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COORDINATION OF LOCOMOTOR AND CARDIORESPIRATORY NETWORKS OF *LYMNAEA STAGNALIS* BY A PAIR OF IDENTIFIED INTERNEURONES

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Summary

1. The morphology and electrophysiology of a newly identified bilateral pair of interneurones in the central nervous system of the pulmonate pond snail *Lymnaea stagnalis* is described.

2. These interneurones, identified as left and right pedal dorsal 11 (L/RPeD11), are electrically coupled to each other as well as to a large number of foot and body wall motoneurones, forming a fast-acting neural network which coordinates the activities of foot and body wall muscles.

3. The left and right sides of the body wall of *Lymnaea* are innervated by left and right cerebral A cluster neurones. Although these motoneurones have only ipsilateral projections, they are indirectly electrically coupled to their contralateral homologues *via* their connections with L/RPeD11. Similarly, the activities of left and right pedal G cluster neurones, which are known to be involved in locomotion, are also coordinated by L/RPeD11.

4. Selective ablation of both neurones PeD11 results in the loss of coordination between the bilateral cerebral A clusters.

5. Interneurones L/RPeD11 are multifunctional. In addition to coordinating motoneuronal activity, they make chemical excitatory connections with heart motoneurones. They also synapse upon respiratory motoneurones, hyperpolarizing those involved in pneumostome opening (expiration) and depolarizing those involved in pneumostome closure (inspiration).

6. An identified respiratory interneurone involved in pneumostome closure (visceral dorsal 4) inhibits L/RPeD11 together with all their electrically coupled follower cells.

7. Both L/RPeD11 have strong excitatory effects on another pair of electrically coupled neurones, visceral dorsal 1 and right parietal dorsal 2, which have previously been shown to be sensitive to changes in the partial pressure of environmental oxygen (P_{O_2}).

8. Although L/RPeD11 participate in whole-body withdrawal responses, electrical

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stimulation applied directly to these neurones was not sufficient to induce this behaviour.

Introduction

Many of the complex types of behaviour exhibited by animals are a result of coordination between two or more motor centres of the central nervous system. Numerous examples are available where such coordination has been demonstrated in both vertebrates (Viala, 1986; Feldman and Ellenberger, 1988; Cohen, 1987) and invertebrates (Hughes and Wiersma, 1960; Ikeda and Wiersma, 1964; MacMillan *et al.* 1983). However, few studies have uncovered the circuitry underlying this coordination, and even fewer have identified specific coordinating neurones (see Bush and Clarac, 1985). Many researchers are currently interested in determining the degree and importance of neural integration between various aspects of behaviours. Some of the questions that need to be answered in this area concern the possible existence of multifunctional neurones (Delcomyn, 1987; Ritzmann *et al.* 1980; Atwood and Wiersma, 1967; Bernard *et al.* 1989; Hooper and Moulins, 1989). For example, do those neurones that coordinate various neural elements of a given behaviour also play an integral role in coordinating seemingly unrelated behaviours, or are coordinating neurones specified for each behaviour? To answer such questions, many researchers have turned to examining the behaviours exhibited by invertebrates, whose 'simple' nervous systems are often very amenable to neurophysiological studies.

In recent years, the freshwater pulmonate snail *Lymnaea stagnalis* (L.) has become a popular subject for studies of such simple behaviour patterns as feeding (Egelhaaf and Benjamin, 1983; Elliott and Benjamin, 1985; McCrohan, 1984; Kyriakides and McCrohan, 1988), reproduction (see Boer *et al.* 1987), whole-body withdrawal (Ferguson, 1984; Benjamin *et al.* 1985), locomotion (Syed, 1988; Syed *et al.* 1988) and respiration (van der Wilt *et al.* 1988; Syed *et al.* 1990). Although in most of these studies the pertinent effector organs and central neurones involved in the specific behaviour have been identified, very little is known regarding the coordination (e.g. spatiotemporal relationships) between these various behaviours, an exception being the work of Kyriakides and McCrohan (1988). The functional integration of the behaviourial repertoire of an animal is a determining factor in survival, particularly in the face of environmental stress. For example, if *Lymnaea* is subjected to a noxious stimulus during normal activity, behaviour such as respiration or locomotion is terminated immediately and the animal withdraws its entire head–foot complex into the shell. This fixed action pattern is called whole-body withdrawal (Ferguson, 1984). To satisfy its respiratory needs, however, a pulmonate snail must expose its pneumostome (respiratory orifice) to the air. In *Lymnaea*, pneumostome opening and closing movements can only be achieved if the body is extended out of the shell, i.e. a behaviour that is in opposition to withdrawal. During respiration, locomotor behaviour is also suppressed. Furthermore, analysis of locomotion in *Lymnaea* has revealed that

well-coordinated movements of the left and right body wall and foot musculature occur in the absence of whole-body withdrawal or respiratory activity (Syed, 1988). Thus, a significant amount of coordination must exist among these three seemingly distinct behaviours. Here we present evidence that a pair of bilateral, electrically coupled interneurones coordinate the activities of locomotor and whole-body withdrawal motoneurones in *Lymnaea*. Furthermore, these two interneurones, identified as left and right pedal dorsal 11 (L/RPeD11), make chemical connections with heart motoneurones (Benjamin *et al.* 1988) and with neurones that participate in respiratory behaviour (Syed and Winlow, 1988*a*,*b*; N. I. Syed, D. Harrison and W. Winlow, in preparation).

Originally, the left and right body wall motoneurones of *Lymnaea*, identified as the left and right cerebral A cluster neurones (L/RCeA cluster), were found to be electrically coupled with their contralateral as well as their ipsilateral homologues (Haydon, 1982; Ferguson, 1984). However, the basis for the coupling between contralateral clusters was puzzling since CeA neurones have only ipsilateral projections. Here we present data demonstrating that L/RPeD11 coordinate the activities of L/RCeA cluster neurones *via* electrotonic coupling. The electrical coupling between LCeA cluster and RCeA cluster neurones was lost after selective ablation of L/RPeD11. However, the L/RCeA cluster neurones remained coupled with their ipsilateral (i.e. neighbouring) cells. We also provide evidence that although L/RPeD11 participate in whole-body withdrawal behaviour, they do not induce this behaviour when stimulated electrically. Furthermore, we demonstrate that the interneurone visceral dorsal 4 (VD4, Janse *et al.* 1985) (=VWI of Benjamin, 1984), which controls the inspiratory phase of the respiratory behaviour (Syed and Winlow, 1988*b*; Syed *et al.* 1990), has inhibitory effects on foot and body wall motoneurones as well as on L/RPeD11. These studies therefore suggest that L/RPeD11 coordinate the activities of locomotor motoneurones which innervate the left and right sides of the body, and that they are also multifunctional interneurones, acting on neuronal networks that control the cardiorespiratory system of *Lymnaea*.

Materials and methods

Specimens of *Lymnaea stagnalis* (L.) were usually obtained from animal suppliers and occasionally collected from the Leeds–Liverpool canal. These snails were maintained at 10–16 °C in aerated pond water obtained locally and fed on lettuce, supplemented with tropical fish food. All experiments were performed on 1–4 g snails bathed in standard snail saline (Benjamin and Winlow, 1981). Isolated brains of *Lymnaea* were prepared for electrophysiological and morphological studies and maintained in snail saline buffered to pH 7.8–7.9 as previously described (Benjamin and Winlow, 1981). Several modified salines were also used. For zero-Ca^{2+}/high-Mg^{2+} saline, calcium in the normal saline was replaced by magnesium, the latter being raised from 2 to 6 mmol l^{-1}. For high-Ca^{2+}/high-Mg^{2+} saline, concentrations of both divalent cations were raised sixfold: Ca^{2+} to

24 mmol l^{-1}, Mg^{2+} to 12 mmol l^{-1}. Individual neurones were impaled with glass microelectrodes (10–20 MΩ) and recordings of neuronal activity were obtained using conventional techniques. Electrophysiological signals were amplified, displayed and recorded by conventional means (Benjamin and Winlow, 1981).

Lucifer Yellow (CH) stains of the neurones were prepared according to the methods of Syed and Winlow (1989). Briefly, microelectrodes were filled with 10 % Lucifer Yellow dissolved in double distilled and deionized water. These dye-filled electrodes had a tip resistance of 20–80 MΩ. Prior to the impalement and withdrawal of electrodes from the neurones a constant holding current of +2 nA was applied to prevent the leakage of the dye from the tip of the electrode. Upon successful penetration of the cells, this current was switched off. The Lucifer Yellow was then injected into the soma by applying a constant −2 nA current for 10–20 min. These preparations were left overnight to allow the spread of dye and were fixed in buffered formalin (formaldehyde, 4 % in 0.1 mol l^{-1} sodium phosphate buffer to pH 7.4) for 3 h. The fixed brains were dehydrated using a series of ascending concentrations of ethanol, then defatted and cleared by incubation in dimethyl sulphoxide and methyl salicylate. Cleared tissues were mounted in FluoroSave (Calbiochem) on depression slides and observed using a Leitz Dialux 20EB incident fluorescence microscope. Successfully injected neurones were photographed using 400 ASA Kodak Ektachrome slide film and drawn using a *camera lucida*.

The effects of sensory inputs on the interneurones were tested using a newly developed semi-intact preparation (Syed, 1988; N. I. Syed, D. Harrison and W. Winlow, in preparation), as were the effects of the interneurones on whole-body withdrawal behaviour. Briefly, animals were anaesthetized by bubbling 2 % halothane into the pond water as described by Girdlestone (1986). Anaesthetized animals were transferred to a chamber designed for these experiments (Syed, 1988). Fine nickel wires were attached to the animals at various points using tissue glue (cyanoacrylate adhesive). All these wires were extended and attached to the wall of the chamber by Plasticine, allowing the animal to be suspended in the saline in a manner similar to the *Tritonia* preparation described by Willows *et al.* (1973). Once firmly suspended, a small mid-dorsal body incision was made and the body wall was gently retracted with blunt hooks attached to the nickel wires. The brain was lifted by inserting a wax-covered spatula held on a micromanipulator (Syed, 1988). One end of a cotton thread was attached to the tension transducer and the other to the foot or body wall musculature of the animal. Upon completion of surgery, the animals were allowed to recover from anaesthesia. After several washes in normal saline, intracellular recordings were made from central neurones and muscle tension was recorded using tension transducers.

Selective photoinactivation of neurones was carried out using previously developed methods (Miller and Selverston, 1979; Bulloch and Kater, 1982; Bulloch *et al.* 1984; Elliott and Kleindienst, 1990). Briefly, the cells were first filled with 10 % Lucifer Yellow and then, using a portable HBO 100 W d.c. mercury arc lamp and a light guide, exposed to a beam of high-intensity blue light. We found

that these Lucifer-Yellow-filled neurones are killed within seconds if they are injected with depolarizing current during the period of blue light exposure. The soma and its main processes began to disintegrate, resting membrane potential was lost and cells became inexcitable (see Results).

