation motor patterns.

During a pause in ventilation, the resting membrane potential of this cell was −49 mV and regular tonic spiking occurred at a rate of 1.5 spikes s^{-1} (Fig. 3A). Depolarizing synaptic potentials were also observed during some pauses in ventilation (Figs 3A,C, 4C). The source of these potentials is unknown. Hyperpolarization of RSi1 with intracellular current inhibited the production of the action potentials (Fig. 3B), whereas spike frequency increased during depolarization of the cell (Fig. 4A; also Fig. 4B,C at the end of the injected current pulse) and on rebound from hyperpolarization (Fig. 3B). Hyperpolarizing current steps applied during forward ventilation had no discernible effect on the ventilatory motor output pattern or on the rate of ventilation (Fig. 3B), although injection of a short pulse of hyperpolarizing current into RSi1 during reversed ventilation could terminate the bout of reverse ventilation (Fig. 3C). In the trial shown here, the ventilation rhythm stopped after application of the current pulse. A pause in ventilation following a spontaneous bout of reverse ventilation is often observed in experiments using this preparation.

Injection of depolarizing current into RSi1 could repeatably elicit the reverse ventilation motor pattern. Fig. 4B shows the effect of a 2.5 nA depolarizing current pulse injected into RSi1 during forward ventilation. After a delay of approximately half the ventilatory period, the reverse motor programme started at the beginning of the next D1 motor neurone burst. This depressor burst lacks D2 motor neurone activity, which is characteristic of the switch to reverse ventilation. When the current step was terminated, the membrane potential of RSi1 remained depolarized during the transitional depressor burst, and repolarized when the ventilation motor output returned to forward ventilation.

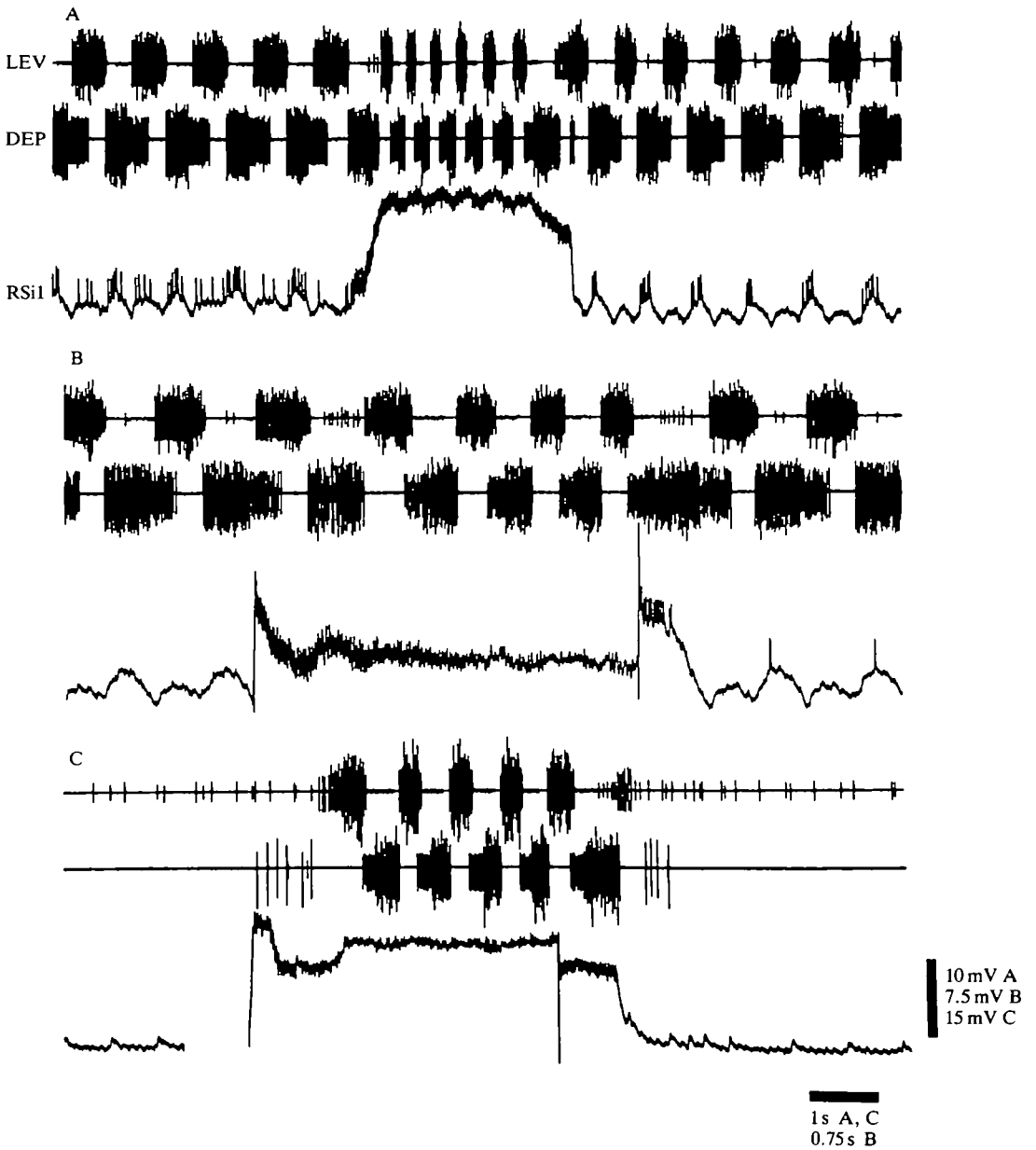

Fig. 4. Depolarization of RSi1 with intracellular current injection will elicit reverse ventilation. (A) A spontaneous switch to reverse ventilation during forward ventilation followed by a return to forward ventilation and (B) the injection of 2.5 nA of depolarizing current during forward ventilation initiates reverse ventilation which persists for the duration of the current step. (C) Depolarization of RSi1 with injected current (3 nA) during a pause in ventilation initiates reverse ventilation without an initial period of forward ventilation. Reverse ventilation ceases after the termination of the current step. The electrometer is unbalanced during the current steps in both B and C and the recording becomes unstable and noisy due to the nonlinear characteristics of the recording electrode during current injection.

Injection of depolarizing current into RSi1 during a pause in ventilation could also elicit reverse ventilation (Fig. 4C). Approximately 2.5 s after initiating a 3 nA depolarizing current step during a ventilatory pause, the reverse motor programme started without any intervening period of forward ventilation. When the current pulse was terminated, the membrane potential of RSi1 remained depolarized for an additional half-cycle of reverse ventilation, and repolarized when the ventilation motor output stopped. The latency to the start of reverse ventilation from the onset of the current pulse was variable when the pulse was applied during a pause in ventilation and always longer then when RSi1 was depolarized during forward ventilation. When RSi1 was depolarized during forward ventilation, the switch to reverse ventilation always occurred after a maximum of one ventilatory cycle had elapsed after the start of the current pulse. The shortest latencies occurred when the depolarization was initiated near the beginning of a forward depressor burst, thereby simulating the pattern of depolarization observed in RSi1 during spontaneous reversals.

RSi1 only depolarized when the motor pattern originating from the hemiganglion containing the interneurone switched from forward to reverse ventilation. There was also a small cyclic modulation of the membrane potential of RSi1 superimposed on the depolarization of this neurone during reverse ventilation (Figs 3C, 4A and 5A). The hyperpolarizing trough of this oscillation occurs in phase with the D1/L2 motor neurone bursts, but it was not possible to test the synaptic sense of this modulation because of the random occurrence and transient nature of bouts of reverse ventilation. No changes were observed in the membrane potential of RSi1 when only the contralateral motor pattern switched from forward to reverse ventilation. Depolarization of RSi1 using high levels of injected current (>6 nA) occasionally induced a switch in motor programme in the contralateral ventilatory CPG. The variability and long latency for this response (3–5 s) indicate that it is probably not due to a direct input from RSi1 to the contralateral CPG.