Results

Location and morphology of interneurones L/RPeD11

The paired interneurones L/RPeD11 are located on the dorsal surface of the left and right pedal ganglia, respectively. These neurones are whitish-orange in colour, have soma diameters of 30–50 μm, and are situated at the pedal end of the pedal–pleural connectives, anterior to the statocyst (Fig. 1). They are the largest cells located within the pedal G clusters (Slade *et al.* 1981). Ionophoretic injections of Lucifer Yellow into neurones L/RPeD11 reveal that they have similar morphologies and that together their processes encircle the lower ganglionic ring (i.e. pedal, pleural, parietal and visceral ganglia) and enter the cerebral ganglia (Fig. 2). Neurone LPeD11 has extensive neuropilar arborizations in all the above ganglia and has two main axons, one running ipsilaterally and the other contralaterally (Fig. 2A). Simultaneous injection of Lucifer Yellow into both left and right PeD11 (Fig. 2B) revealed that, although these neurones do not have main axons in the superior, median or inferior pedal nerves (see Slade *et al.* 1981), a small axonal branch of each cell does project towards (but does not enter) the superior pedal nerve.

Synaptic connections of L/RPeD11

Electrical connections between interneurones L/RPeD11 and locomotor and body wall motoneurones

Motoneurones to the foot lie within the pedal G clusters (L/RPeG cluster) (McCrohan and Winlow, 1985; Winlow and Haydon, 1986; Haydon and Winlow, 1986). Cerebral A cluster (CeA) neurones innervate the body wall musculature (Haydon, 1982) and are termed whole-body withdrawal motoneurones (Ferguson, 1984; Benjamin *et al.* 1985). The CeA cluster neurones are a group of 20–25 orange-coloured cells (30–40 μm soma diameter) located in each cerebral ganglion (Fig. 1). They have axons in the (ipsilateral) superior and inferior cervical nerves of the pedal ganglia and extensive branches in ipsilateral cerebral, pleural and pedal ganglia (Fig. 3A.B).

Functional electrical connections are present between neurones L/RPeD11 and PeG clusters (Fig. 4A,B). Most ipsilateral CeA cluster neurones are known to be electrically coupled to each other (Haydon, 1982; Ferguson, 1984; Benjamin *et al.* 1985). Furthermore, L/RCeA cluster neurones are also known to be electrically coupled to their contralateral homologues (Ferguson, 1984). We found that interneurones L/RPeD11 were also electrically coupled to L/RCeA cluster neurones (Fig. 4C). In the present study, to rule out the possibility of chemical

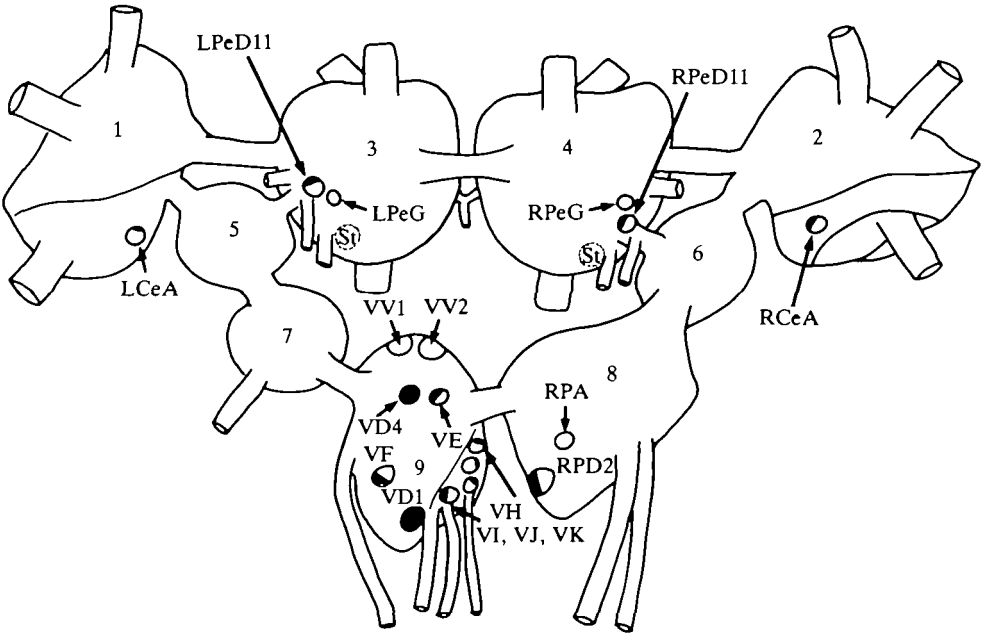


Fig. 1. Diagrammatic representation of the central ring ganglia of *Lymnaea stagnalis* showing the location of neurones examined in the present study. Individually identifiable neurones are numbered (e.g. L/RPeD11, VD1, VD4, etc.), while identifiable neuronal clusters are given a letter (e.g. L/RCeA; VH, I, J, K cells, etc.) according to the convention of Slade *et al.* (1981) and Kyriakides *et al.* (1989). The boundaries of the pedal clusters are given by Kyriakides *et al.* (1989), whereas the boundaries of the cerebral A cluster neurones are shown by lines drawn across the cerebral ganglia. Ganglia are numbered as follows: left and right cerebral ganglia (1, 2); left and right pedal ganglia (3, 4); left and right pleural ganglia (5, 6); left and right parietal ganglia (7, 8); visceral ganglion (9). Identified neurones and neuronal clusters: left and right pedal dorsal 11 (L/RPeD11); left and right cerebral A cluster neurones (L/RCeA); visceral dorsal 1 and 4 (VD1 and VD4); visceral E group neurone (VE); visceral H, I, J, K cells (VH, VI, VJ, VK cells); right parietal A group (RPA group); right parietal dorsal 2 (RPD2); visceral ventral 1 and 2 (VV1 and VV2); visceral F group neurone (VF). Statocyst organs (St) are located in the pedal ganglia. Not drawn to scale.

synaptic transmission, all experiments where the presence of electrical coupling is demonstrated were performed in zero-Ca^{2+}/high-Mg^{2+} salines.

Electrical coupling between L/RCeA cluster neurones is lost after selective ablation of neurones L/RPeD11

As described above, L/RCeA cluster neurones have only ipsilateral projections, yet they were found to be electrically coupled to their contralateral homologues. Interneurones L/RPeD11, in contrast, not only have ipsilateral and contralateral projections but are also electrically coupled to both L/RCeA cluster neurones. To

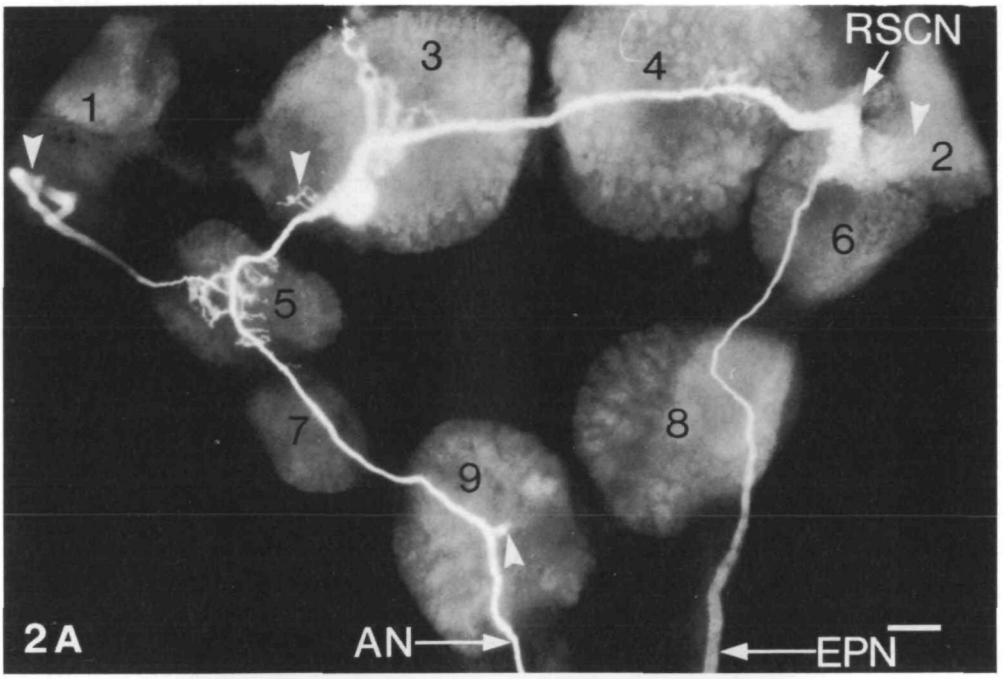
test whether the electrical coupling between L/RCeA cluster neurones was *via* these interneurones, we selectively ablated either LPeD11 or RPeD11 by intracellular injection of 10% Lucifer Yellow followed by exposure of the preparations to blue light for 5–10 min (Bulloch *et al.* 1984; Elliott and Kleindienst, 1990). The effectiveness of this photoablation was checked in two ways: (1) visually by observation of the immediate disintegration and fragmentation of the cell body, and (2) electrophysiologically, by the loss of electrical activity and resting membrane potential (Fig. 5). Normally, the loss of resting membrane potential occurred in 5–10 min, but this could be achieved immediately by depolarising the injected cell (Fig. 5). When either left or right PeD11 was selectively killed by this procedure, the electrical coupling between L/RCeA cluster neurones persisted (Fig. 6A). However, when neurones L/RPeD11 were both photo-inactivated, the electrical coupling between L/RCeA cluster neurones was lost (Fig. 6B). Cerebral A cluster neurones within the ipsilateral ganglion, however, remained coupled even after the removal of both interneurones from the circuit (Fig. 6C). These data suggest that the activities of the contralateral cerebral A cluster neurones are coordinated *via* neurones L/RPeD11.

Chemical connections of neurones L/RPeD11 with visceral neurones of unknown function

In addition to their electrical connections, interneurones L/RPeD11 also make chemical synapses with a wide variety of neurones. The giant cells visceral ventral 1 and 2 (VV1, VV2), (Benjamin and Winlow, 1981) were found to have axonal branches in various visceral and parietal nerves. In addition, they were found to project to the periphery *via* the pedal nerves, which innervate foot and body wall musculature (Fig. 7A). Some of the visceral F group neurones also had axon projections in various pedal nerves (Fig. 7B) and received inputs in common with locomotor motoneurones (N. I. Syed, unpublished data). The function of VV1, VV2 and visceral F group neurones is unknown, but electrical stimulation or spontaneous action potentials in either left or right PeD11 produced 1:1 inhibitory postsynaptic potentials (IPSPs) in VV1 and VV2 (not shown here) and excitatory postsynaptic potentials (EPSPs) in VF cells (Fig. 8A,B). These connections between L/RPeD11 and VV1 and VV2 and VF group neurones were significantly blocked when bathing solution Ca^{2+} was replaced with Mg^{2+} (Fig. 8A,B), providing evidence for the chemical nature of these connections. Evidence that these connections might be monosynaptic is also provided in Fig. 8, where these connections are shown to persist in high-Ca^{2+}/high-Mg^{2+} salines.