All other penetrations of interneurone RSi1 did not reveal the presence of attenuated action potentials as shown in the previous figures. In these experiments, a low-amplitude membrane potential oscillation in-phase with the forward ventilatory motor programme was present, the cell depolarized when the motor programme switched to reverse ventilation (Fig. 5A) and injection of 2–4 nA of depolarizing current elicited reverse ventilation (Fig. 5B). The membrane potential of RSi1 in this experiment was −56 mV during a pause in ventilation and ranged from −48 to −56 mV in the seven penetrations of this cell in different preparations. Note that in the experiment shown in Fig. 5, the oscillation in membrane potential during forward ventilation is not identical to the modulation observed in the previous figures. This difference is probably due to the relatively more hyperpolarized resting potential of RSi1 in this experiment. It is also possible that this difference is a manifestation of localized inputs to RSi1, given the large distance between penetration sites noted below. In some cases, what appeared to be small postsynaptic potentials or extremely attenuated action potentials of

approximately 1 mV amplitude were observed in RSi1 and the frequency of occurrence of these potentials increased during the depolarization associated with reverse ventilation (Fig. 5A). The morphology of all these neurones was essentially identical to the cell shown in Fig. 6, that is the soma was in the same position relative to external features of the ganglion, the largest diameter neurite was positioned laterally and oriented dorso-ventrally in the neuropile, there were three groups of processes extending towards the midline in different dorso-ventral planes, and the longest of these process projected posteriorly and ventrally.

These findings indicate that RSi1 possesses a spike-initiating region, possibly associated with one of the branches projecting towards the midline of the ganglion, and that the data presented in Figs 2–4 represent a fortuitous penetration of RSi1 close to a spike-initiating region. The recordings from RSi1 shown in Figs 2–4 were made from a relatively medial penetration of the cell from a dorsal approach