Chemical connections of L/RPeD11 with heart motoneurones

Intracellular stimulation of L/RPeD11 also produced EPSPs of constant latency in all visceral E (VE) group neurones examined, including two cells that are electrically coupled to each other and that have been described as heart motoneurones (Benjamin *et al.* 1988) (Fig. 8C). The connections between VE group neurones and L/RPeD11 were also significantly reduced by replacing the


RSCN
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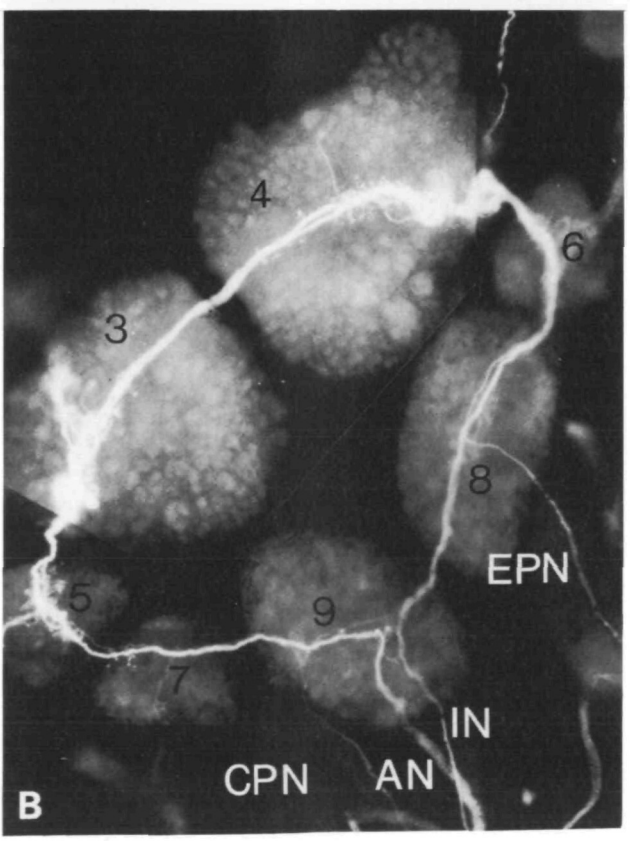
4
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B

Fig. 2. The morphology of interneurones L/RPeD11 revealed by the injection of Lucifer Yellow. (A) Lucifer Yellow CH was injected ionophoretically into LPeD11. This neurone was found to project axons both ipsilaterally and contralaterally, almost encircling the lower ganglionic ring. These main axons have three peripheral projections (arrows). One of these projections is *via* the right superior cervical nerve (RSCN), which emanates from the right pedal ganglion and innervates the right body wall. The other two projections are *via* the anal (AN) and external parietal nerves (EPN), which innervate the pneumostome and mantle cavity musculature, respectively (Slade *et al.* 1981). The axonal projections of both cells have branches in nearly all the ganglia shown, but arborisations are most extensive in neuropile areas where somata or axonal branches of L/RPeG cluster and visceral H, I, J and K cells are located (arrowheads). Ganglia are numbered as in Fig. 1. (B) Simultaneous injections of Lucifer Yellow into both left and right PeD11 show that together these interneurones encircle the lower ganglionic ring. Unlike LPeD11 (A), which projects down the external parietal nerve (EPN) and the anal nerve (AN), RPeD11 projects to the periphery *via* the intestinal nerve (IN), the anal nerve and the cutaneous pallial nerve (CPN). Scale bar, 50 μm.

Ca^{2+} in normal saline with other divalent cations, such as Mg^{2+} (Fig. 8C) or Co^{2+}. Furthermore, the connections between PeD11 and VE group neurones persist in high-Ca^{2+}/high-Mg^{2+} saline (Fig. 8C), suggesting the possibility of a monosynaptic pathway.

Chemical connections of L/RPeD11 with respiratory motoneurones

It has recently been shown that a visceral J cell (VJ cell) is a pneumostome opener muscle motoneurone, while a visceral K cell (VK cell) is a pneumostome closer muscle motoneurone (N. I. Syed, D. Harrison and W. Winlow, in preparation). Electrical stimulation of RPeD11 produced IPSPs in the VJ cell and EPSPs in the VK cell (Fig. 9A,B). Similarly, stimulation of neurones L/RPeD11 produced EPSPs in VG group neurones (Fig. 9C), a group that receives inhibitory inputs during pneumostome opening (Syed, 1988), but whose exact function is not yet known. These connections were also blocked in zero-Ca^{2+}/high-Mg^{2+} saline but not in high-Ca^{2+}/high-Mg^{2+} saline (Fig. 9A,B and C).

Several right parietal A group neurones (RPA group) (Benjamin and Winlow, 1981) have been found to be motoneurones to the mantle cavity musculature (N. I. Syed, D. Harrison and W. Winlow, in preparation). Neurones L/RPeD11 were found to have electrical coupling with two of these RPA group neurones (Fig. 10A). Another pair of electrically coupled neurones present in the visceral and parietal ganglia are visceral dorsal 1 (VD1) and right parietal dorsal 2 (RPD2) (Boer *et al.* 1979; Benjamin and Winlow, 1981; Benjamin and Pilkington, 1986). Electrical stimulation of RPeD11 had excitatory effects on both VD1 and RPD2 (Fig, 10A). The RPA group neurone, which was found to be electrically coupled to RPeD11, had no effect on VD1 and RPD2 (Fig. 10B). All the connections found between neurones L/RPeD11 and the respiratory neurones were reversibly blocked when the Ca^{2+} in the normal saline was replaced with Mg^{2+} (Fig. 11) or

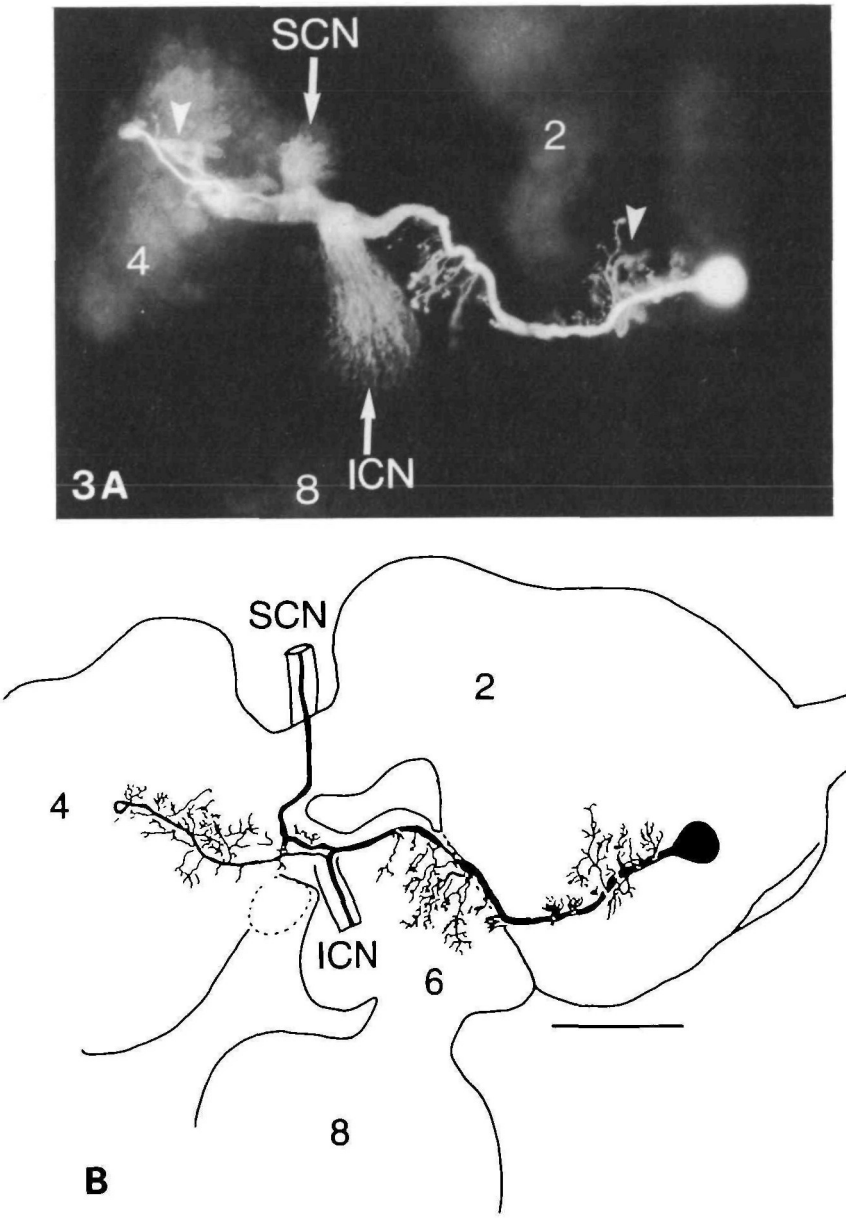


Fig. 3. The morphology of an RCeA cluster neurone. (A) The injection of Lucifer Yellow into an RCeA cluster neurone revealed that this neurone has peripheral projections *via* the superior and inferior cervical nerves (SCN, ICN, arrowheads) and extensive neurites in the right cerebral, pleural and pedal ganglia (arrows). Note that this cell, in common with all cerebral A cluster neurones, has only ipsilateral projections and thus does not cross the cerebral or pedal commissures. (Ganglia are numbered as in Fig. 1.) (B) *Camera lucida* drawing of A. Scale bar, 100 μm.

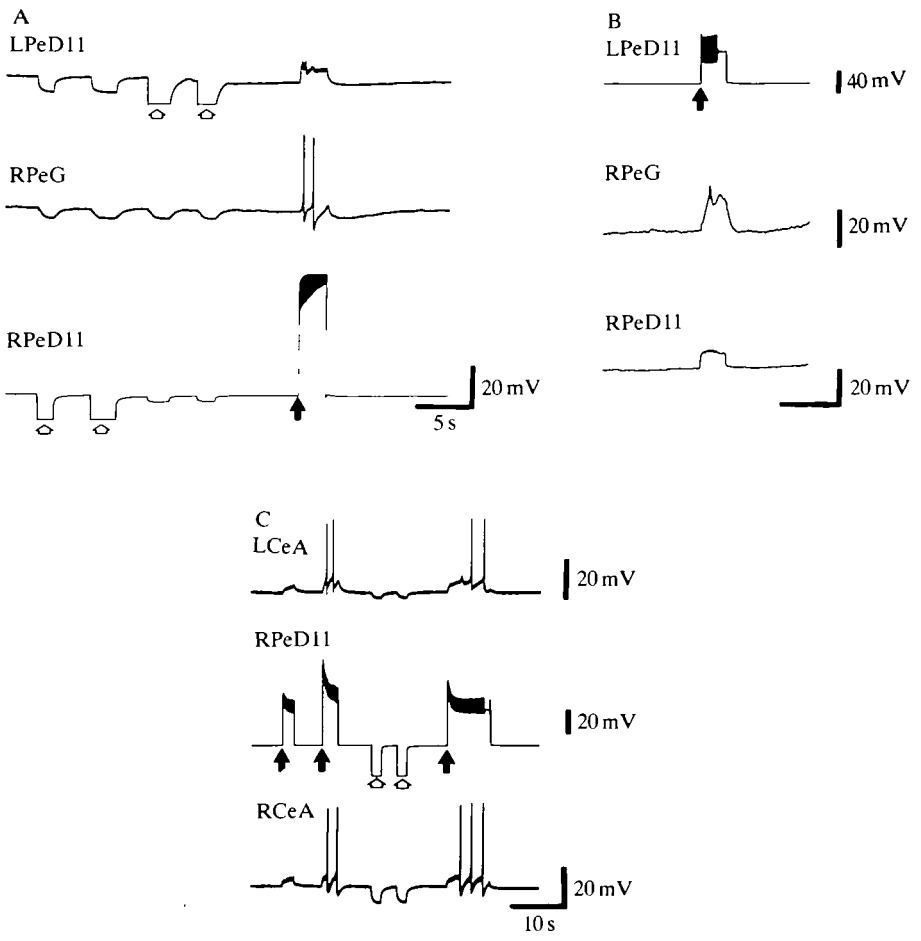


Fig. 4. The presence of electrical coupling between interneurones L/RPeD11 and various motoneurones as demonstrated in zero-Ca^{2+} saline. (A) Injection of hyperpolarising current into either RPeD11 or LPeD11 (open arrows) caused the hyperpolarisation of an RPeG cluster neurone. Similarly, injection of depolarising current into RPeD11 (filled arrows) caused depolarisation accompanied by firing of the RPeG neurone. (B) Injection of depolarising current into LPeD11 could also be recorded from both an RPeG cluster neurone and interneurone RPeD11. (C) Evidence for electrical coupling between interneurone RPeD11 and L/RCeA cluster neurones. Injection of hyperpolarising (open arrows) or depolarising current pulses of increasing strength and duration (filled arrows) into RPeD11 caused the hyperpolarisation or depolarisation of L/RCeA cluster neurones.

Co^{2+}. These divalent cation manipulations did not affect the electrical coupling between RPeD11 and RPA group neurones (Fig. 11).

Inhibitory effects of respiratory interneurone VD4 on L/RPeD11

In *Lymnaea*, during respiratory movements the locomotor and whole-body

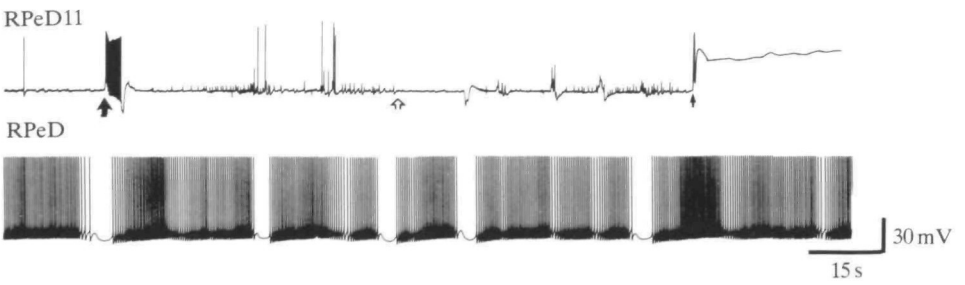


Fig. 5. Selective photoablation of interneurone RPeD11. Simultaneous intracellular recordings were made from RPeD11 and an adjacent pedal D cluster neurone (RPeD) (Slade *et al.* 1981). The microelectrode used to impale RPeD11 contained 10 % Lucifer Yellow, and the dye was injected as described earlier (see Materials and methods). To test that the cell was healthy prior to its ablation, RPeD11 was electrically stimulated (at filled arrow). This stimulation induced several action potentials; furthermore, spontaneous action potentials were also apparent. The preparation was then exposed to blue light (at open arrow). Within 15 s, spontaneous action potentials were lost. When stimulated electrically (at small arrow), the interneurone RPeD11 failed to respond and lost its resting membrane potential. Note that the exposure to blue light had no effect on the RPeD cluster neurone, which had not been injected with Lucifer Yellow.

withdrawal motor activity must be inhibited (N. I. Syed, D. Harrison and W. Winlow, in preparation). Identified interneurone VD4 (Janse *et al.* 1985) (VWI of Benjamin, 1984) is implicated in respiratory behaviour (Syed and Winlow, 1988*a*,*b*; Syed *et al.* 1990; N. I. Syed and W. Winlow, in preparation). Stimulation of VD4 inhibited the electrically coupled interneurones L/RPeD11 and also the motoneurones coupled to them, i.e. L/RPeG and L/RCeA cluster neurones (Fig. 12). The effects of VD4 on most follower cells are slow and difficult to resolve as unitary 1:1 responses. Both the electrical and chemical connections of L/RPeD11 with their follower cells described in the present study are summarized in Fig. 13.

Role of L/RPeD11 in whole-body withdrawal behaviour

Previously, L/RCeA cluster neurones of *Lymnaea* were described as whole-body withdrawal motoneurones (Benjamin *et al.* 1985). In the present study, however, these CeA cluster neurones were found to be electrically coupled to interneurones L/RPeD11. We therefore investigated the role of these interneurones in whole-body withdrawal behaviour. To test the relationship between the withdrawal behaviour and the activity in L/RPeD11, photic or mechanical stimuli, which induced withdrawal behaviour, were applied to semi-intact preparations, either by illumination of the head–foot complex or by pressure application with a blunt glass rod held on a micromanipulator. The results obtained from these experiments showed that although L/RPeD11 received excitatory inputs during induced withdrawal behaviour, strong electrical stimu

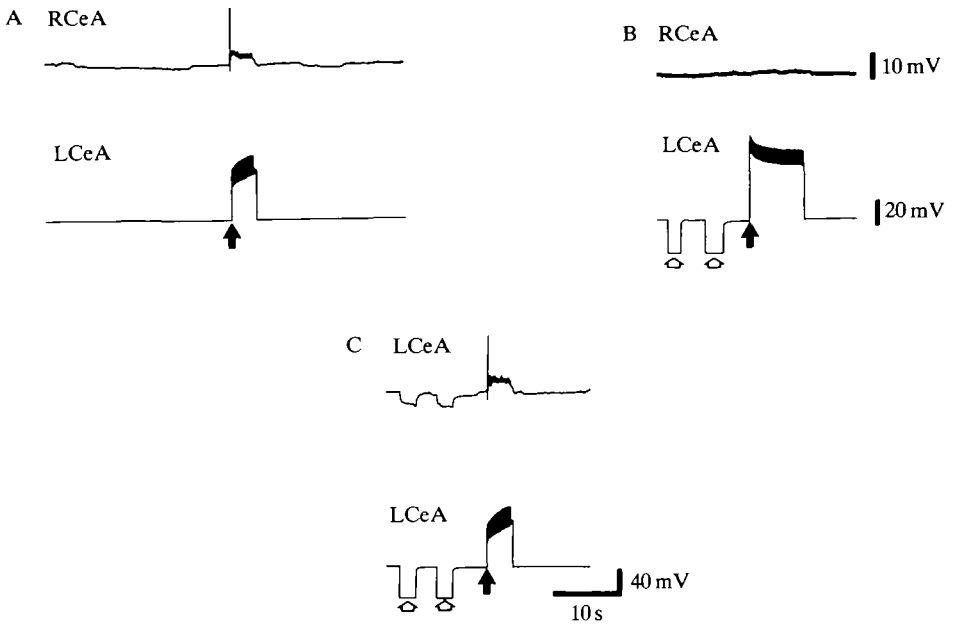


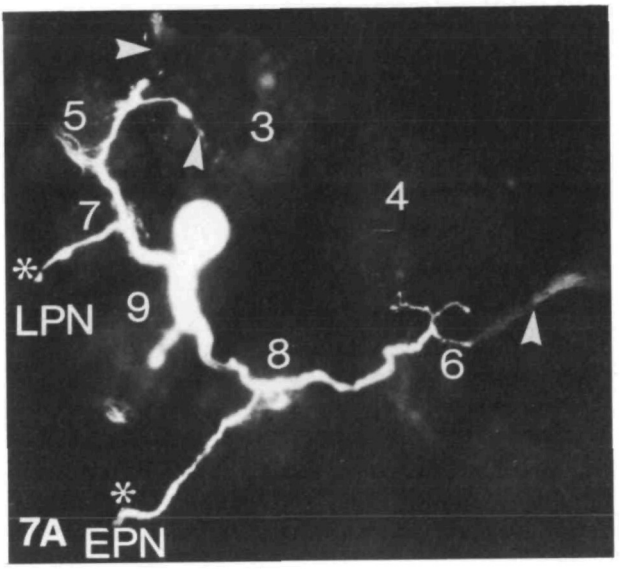
Fig. 6. Loss of electrical coupling between left and right CeA cluster neurones after ablation of interneurones L/RPeD11. (A) Photoablation of LPeD11 alone did not result in the loss of coupling between L/RCeA cluster neurones. (B) When interneurones L/RPeD11 were both ablated the coupling between L/RCeA cluster neurones was lost. Injection of either hyperpolarising (open arrows) or depolarising currents into an LCeA cluster neurone (filled arrow) could not be recorded from the contralateral RCeA cluster neurone, whereas the LCeA cluster neurone remained coupled to another (ipsilateral) CeA cluster neurone even when interneurones L/RPeD11 were both ablated (C).

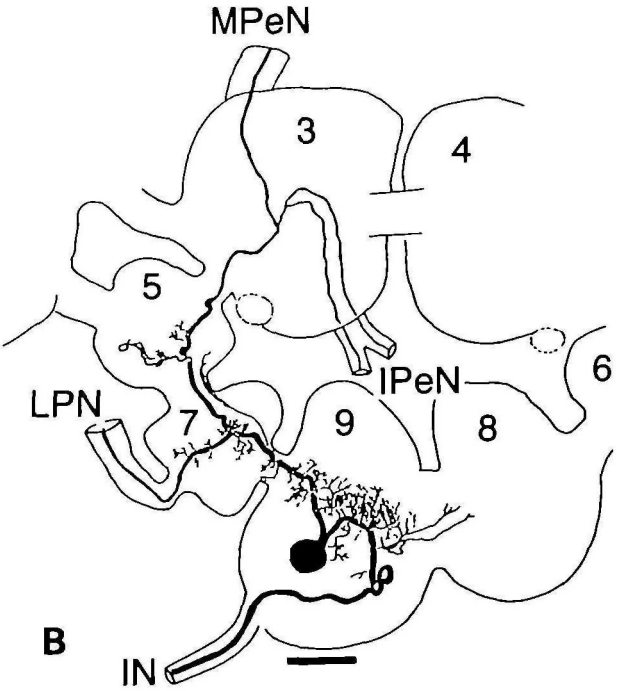
lation of these cells did not cause whole-body withdrawal (Fig. 14), as was suggested for the electrically coupled L/RCeA cluster neurones by Benjamin *et al.* (1985).

Discussion

The role of electrically coupled systems of neurones

Most rhythmic behaviour, such as locomotion and respiration, requires coordination between various parts of the body so that movements or postures achieved are effective and useful. Probably the most effective and prompt way to coordinate various neural elements is *via* electrotonic coupling. The propagation of action potentials in these electrically coupled systems is faster than chemical transmission and, therefore, allows the animals to respond rapidly during a stereotyped behaviour (Marder, 1984). In the invertebrates, a number of preparations have been observed where extensive electrotonic coupling among neurones comprising a rhythm generator forms a positive feedback loop. This feedback, in turn, causes





a synchronised discharge of the interconnected neurones (Farmer, 1970; Getting, 1974; Kaneko *et al.* 1978; Friesen, 1985; Nusbaum *et al.* 1987; Syed *et al.* 1988; Koester, 1989).