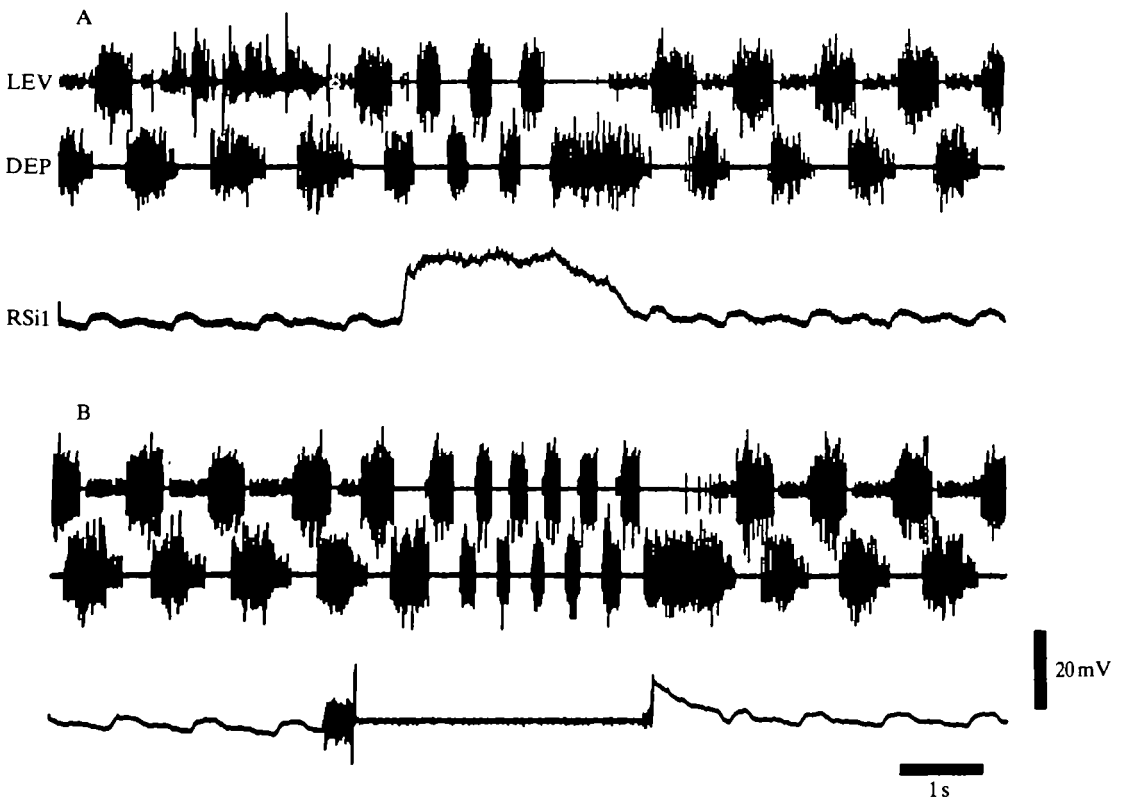

Fig. 5. Intracellular recording from RSi1 in a different preparation from that shown in Figs 2–4. (A) No action potentials were evident in this penetration of RSi1 during forward ventilation and during a spontaneous switch to reverse ventilation. The irregular 'activity' early in the levator trace is due to the break-in voice channel which is shared with the data recording. (B) Injection of depolarizing current (3 nA, bridge is unbalanced and tape recorder amplifier is saturated) into this cell elicits a period of reverse ventilation which persists for the duration of current step.