Such synchrony becomes more important if the survival of the animal is at stake. When confronted with a noxious stimulus, *Lymnaea* retracts its entire head–foot complex into the shell; a behaviour that can be considered an escape response

Fig. 7. Morphologies of VV1 and a VF group neurone. (A) Photomicrograph of identified neurone VV1 after the injection of the dye Lucifer Yellow CH. This neurone has axon projections in a large number of pedal nerves, which innervate either foot or body wall musculature (arrowheads). In addition, VV1 projects to the periphery *via* left parietal (LPN) and external parietal nerves (EPN, asterisks), which innervate the mantle cavity musculature. Ganglia are numbered as in Fig. 1. (B) *Camera lucida* drawing of a visceral F (VF) group neurone. Injection of Lucifer Yellow showed this neurone to have axon projections in the intestinal nerve (IN) and left parietal nerve (LPN), which innervate the mantle cavity area, and also the median and inferior pedal nerves (MPeN, IPeN), which innervate the foot musculature. The VF cell has extensive neuritic branches, several of which extend to the right parietal ganglion, while others are concentrated in the neuropile areas of the visceral, left parietal and pleural ganglia (arrows). Scale bar, 100 μm.

This is achieved by the abrupt cessation of locomotion and respiratory activity, followed by a simultaneous contraction of body wall and foot muscles. Therefore, from a hierarchical point of view, the whole-body withdrawal behaviour takes priority over other behaviours, such as locomotion or respiration. Since the effector organs involved in these different behaviours are the same, an interaction and coordination between neural elements controlling these muscles must exist.

In *Lymnaea* we have identified a pair of electrically coupled interneurones that are also electrically coupled to foot and body wall motoneurones, thus forming an integrated network that may serve to coordinate locomotory and respiratory motor activities. In addition, these interneurones modulate cardiorespiratory activity *via* chemical connections with appropriate motoneurones. We believe that the electrical coupling between homologous contralateral groups of motoneurones is maintained *via* L/RPeD11 because the selective ablation of both interneurones results in the loss of connectivity between the motoneurones. Such a loss of connectivity is not without physiological significance, for it would allow the two sides of the body to function independently. Dysynchrony between left and right sides of the body is evident in turning and twisting movements of *Lymnaea*, although no direct electrophysiological studies have been carried out to show decoupling of motor centres. In an electrically coupled network, such as that described in the present study, the decoupling between L/RCeA cluster neurones could be efficiently achieved through modulating the activity of interneurones L/RPeD11. A mechanism (e.g. inhibitory synaptic input) that would selectively inactivate these two interneurones would allow the contralateral CeA clusters to act independently.

As an electrically coupled network, these *Lymnaea* neurones have features in common with the neural network that underlies the escape behaviour of crayfish and hermit crabs. In crayfish, giant fibres cause synchronous excitation of the abdominal flexor motoneurones *via* electrotonic coupling (See Wine and Krasne, 1982). Similarly, in the hermit crab, each giant fibre drives the ipsilateral segmental giant neurones through electrical coupling (Heitler and Fraser, 1987). In *Lymnaea*, another pair of electrically coupled interneurones, the cerebral giant

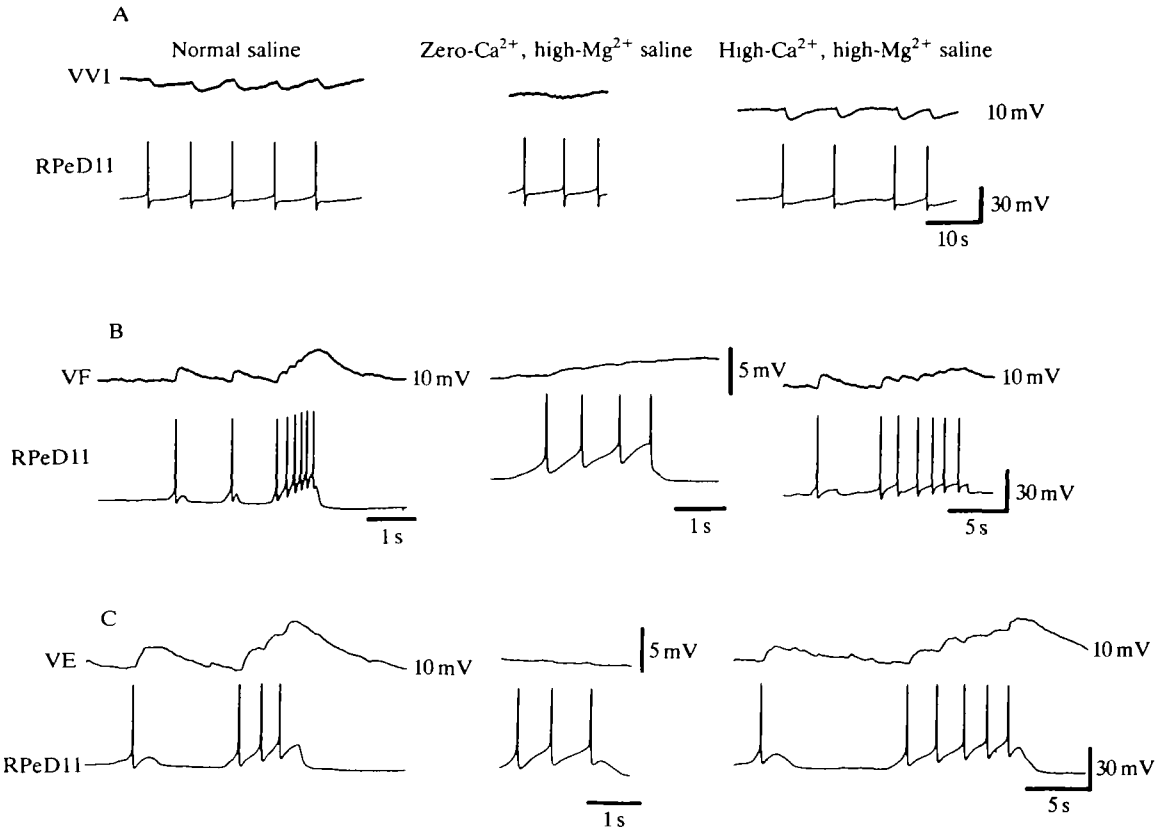


Fig. 8. Chemical and monosynaptic nature of connections between RPeD11 and VV1, VF and VE neurones. (A) Simultaneous intracellular recordings were made from RPeD11 and a VV1 neurone. Spontaneous action potentials in RPeD11 in normal saline produced 1:1 IPSPs in its follower VV1 neurone. These synaptic potentials were significantly reduced when Ca^{2+} in the normal saline was replaced with Mg^{2+}, but persisted when bathed in high-Ca^{2+}/high-Mg^{2+} saline. Induced action potentials in RPeD11 also produced 1:1 EPSPs in both (B) VF and (C) VE group neurones when preparations were bathed in normal saline. These synaptic potentials were significantly reduced in zero-Ca^{2+} saline but were unaffected in high-Ca^{2+}/high-Mg^{2+} saline, suggesting that these connections are chemical and probably monosynaptic.

cells (CGCs) has been shown to coordinate the buccal motor output underlying rhythmic feeding behaviour. However, the connections between CGCs and buccal interneurones and motoneurones are known to be chemical in nature (see McCrohan and Winlow, 1985). Similar CGC and buccal neurone circuitry has been described for other gastropods, such as *Planorbis corneus* (Berry and Pentreath, 1976), *Aplysia californica* (Weiss *et al.* 1978) and *Philine aperta* (Barber, 1983). In *Helisoma trivolvis*, which is closely related to *Planorbis* and *Lymnaea*, the CGCs are not electrically coupled to each other (Granzow and Kater, 1977), but other methods of coordination between these CGCs (e.g. *via* other interneurones) may exist. Recently, Kyriakides and McCrohan (1988) have suggested that, in

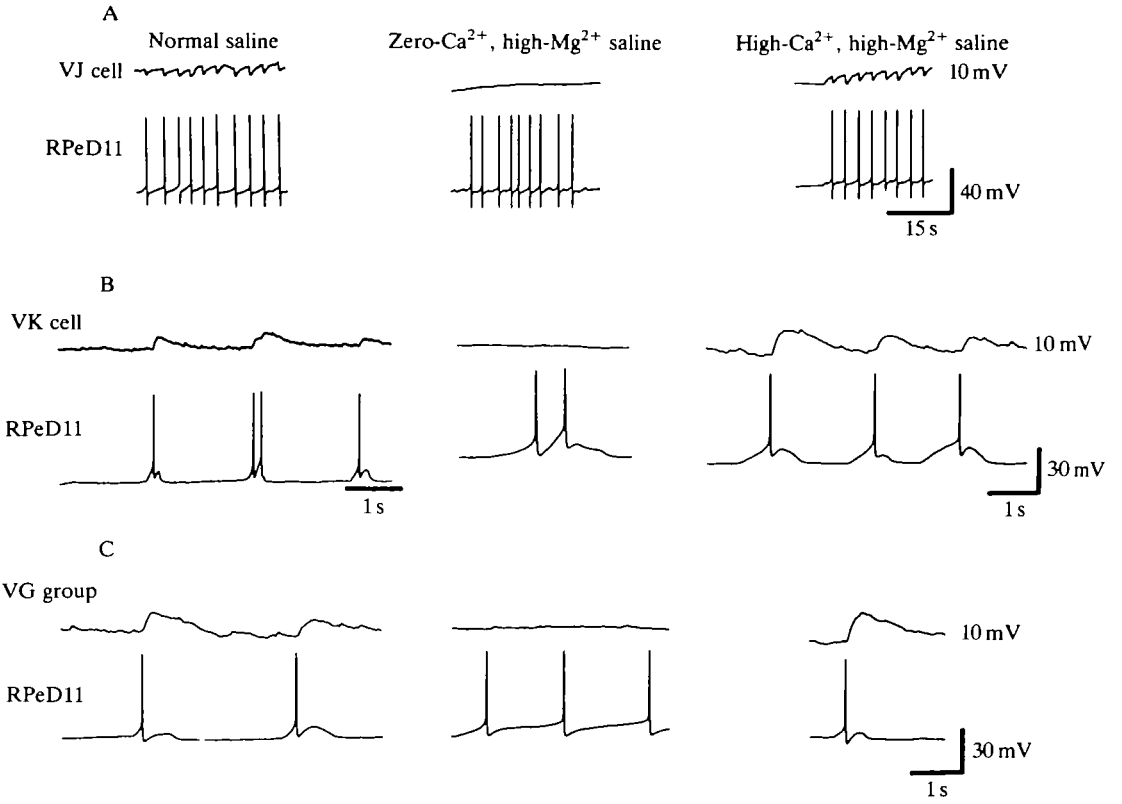


Fig. 9. Chemical and monosynaptic nature of connections between RPeD11 and respiratory motoneurones. In preparations maintained in normal saline, spontaneous action potentials in RPeD11 produced 1:1 IPSPs in a VJ cell (A) and 1:1 EPSPs in both a VK cell (B) and a VG group neurone (C). All these synaptic connections were reversibly reduced in amplitude in a zero-Ca^{2+} saline, but remained unaffected in high-Ca^{2+}/high-Mg^{2+} saline.