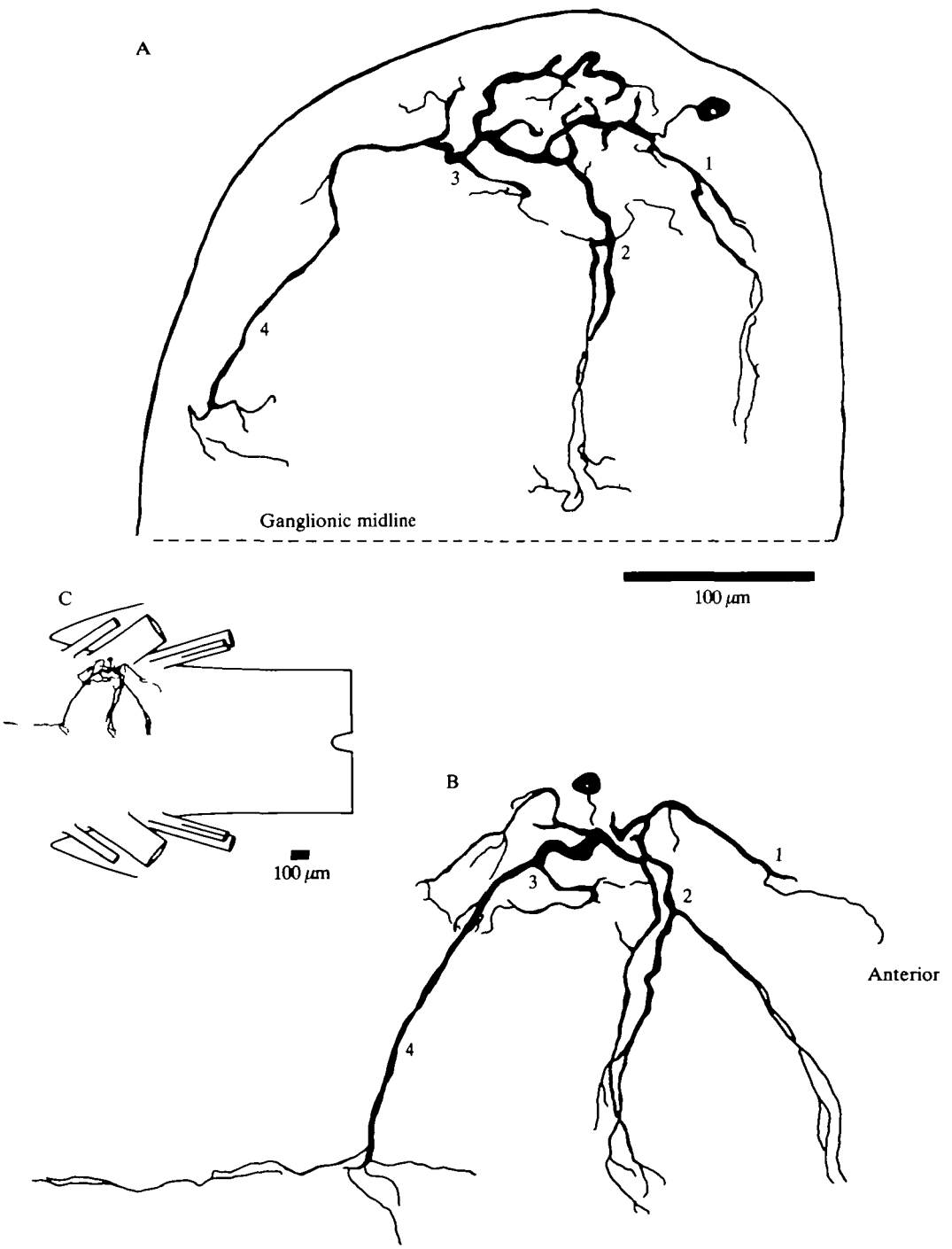

Fig. 6

Fig. 6. Structure of interneurone RSi1. The neurone is shown from the frontal (A) and dorsal (B) aspects. The inset (C) is a low-power dorsal view with the anterior portion of the thoracic ganglion shown in outline. The frontal view is presented looking from posterior to anterior with the dotted line indicating the ganglionic midline, and the dorsal view is oriented with anterior to the right of the figure. The numbers in A and B indicate corresponding points in the cell.

(Fig. 6B, medial to points 2 and 3), whereas all other penetrations were made in the large-diameter neurite of this cell near the lateral margin of the ventilatory neuropile (Fig. 6A, in the area bounded by points 2 and 3 and the lateral edge of the ganglion) from a lateral approach. This lateral neurite, therefore, does not appear to be able to generate action potentials and was never observed to do so during forward or reverse ventilation, on initial penetration of the cell, on rebound from hyperpolarization, or during the injection of depolarizing current into this neurone.

A phase–response curve (Fig. 7) demonstrates the ability of brief intracellular current pulses injected into RSi1 to reset the ventilatory rhythm at different phases of the ventilatory cycle. Hyperpolarizing pulses applied at any time produced no phase shift in the motor output, while depolarizing pulses produced a maximum

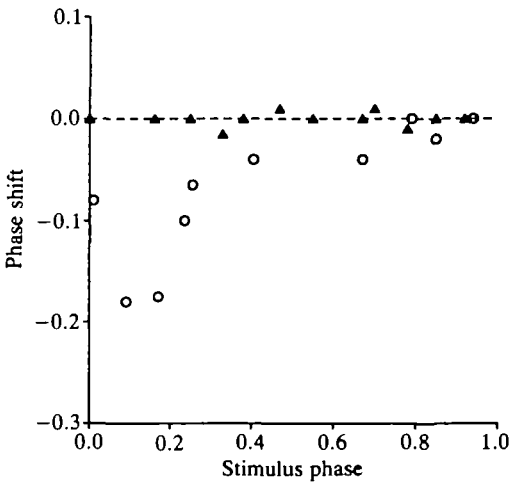

Fig. 7. Phase–response curve indicating the phase shift in the ventilatory output after the injection of hyperpolarizing (▲) and depolarizing (○) current pulses (150 ms duration, 3 nA amplitude) into RSi1. The start of the ventilatory cycle was arbitrarily taken to be the start of the depressor motor burst. The phase shift in the motor output pattern was calculated from the formula:

$$\text{phase shift} = (\bar{t}_b - t_s)/\bar{t}_b,$$

where $\bar{t}_b$ is the mean duration of the ventilatory cycle before the stimulus pulse (N=2–6, where N is the number of ventilatory cycles averaged) and t_s is the duration of the ventilatory cycle when the stimulus pulse was applied. Positive values of phase change therefore denote a phase advance and negative values a phase delay of the ventilatory motor pattern.