Lymnaea, coordination of the buccal feeding rhythm with foot and body wall movements may occur through inputs originating from buccal ganglia interneurones. Coordination of various behaviours may, therefore, involve a number of interneurones distributed among the different central ganglia to form a complex higher-order network. Modulation of the synaptic connections within such a network would allow certain behaviours to have priority over others or allow switching between behavioural states (Hooper and Moulins, 1989; Harris-Warrick and Johnson, 1989; DiCaprio, 1990; see also Selverston, 1989).

L/RPeD11 are multifunctional

Interneurones L/RPeD11 are not only electrically coupled to the withdrawal motoneurones, but also make chemical connections with cardiorespiratory motoneurones. The VJ and VK cells of the visceral ganglion have been shown to be the motoneurones to the pneumostome opener muscle and pneumostome closer

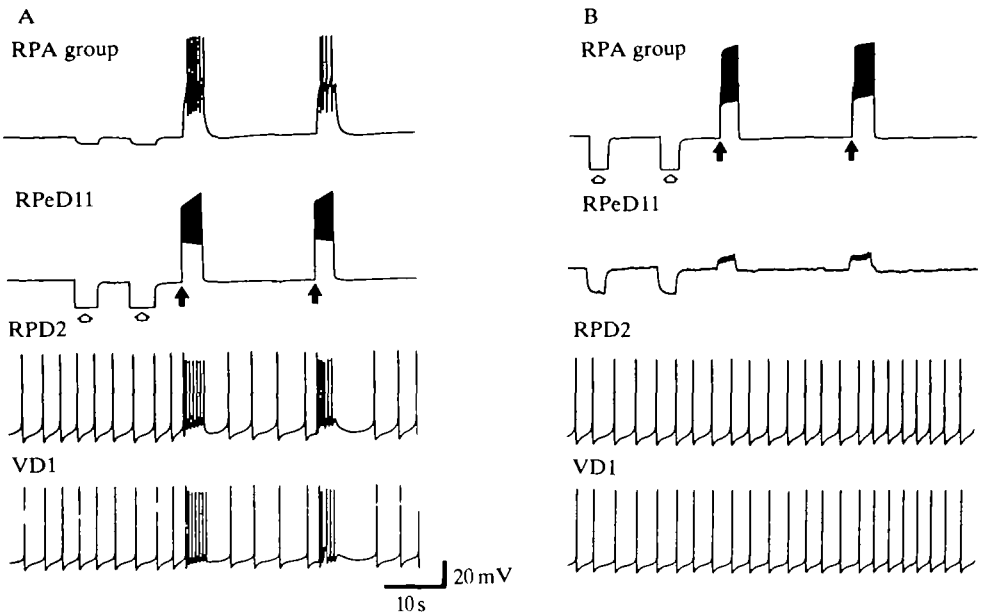


Fig. 10. Electrical and chemical connections between RPeD11 and respiratory neurones. (A) Here RPeD11 is shown to be electrically coupled to one of the RPA group neurones. This is demonstrated by passing hyperpolarising (open arrows) and depolarising (filled arrows) current pulses between these neurones. In addition, RPeD11 made chemical connections with another pair of electrically coupled neurones, VD1 and RPD2. Since both VD1 and RPD2 are strongly electrically coupled to each other, it was not possible to obtain a unitary response. (B) The connections between RPeD11 and VD1 and RPD2 are exclusive and cannot be induced by stimulation of RPA group neurones, which are electrically coupled to RPeD11. Injection of hyperpolarising (open arrows) or depolarising (filled arrows) current pulses into an RPA group neurone could be recorded from RPeD11, showing the presence of electrical coupling. However, the injection of depolarising current into the RPA group neurone had no effect on VD1 and RPD2.

muscle, respectively (N. I. Syed, D. Harrison and W. Winlow, in preparation). These motoneurones and others receiving common synaptic inputs (e.g. VG cells) are driven by respiratory interneurones and fire alternating bursts of action potentials during spontaneously occurring respiratory behaviour (N. I. Syed, D. Harrison and W. Winlow, in preparation). The electrical stimulation of L/RPeD11 caused the inhibition of VJ cells while exciting VK and VG cells. These findings suggest that when L/RPeD11 are spontaneously active, such as during locomotion or withdrawal behaviour, they should have an inhibitory effect on respiratory motor output, as is found during normal behaviour. In addition to these chemical connections, interneurones L/RPeD11 make electrical connections with mantle cavity muscle motoneurones (RPA group neurones), which are also involved in the respiratory behaviour (N. I. Syed, D. Harrison and W. Winlow, in preparation).

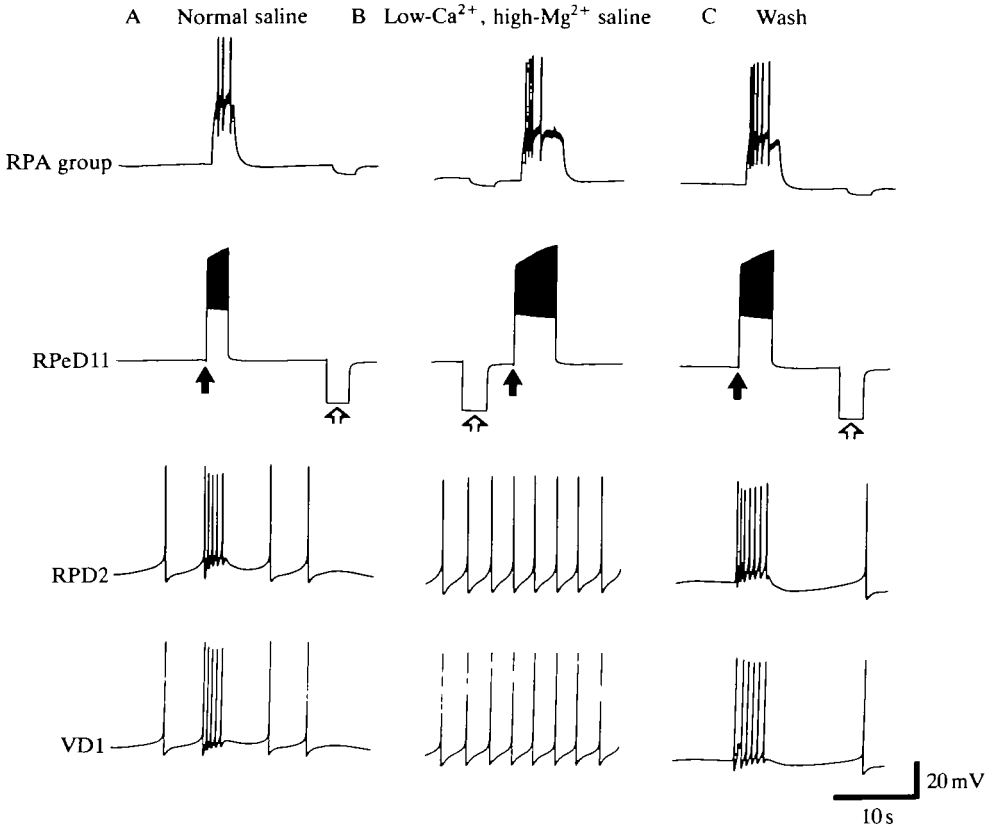


Fig. 11. Chemical nature of connections between RPeD11 and VD1 and RPD2. The connections between RPeD11 and VD1 and RPD2 recorded in normal saline (A) were blocked (B) when Ca^{2+} in the bathing saline was replaced by Mg^{2+}. Upon returning to the normal saline (C) these connections were re-established. Note that the electrical coupling between RPeD11 and the RPA group neurone remained unaffected. Injection of current into RPeD11 is indicated by either open (hyperpolarising current) or filled (depolarising current) arrows.

In locusts, a pair of interneurones that makes simultaneous synaptic contact with flight and respiratory motoneurones has previously been described by Burrows, (1975*a*,*b*, 1982). These interneurones not only make extensive chemical connections with 30 flight motoneurones but also synapse upon 20 ventilatory motoneurones (Burrows, 1975*a*,*b*, 1982). These locust interneurones have reciprocal effects on antagonistic ventilatory motoneurones, depolarizing those that spike during expiration and hyperpolarizing those that spike during inspiration (Burrows, 1975*b*). In addition to these similarities with the locust interneuronal network, L/RPeD11 of *Lymnaea* excite heart motoneurones (Benjamin *et al.* 1988). This excitation could serve to increase the cardiac output, particularly when there is an increased demand for blood supply during locomotion. Another pair of electrically coupled neurones of *Lymnaea*, VD1 and RPD2, receives inputs during

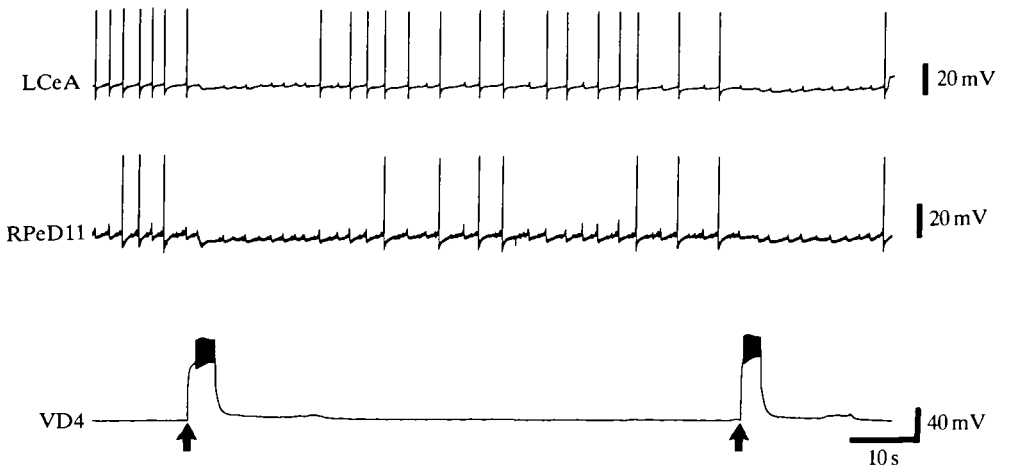


Fig. 12. Inhibition of RPeD11 and a LCeA cluster neurone by the respiratory interneurone VD4. The electrical stimulation of VD4 (at arrows) inhibited both the LCeA cluster neurone and RPeD11. The unitary attenuated EPSPs seen in both neurones are produced by inputs from electrically coupled neurones.

respiratory behaviour (Syed and Winlow, 1988*a*,*b*; N. I. Syed and W. Winlow, in preparation). Both VD1 and RPD2 are sensitive to changes in external P_{O_2} (Janse *et al.* 1985; van der Wilt *et al.* 1988) and are follower cells of L/RPeD11. The actions of L/RPeD11 on VD1 and RPD2 are exclusive and cannot be induced *via* stimulation of other electrically coupled cells (e.g. RPA group neurones). The interneurones L/RPeD11 also make chemical connections with several other neurones (e.g VV1 and VF group neurones), which receive inputs in common with locomotor and respiratory motoneurones (Syed, 1988), but whose exact functions remain to be determined. All these results support a multifunctional role for interneurones L/RPeD11 in integrating cardiorespiratory output with other motor behaviours.

L/RPeD11 and whole-body withdrawal behaviour

Recent investigations of the whole-body withdrawal system of *Lymnaea* provided evidence for motoneuronal function, and L/RCeA cluster neurones were described as the largest group of withdrawal motoneurones (Ferguson, 1984; Benjamin *et al.* 1985). According to Haydon (1982) and Winlow and Haydon (1986), the L/RCeA cluster neurones are left and right body wall motoneurones. Using our newly developed semi-intact preparation, we demonstrated that L/RCeA cluster neurones and cells coupled to them are spontaneously active during locomotion. These electrically coupled neurones fire synchronous discharges of action potentials during terrestrial locomotion (Syed, 1988). In experiments described here, we have demonstrated that the induction of whole-body withdrawal behaviour *via* mechanical stimulation of the head–foot comple

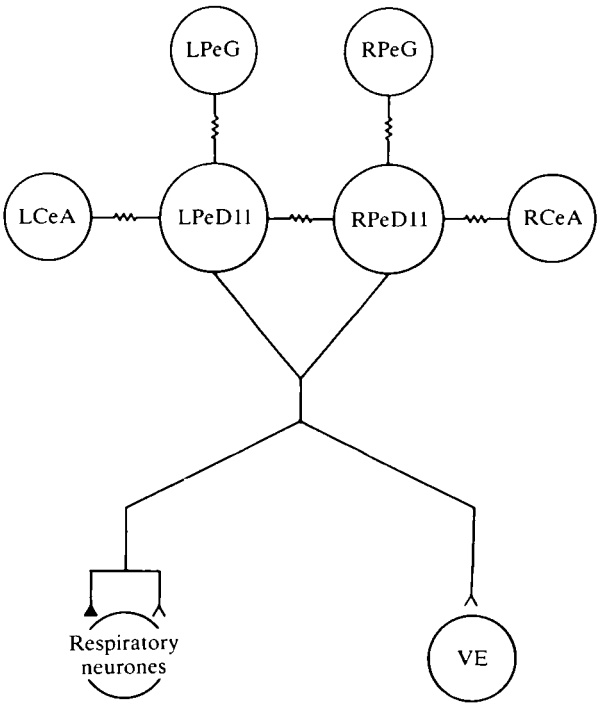


Fig. 13. Summary diagram showing the electrical connections between L/RPeD11, L/RPeG and L/RCeA cluster neurones and their effects on cardiorespiratory neurones. Interneurones L/RPeD11 are electrically coupled to each other, to L/RPeG cluster neurones (foot motoneurones) and also to L/RCeA cluster neurones (left and right body wall motoneurones). In addition, this electrically coupled network has excitatory effects (open symbol) on heart motoneurones (VE group cells) (Syed, 1988) and either excites or inhibits (closed symbol) those cells involved in respiratory behaviour.

excites previously quiescent L/RPeD11 (Fig. 13) and other motoneurones coupled to them (not shown here). Nevertheless, strong electrical stimulation of L/RPeD11 neurones did not induce whole-body withdrawal. We believe that, since the earlier experiments were carried out on severely restrained or extensively dissected animals, it is likely that the presence of motor activity in appropriate muscles and motor nerves was taken as an indication that the animal was engaged in withdrawal behaviour. From experiments described here it is apparent that, although L/RPeD11 do participate in whole-body withdrawal behaviour, their prime function appears to be the coordination of foot and body wall musculature, as observed during locomotion (Syed, 1988). Since whole-body withdrawal in *Lymnaea* represents a form of escape behaviour, it is not surprising that some elements of the neural circuitry resemble those involved in escape responses of other animals. As mentioned before, electrotonic coupling plays an important role in the escape tail-flip of various crustaceans (Wine and Krasne, 1982; Heitler and Fraser, 1987). Interneuronal networks are involved in most escape responses,

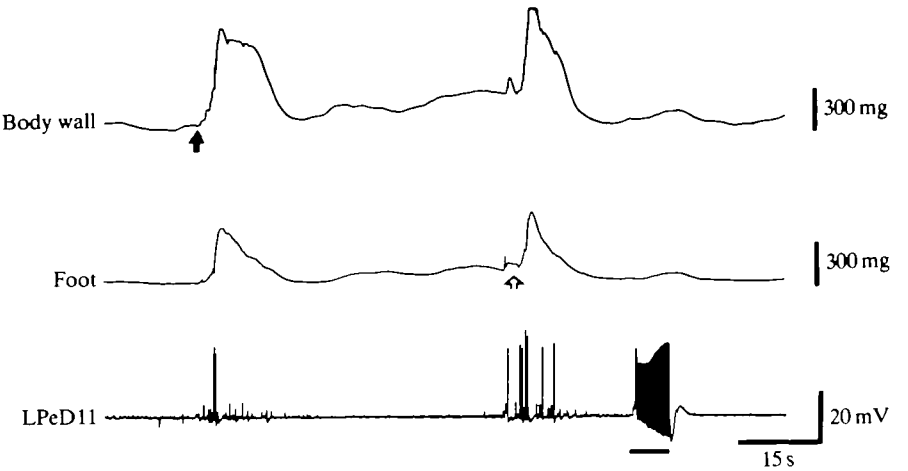


Fig. 14. Role of LPeD11 in the whole-body withdrawal behaviour. In a semi-intact preparation, simultaneous muscle tension and intracellular recordings were made from body wall musculature and foot musculature and interneurones LPeD11. Mechanical stimulation of the body wall (at filled arrow) or foot musculature (open arrow) using a glass rod caused whole-body withdrawal, i.e. the longitudinal contraction of the head–foot complex into the shell. Interneurone LPeD11 received excitatory inputs during these withdrawal movements. Electrical stimulation of LPeD11 (at bar) did not induce whole-body withdrawal. However, it did cause slight contraction of both muscles.

including escape swimming in leeches (Stent *et al.* 1978; Stent and Kristan, 1981) and molluscs, such as *Tritonia diomedia* (Getting, 1988, 1989) and *Clione limacina* (Arshavsky *et al.* 1985; Satterlie, 1989). In *Clione* it is interesting that the circuitry underlying both slow and fast swimming involves considerable electrotonic coupling between identified interneurones and motoneurones. Chemical connections are also important, especially in switching between the two swimming patterns (Satterlie, 1989). The role played by interneurones L/RPeD11 in *Lymnaea* whole-body withdrawal is not yet clear, but they may help to filter sensory inputs so that the appropriate motor programme (withdrawal, locomotion, etc.) can be triggered.

In conclusion, a pair of electrically coupled interneurones has been shown to be electrically coupled to both foot and body wall motoneurones, providing a pathway by which contralateral motoneurones can be coordinated and modulated. These interneurones are also involved in modulating the cardiorespiratory system through chemical connections with heart motoneurones and respiratory interneurones. Furthermore, although interneurones L/RPeD11 participate in whole-body withdrawal, their prime function appears to be the coordination of locomotor outputs. Left and right PeD11 thus provide an example of interneurones specialized to serve both coordinating and multifunctional roles.

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References

ARSHAVSKY, Y. I., BELOOZEROVA, I. N., ORLOVSKY, G. N., PANCHIN, Y. V. AND PAVLOVA, G. A. (1985). Control of locomotion in marine mollusc *Clione limacina*. III. On the origin of locomotory rhythm. *Expl Brain Res.* **58**, 273–284.

ATWOOD, H. L. AND WIERSMA, C. A. G. (1967). Command interneurones in crayfish nervous system. *J. exp. Biol.* **46**, 249–261.

BARBER, A. (1983). Properties of the serotonergic cerebral ganglion neurones of the gastropod mollusc, *Philine aperta*. *Comp. Biochem. Physiol.* **76**C, 135–149.

BENJAMIN, P. R. (1984). Interneuronal network acting on snail neurosecretory neurones (yellow cells and yellow green cells of *Lymnaea*). *J. exp. Biol.* **113**, 165–185.

BENJAMIN, P. R., BUCKETT, K. R. AND PETERS, M. (1988). Neurones containing FMRFamide-like peptides in the model invertebrate system, *Lymnaea*. *Symp. biol. Hung.* **36**, 247–259.

BENJAMIN, P. R., ELLIOTT, C. J. H. AND FERGUSON, G. P. (1985). Neural network analysis in the snail brain. In *Model Neural Networks and Behaviour* (ed. A. Selverston), pp. 87–108. New York: Plenum Press.

BENJAMIN, P. R. AND PILKINGTON, J. B. (1986). The electrotonic location of low-resistance intercellular junctions between a pair of giant neurones in the snail *Lymnaea*. *J. Physiol., Lond.* **370**, 111–126.

BENJAMIN, P. R. AND WINLOW, W. (1981). The distribution of three wide-acting synaptic inputs to identified neurones in the isolated brain of *Lymnaea stagnalis* (L.). *Comp. Biochem. Physiol.* **70**A, 293–307.

BERNARD, F., MCANELLY, M. L. AND LARIMER, J. L. (1989). Abdominal positioning interneurones in crayfish: participation in behavioral acts. *J. comp. Physiol.* **165**, 461–470.

BERRY, M. S. AND PENTREATH, V. W. (1976). Properties of a symmetric pair of serotonin-containing neurones in the cerebral ganglia of *Planorbis*. *J. exp. Biol.* **65**, 361–380.

BOER, H. H., GERAERTS, W. P. M. AND JOOSSE, J (eds) (1987). *Neurobiology, Molluscan Models*. Mon. Kon. Ned. Akad. Wetensch. Amsterdam: North Holland Publ. Co.

BOER, H. H., SCHOT, L. P. C., ROUBOS, E. W., TER MAAT, A., LODDER, J. C., REICHELT, D. AND SWAAB, D. F. (1979). ACTH-like immunoreactivity in two electrotonically coupled giant neurones in the pond snail, *Lymnaea stagnalis*. *Cell Tiss. Res.* **202**, 231–240.

BULLOCH, A. G. M. AND KATER, S. B. (1982). Neurite outgrowth and selection of new electrical connections by adult *Helisoma* neurons. *J. Neurophysiol.* **48**, 569–583.

BULLOCH, A. G. M., KATER, S. B. AND MILLER, H. R. (1984). Stability of new electrical connections between adult *Helisoma* neurones is influenced by preexisting neuronal interactions. *J. Neurophysiol.* B **52**, 1094–1105.

BURROWS, M. (1975*a*). Co-ordinating interneurones of the locust which convey two patterns of motor commands: their connexions with flight motoneurones. *J. exp. Biol.* **63**, 713–733.

BURROWS, M. (1975*b*). Co-ordinating interneurones of the locust which convey two patterns of motor commands: their connexions with ventilatory motoneurones. *J. exp. Biol.* **63**, 735–753.

BURROWS, M. (1982). Interneurones coordinating the ventilatory movements of the thoracic spiracles in the locust. *J. exp. Biol.* **97**, 385–400.