phase delay of 0.2 cycles when applied immediately after the start of the depressor burst. The amount of phase delay decreased as the stimulus phase increased, until pulses injected during the levator burst had little or no effect on forward ventilation. This phase-dependent ability of depolarizing current to reset forward ventilation indicates that RSi1 has access to the ventilatory CPG during specific parts of the ventilatory cycle only. The inability of hyperpolarizing pulses or current steps (Fig. 3B) to perturb or alter the ventilatory motor output indicates that RSi1 is not involved in the production or maintenance of the forward ventilation motor pattern.

The structure of RSi1 is shown from both dorsal and frontal aspects in Fig. 6. The cell soma is located along the dorsolateral margin of the ventilatory neuropile. The largest (lateral) neurite ranges from 6 to 15 μm in diameter and is positioned centrally along the dorsoventral extent of the ventilatory neuropile at its lateral margin. A large secondary process leaves this neurite to run ventrally and posteriorly towards the midline of the ganglion. Two additional groups of finer processes branch from the lateral neurite dorsally and extend to the midline. None of these branches was ever observed to cross the midline of the ganglion.

Discussion

A single, identifiable interneurone has been described which is at least partly responsible for the temporal reorganization of the components of the ventilatory CPG to produce two distinct motor programmes. During a spontaneous switch to reverse ventilation, RSi1 starts to depolarize at the beginning of the D1 depressor burst, which precedes the forward-to-reverse switch, and reaches its maximum depolarized level at the end of this transitional burst. In addition, when the switch to reverse ventilation occurred less abruptly than shown in Figs 2–5, the rate of depolarization of RSi1 was observed to decrease as the duration of the transitional depressor motor neurone burst increased. Depolarization of RSi1 with intracellular current injection elicited a period of reverse ventilation which persisted for the duration of the current step. When the amplitude of the injected current was greater than 3 nA, the reverse motor programme was evoked in all trials. Lower levels of injected current did not reliably elicit reverse ventilation, but did decrease the rate of the forward ventilatory rhythm. The latency to the start of reverse ventilation after the start of the depolarizing pulse was variable, and was apparently dependent on the phase of the ventilatory cycle at which the current step started. The shortest latencies were observed when the depolarization was initiated at the beginning of the depressor burst, in which case it induced the reverse motor programme during the same cycle. In contrast, when the depolarizing current was applied during a levator burst, the forward ventilation programme continued until the next depressor burst before reverse ventilation began.

A phase–response curve (Fig. 7) demonstrates the ability of brief depolarizing current pulses injected into RSi1 to reset the ventilatory rhythm, whereas hyperpolarizing pulses applied at any time produced no phase shift in the motor

output. The inability of hyperpolarizing current pulses to alter the ventilatory motor output suggests that RSi1 is not a necessary component of the ventilatory CPG during forward ventilation. The phase-dependent ability of depolarizing current pulses injected into RSi1 to reset ventilation demonstrates that RSi1 has access to the ventilatory CPG only during a specific portion of the ventilatory cycle. The point of maximum phase shift produced by perturbation of RSi1 also occurs near the phase of the ventilatory cycle where RSi1 normally depolarizes during a spontaneous switch to reverse ventilation. This correspondence between the timing of the maximum phase shift produced by this interneurone and the transition point for the switch from forward to reverse ventilation is presumably a property of the circuitry of the ventilatory CPG.

The stereotyped transition between motor programmes may reflect an optimum motor pattern for the efficient operation of the ventilatory pump. The forward-to-reverse transition occurs between the depressor and levator bursts, which is at the minimum point of negative pressure generation in the pumping chamber (Wilkens and Young, 1975). The motor programme switch therefore occurs at a point of nearly equal pressure between the pumping chamber and the branchial chamber. Motor programme switching at this phase of the ventilatory cycle would minimize the backwash of fluid into the pumping chamber due to a pressure pulse, which could cause a mechanical perturbation of the SG blade.

The depolarization of RSi1 during reverse ventilation does not appear to be due to a plateau potential (Russell and Hartline, 1978, 1982) in this neurone. Depolarization of RSi1 during pauses in ventilation with currents sufficient to initiate the motor programme switch in a rhythmically active preparation did not elicit a plateau response in RSi1. Since tonic hyperpolarization of RSi1 from other ventilatory neurones could possibly inhibit a plateau response, depolarizing pulses were also applied when superimposed on a tonic injection of low-amplitude depolarizing current. Depolarizing pulses applied under these conditions also failed to trigger a plateau potential in RSi1.

The possible functional role of interneurone RSi1 in ventilatory motor programme switching may be addressed by considering the changes observed in the activity of other ventilatory neurones during the switch from forward to reverse ventilation. In particular, some of the members of two classes of ventilatory interneurones exhibit marked changes in their membrane potential which are correlated with the switch between the two modes of ventilation.

The frequency-modulating interneurones are able to control the frequency of the ventilatory rhythm in a graded manner (DiCaprio and Fourtner, 1988). During a spontaneous switch to reverse ventilation, the membrane potential of one of these interneurones, FMi2, hyperpolarizes when the motor output switches to reverse ventilation (DiCaprio and Fourtner, 1988, Fig. 7).

Eight nonspiking interneurones have been identified and classified as central pattern generator interneurones (DiCaprio, 1989). The membrane potential of each of these interneurones oscillates in phase with the motor output during forward and reverse ventilation, and the amplitude of the oscillation in six of the

eight cells is essentially identical during forward and reverse ventilation. However, the amplitude of the membrane potential oscillation in CPGi1 increases while that of CPGi5 decreases when the ventilatory rhythm switches from the forward to the reverse motor programme (DiCaprio, 1989, Figs 25 and 29).

In addition, the levator L2 and depressor D2 muscles are activated by two separate populations of motor neurones during forward and reverse ventilation (Young, 1975; Simmers and Bush, 1983*b*). Simmers and Bush (1983*a*,*b*) have described the intracellular activity of ventilatory motor neurones in forward and reverse ventilation and have concluded that the ventilatory motor neurones are primarily driven by chemically mediated cyclic inhibitory inputs during ventilation.

What are the possible interactions between RSi1 and these elements of the ventilatory system and their modes of action? The most obvious difference between forward and reverse ventilation is the inhibition of L2 and D2 motor neurones, which are active during forward ventilation, and their replacement by a distinct set of motor neurones which drive these muscle groups during reverse ventilation. Reversal motor neurones are driven by a cyclic inhibition which is subthreshold for the generation of action potentials during forward ventilation (Simmers and Bush, 1983*b*). Depolarization of a reversal motor neurone with intracellular current during forward ventilation results in these neurones firing in bursts. These bursts occur at the phase of the ventilatory cycle which is appropriate for reverse ventilation, i.e. an L2 reversal motor neurone will fire before the L1 burst when depolarized during forward ventilation.

A tonic excitation of the population of reversal motor neurones by RSi1 could, therefore, be responsible for the activation of these cells during reverse ventilation. For example, excitation of reversal motor neurones by an electrical synaptic connection from RSi1 would be consistent with the finding that the reversal potential of the cyclic drive to reversal motor neurones is more negative than their membrane potential oscillation during both forward and reverse ventilation (Simmers and Bush, 1983*a*,*b*). A tonic excitation of reversal motor neurones produced by a chemical synaptic input would not be expected to produce a hyperpolarized reversal potential during the reverse motor programme.

In a similar manner, it is proposed that forward motor neurones are inhibited by RSi1 during reverse ventilation *via* a chemical synaptic connection. These motor neurones are driven by cyclic inhibition during forward ventilation and the reversal potential for the membrane potential oscillation in forward motor neurones during forward and reverse ventilation is also hyperpolarized with respect to this oscillation (Simmers and Bush, 1983*a*,*b*). Chemically mediated inhibition of forward motor neurones during reverse ventilation would, therefore, be consistent with these observations.