BUSH, B. M. H. AND CLARAC, F. (eds) (1985). *Coordination of Motor Behaviours*. (*Soc. exp. Biol. Series.* vol. **24**). Cambridge: Cambridge University Press.

COHEN, A. H. (1987). Intersegmental coordinating system of the lamprey central pattern generator for locomotion. *J. comp. Physiol.* A **160**, 181–193.

DELCOMYN, F. (1987). Motor activity during scratching and walking movements of cockroach legs. *J. exp. Biol.* **133**, 111–120.

DICAPRIO, R. A. (1990). An interneurone mediating motor programme switching in the ventilatory system of the crab. *J. exp. Biol.* **154**, 517–535.

EGELHAAF, M. AND BENJAMIN, P. R. (1983). Coupled neuronal oscillators in the snail *Lymnaea stagnalis*: endogenous cellular properties and network interactions. *J. exp. Biol.* **102**, 93–114.

ELLIOTT, C. J. H. AND BENJAMIN, P. R. (1985). Interactions of pattern generating interneurones controlling feeding in *Lymnaea stagnalis*. *J. Neurophysiol.* **54**, 1396–1411.

ELLIOTT, C. J. H. AND KLEINDIENST, H.-U. (1990). Photoinactivation of neurones in the pond snail, *Lymnaea stagnalis*: estimation of a safety factor. *Brain Res.* **524**, 149–152.

FARMER, W. M. (1970). Swimming gastropods (Opisthobranchia and Prosobranchia). *Veliger* **13**, 73–89.

FELDMAN, J. L. AND ELLENBERGER, H. H. (1988). Central coordination of respiratory and cardiovascular control in mammals. *A. Rev. Physiol.* **50**, 593–606.

FERGUSON, G. P. (1984). Neurophysiological analysis of whole body withdrawal in *Lymnaea stagnalis*. PhD thesis, University of Sussex, Sussex, UK.

FRIESEN, W. O. (1985). Neuronal control of leech swimming movements: interactions between cell 60 and previously described oscillator neurones. *J. comp. Physiol.* A. **156**, 231–242.

GETTING, P. (1988). Comparative analysis of invertebrate central pattern generators. In *Neural Control of Rhythmic Movements* (ed. A. H. Cohen, S. Rosignol, S. Grillner), pp. 101–128. New York: John Wiley.

GETTING, P. (1989). Emerging principles governing the operation of neural networks. *A. Rev. Neurosci.* **12**, 185–204.

GETTING, P. A. (1974). Modification of neurone properties by electrostatic synapses. I. Input resistance, time constant, and integration. *J. Neurophysiol.* **37**, 846–857.

GIRDLESTONE, D. (1986). Electrophysiological studies of the actions of general anaesthetics on identified molluscan neurones and neuronal networks. PhD thesis, University of Leeds, Leeds, UK.

GRANZOW, B. AND KATER, S. B. (1977). Identified higher order neurones controlling feeding motor program of *Helisoma*. *Neuroscience*. **2**, 1049–1063.

HARRIS-WARRICK, R. M. AND JOHNSON, B. R. (1989). Motor pattern networks: Flexible foundations for rhythmic pattern production. In *Perspectives in Neural Systems and Behaviours* (ed. T. C. Thomas and B. K. Darcy), pp. 51–71. New York: Alan R. Liss.

HAYDON, P. G. (1982). An electrophysiological study of the nervous control of locomotion in the pond snail *Lymnaea stagnalis* (L.). PhD thesis, University of Leeds, Leeds, U.K.

HAYDON, P. G. AND WINLOW, W. (1986). Shell movements associated with locomotion in *Lymnaea* are driven by a central pattern generator. *Comp. Biochem. Physiol.* **83**A, 23–25.

HEITLER, W. J. AND FRASER, K. (1987). Interactions of the giant fibres and motor giant neurones of the hermit crab. *J. exp. Biol.* **133**, 353–370.

HOOPER, S. L. AND MOULINS, M. (1989). Switching of a neuron from one network to another by sensory induced changes in membrane properties. *Science* **244**, 1587–1589.

HUGHES, G. M. AND WIERSMA, C. A. G. (1960). The co-ordination of swimmeret movements in the crayfish *Procambarus clarkii* (Girard). *J. exp. Biol.* **37**, 657–670.

IKEDA, K. AND WIERSMA, C. A. G. (1964). Autogenic rhythmicity in the abdominal ganglia of the crayfish: the control of swimmeret movements. *Comp. Biochem. Physiol.* **60**A, 459–465.

JACKLET, J. W (ed.) (1989). *Cellular and Neuronal Oscillators*. New York: Marcel Dekker.

JANSE, C., VAN DER WILT, C. J., VAN DER PLAS, J. AND VAN DER ROEST, M. (1985). Central and peripheral neurones involved in oxygen perception in the pulmonate snail *Lymnaea stagnalis* (Mollusca, Gastropoda). *Comp. Biochem. Physiol.* **82**A, 459–467.

KANEKO, C. R. S., MERICKEL, M. AND KATER, S. B. (1978). Centrally programmed feeding in *Helisoma*: identification and characteristics of an electrically coupled premotor neuron network. *Brain Res.* **146**, 1–21.

KOESTER, J. (1989). Chemically and electrically coupled interneurones mediating respiratory pumping in *Aplysia*. *J. Neurophysiol.* **62**, 1113–1126.

KYRIAKIDES, M. A. AND MCCROHAN, C. R. (1988). Central coordination of buccal and pedal neuronal activity in the pond snail *Lymnaea stagnalis*. *J. exp. Biol.* **136**, 103–123.

KYRIAKIDES, M., MCCROHAN, C. R., SLADE, C. T., SYED, N. I. AND WINLOW, W. (1989). The

morphology and electrophysiology of the neurones of the pedal ganglia of *Lymnaea stagnalis* (L.) *Comp. Biochem. Physiol.* **93**A, 861–876.

MACMILLAN, D. L., ALTMAN, J. S. AND KIEN, J. (1983). Intersegmental coordination in the crayfish swimmeret system reconsidered. *J. exp. Zool.* **288**, 157–162.

MARDER, E. (1984). Roles for electrical coupling in neural circuits as revealed by selective neuronal deletions. *J. exp. Biol.* **112**, 147–167.

MCCROHAN, C. AND WINLOW, W. (1985). Interganglionic coordination and bilateral symmetry in the nervous system of gastropod molluscs. In *Coordination of Motor Behaviour* (ed. B. M. H. Bush and F. Clarac), *Soc. exp. Biol. Seminar Series* **24**, pp. 33–62. Cambridge: Cambridge University Press.

MCCROHAN, C. R. (1984). Initiation of the feeding motor output by an identified interneurone in the snail, *Lymnaea stagnalis*. *J. exp. Biol.* **113**, 351–366.

MILLER, J. P. AND SELVERSTON, A. I. (1979). Rapid killing of single neurones by irradiation of intracellularly injected dyes. *Science* **206**, 702–704.

NUSBAUM, M. P., FRIESEN, W. O., KRISTAN, W. B., JR AND PEARCE, R. A. (1987). Neuronal mechanisms generating the leech swimming rhythm: swim-initiator neurons excite the network of swim oscillator neurons. *J. comp. Physiol* A**161**, 355–366.

RITZMANN, R. E., TOBIAS, M. L. AND FOURTNER, C. R. (1980). Flight activity *via* giant interneurones of the cockroach: evidence for bifunctional trigger interneurones. *Science* **210**, 443–445.

SATTERLIE, R. A. (1989). Reciprocal inhibition and rhythmicity: swimming in a pteropod mollusk. In *Neuronal and Cellular Oscillators* (ed. J. W. Jacklet), pp. 151–171. New York: Marcel Dekker.

SELVERSTON, A. (1989). Twitching and switching. *Nature* **341**, 690–691.

SLADE, C. T., MILLS, J. AND WINLOW, W. (1981). The neuronal organization of the paired pedal ganglia of *Lymnaea stagnalis* (L.) *Comp. Biochem. Physiol.* **69**A, 789–803.

STENT, G. S. AND KRISTAN, W. B. (1981). Neural circuits generating rhythmic movements. In *Neurobiology of the Leech* (ed. K. J. Muller, J. G. Nicholls and G. S. Stent), pp. 197–226. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

STENT, G. S., KRISTAN, W. B. JR, FRIESEN, W. O., ORT, C. A., POON, M. AND CALABRESE, R. L. (1978). Neuronal generation of leech swimming movements. An oscillatory network of neurones driving a locomotory behaviour has been identified. *Science* **200**, 1348–1357.

SYED, N. I. (1988). Neural control of locomotion in *Lymnaea*. PhD Thesis, University of Leeds, Leeds, UK.

SYED, N. I., BULLOCH, A. G. M. AND LUKOWIAK, K. (1990). *In vitro* reconstruction of the respiratory central pattern generator of the mollusk *Lymnaea*. *Science* **250**, 282–285.

SYED, N. I., HARRISON, D. AND WINLOW, W. (1988). Locomotion in *Lymnaea*: Role of serotonergic A cluster neurones. *Symp. biol. hung.* **36**, 387–402.

SYED, N. I. AND WINLOW, W. (1988*a*). A pair of electrically coupled interneurones coordinating locomotor, respiratory and cardiac neuronal networks in *Lymnaea*. *J. Physiol., Lond.* **400**, 35p.

SYED, N. I. AND WINLOW, W. (1988*b*). The role of central neurones in respiratory behaviour in *Lymnaea*. *J. Physiol., Lond.* **403**, 62p.

SYED, N. I. AND WINLOW, W. (1989). Morphology and electrophysiology of neurones innervating the ciliated locomotor epithelium in *Lymnaea stagnalis* (L.). *Comp. Biochem. Physiol.* **93**A, 633–644.

VAN DER WILT, C. J., VAN DER ROEST, M. AND JANSE, C. (1988). The role of two peptidergic giant neurones in modulation of respiratory behaviour in the pond snail, *Lymnaea stagnalis*. *Symp. biol. hung.* **36**, 377–386.

VIALA, D. (1986). Evidence for direct reciprocal interactions between the central rhythm generators for spinal 'respiratory' and locomotor activities in the rabbit. *Expl Brain Res.* **63**, 225–232.

WEISS, K. R., COHEN, J. AND KUPFERMANN, I. (1978). Modulatory control of buccal musculature by a serotonergic neuron (metacerebral cell) in *Aplysia*. *J. Neurophysiol.* **41**, 181–203.

WILLOWS, A. O. D., DORSETT, D. A. AND HOYLE, G. (1973). The neuronal basis of behaviour in *Tritonia*. I. Functional organization of the central nervous system. *J. Neurobiol.* **4**, 207–237.

WINE, J. J. AND KRASNE, F. B. (1982). The cellular organisation of crayfish escape behaviour. In *The Biology of Crustacea*, vol. 4, *Neural Integration and Behaviour* (ed. D. C. Sandemann and H. L. Atwood), pp. 242–292. New York: Academic Press.

WINLOW, W. AND HAYDON, P. G. (1986). A behavioral and neuronal analysis of the locomotory system of *Lymnaea stagnalis*. *Comp. Biochem. Physiol.* **83**A, 13–21.