The changes in membrane potential of CPGi1, CPGi5 and FMi2 observed during reverse ventilation could also be mediated by appropriate synaptic inputs from RSi1. Interneurone CPGi1 oscillates in-phase with ventilation and this oscillation is the result of a cyclic inhibition during forward ventilation (DiCaprio,

1989). The depolarization of CPGi1 by an electrical connection from RSi1 could, therefore, produce the increase in oscillation amplitude seen during reverse ventilation. Likewise, the decreased amplitude of the membrane potential oscillation of interneurone CPGi5 and the tonic hyperpolarization of interneurone FMi2 during reverse ventilation could be due to a chemically mediated inhibition of these neurones by RSi1.

The confirmation of the interactions proposed above must await a direct assessment using paired intracellular recordings from RSi1 and other ventilatory neurones. The structure of RSi1 is appropriate for these proposed connections, as it is located in the ventilatory neuropile along with the CPG interneurones and the dendritic fields of the ventilatory motor neurones. In addition, the anterior branches of RSi1 are close to the area where the FM interneurones span the width of the thoracic ganglion. The branch of RSi1 projecting posteriorly could also provide a possible pathway for the correlation between the onset of reverse ventilation and tachycardia (Cumberlidge and Uglow, 1977; Taylor *et al.* 1973), as the cardiac accelerator and inhibitor neurones lie posterior to the ventilatory neuropile.

Two lines of evidence argue that whereas RSi1 may be a primary component of the switching mechanism, and can obviously elicit the complete reverse motor programme, changes in other ventilatory neurones and interactions between these elements are independent components of the normal physiological switch between motor patterns. First, during spontaneous episodes of reverse ventilation, the rate of ventilation is characteristically higher than the rate in the preceding period of forward ventilation. In the same preparation, when RSi1 was depolarized by current injection, the rate of reverse ventilation was not always as high as that observed during spontaneous switches. For example, in Fig. 4, a spontaneous bout of reverse ventilation occurred at 150 cycles min^{-1}, while depolarization of RSi1 evoked reverse ventilation at 75 cycles min^{-1}.

Second, in preliminary experiments, axons have been penetrated in the circumoesophageal connective that spike when the connective is stimulated and which will elicit a switch to reverse ventilation when depolarized by current injection. These axons were found to arborize and terminate in the ventilatory neuropile. The depolarization of such a neurone immediately evoked reverse ventilation, whether applied during pauses in ventilation or during forward ventilation, without the delay associated with the intracellular injection of depolarizing current into RSi1. In addition, the latency from the injection of depolarizing current into RSi1 to the initiation of reverse ventilation was always greater in a quiescent preparation than when the same current was applied during forward ventilation.

These observations indicate that RSi1 is only one element of the neuronal circuitry responsible for reverse ventilation. Descending control to the ventilatory system may involve distributed inputs to FM interneurone(s) and/or CPG interneurones, in addition to RSi1. There is no direct evidence for inputs from descending ventilatory 'command' neurones (Wilkens *et al.* 1974) to RSi1, as

postsynaptic potentials were not observed in RSi during stimulation of the connective. The stimulation of the entire intact connective, however, was never an adequate or an effective stimulus for motor programme switching in these experiments. Paired intracellular recordings from RSi1 and descending neurones or more specific stimulation of small bundles of axons in the connective will be required to investigate this possibility further. The hypothesis that RSi1 is an essential element of the switching mechanism has not been established in this study, but could possibly be determined by the photoinactivation (Miller and Selverston, 1979) of RSi1 after injection of Lucifer Yellow into this cell.

I would like to thank Dr C. R. Fourtner for comments on an initial draft of this work, which was supported by grants from the National Institutes of Health (NS25002), the Whitehall Foundation and the Ohio University College of Osteopathic Medicine.

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