PHYLOGENETIC PLASTICITY OF CRUSTACEAN STOMATOGASTRIC CIRCUITS

I. PYLORIC PATTERNS AND PYLORIC CIRCUIT OF THE SHRIMP PALAEMON SERRATUS

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Summary

Recordings from the muscles of the pyloric chamber of the shrimp Palaemon display a rhythmic pattern which is either monophasic or biphasic, and is different from the triphasic pyloric pattern of large decapods. Identification of the pyloric neurones in the stomatogastric ganglion and study of their synaptic relationships indicate that the pyloric circuit of Palaemon is very similar to the pyloric circuit of large decapods. It is concluded that homologous neuronal circuits in related species, although similar in terms of their ‘wiring diagram’, are able to produce significantly different patterned outputs.

Introduction

Our understanding of the cellular activity which underlies behaviour has been considerably improved by study of selected preparations, usually of invertebrates, as simple models of the nervous system (Bullock, 1986). With in vitro preparations of isolated centres, it is sometimes possible to record activity patterns which are directly comparable to those observed in the intact animal (Delcomyn, 1980). Using such preparations, with their limited number of neurones, the network which organizes a given motor output can be studied in terms of both synaptic relationships and intrinsic properties of the constituent neurones. Until now the most significant results have been obtained for elementary rhythmic behaviour such as swimming in Tritonia (Getting, 1983a,b), heart beat in the leech (Calabrese & Peterson, 1983), feeding in the snail (Benjamin, 1983) and foregut movements associated with feeding in Crustacea (Selverston & Moulins, 1987).

To obtain some idea of the general rules that can govern the organization of a nervous system, it is tempting to make mechanistic comparisons between these different networks, and some common ‘building blocks’ can be recognized in most cases (Getting, 1988). However, the rules that govern the arrangement of these building blocks within each network are not obvious. Possibly a more useful

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approach is to make comparisons of homologous networks belonging to related species but which produce significantly different motor patterns. This would permit us to ascertain how a given network can be modified in the course of evolution and to determine which components are stable and which can be modified.

It is using this comparative perspective that we have currently investigated the stomatogastric system of decapod crustaceans. In this system, the network which organizes the rhythmic movements of the pyloric chamber in lobsters is probably one of the best understood networks for central pattern generation (Miller, 1987). It is responsible for a triphasic pattern of rhythmic contraction involving the dilator muscles, the anterior constrictor and the posterior constrictor muscles of the pylorus. The sequential activation of these muscles can be shown with in vivo recordings (Rezer & Moulins, 1983) and the underlying motor output patterns can be recorded in vitro (Maynard, 1972; Maynard & Selverston, 1975). The 13 motoneurones that innervate these muscles have been identified in the stomatogastric ganglion in which, along with a solitary interneurone (AB), they are interconnected in a discrete network. The synaptic connectivity within this network and the properties of the neurones themselves have been studied extensively and we now have a good idea of how the triphasic pyloric pattern is produced. In other decapods such as shrimps, however, although it is possible to identify homologous stomatogastric muscles, specifically pyloric muscles (Meiss & Norman, 1977), the feeding behaviour appears to be different and the anatomy of the foregut is considerably modified. To investigate how the pyloric pattern is organized in these animals, the present investigation has been carried out on a newly developed in vitro preparation of the stomatogastric system of the shrimp Palaemon serratus (Pennant, 1777) (Meyrand & Moulins, 1986). The results presented in this paper lead to the conclusion that, despite considerable differences in the pyloric patterns of shrimp and lobsters, the homologous pyloric networks themselves are remarkably similar. In the subsequent paper (Meyrand & Moulins, 1988), we report the effects of extrinsic inputs projecting onto the pyloric network of Palaemon and, by comparison with the pyloric system of large decapods (see Nagy & Moulins, 1987), we show how different extrinsic influences impinging on similar neuronal networks can produce substantially different outputs.

Materials and methods

All experiments \((N = 90)\) were performed on female shrimp Palaemon serratus, caught in the Atlantic Ocean by local fishermen at Royan (France). The animals were maintained in the laboratory in large tanks of running sea water. These tanks were continuously aerated and held at a temperature of 15 °C.

In a few experiments, the pyloric pattern was recorded from semi-intact preparations. Intracellular recordings were made from the pyloric muscles using glass microelectrodes inserted through a small hole in the dorsal cuticle of the cephalothorax.
The preparation used most was the stomatogastric nervous system muscle preparation of Meyrand & Moulins (1986). This preparation was developed for this animal from the in vitro preparation used routinely to record from the stomatogastric nervous system of lobsters and other large Crustacea (see combined preparation of Selverston et al. 1976). It consisted of the stomatogastric nervous system left attached to the intact pyloric chamber. After opening the cephalothorax dorsally, the stomach and the pyloric chamber were removed, and the ventral nerve cord was cut on each side of the commissural ganglion (CoG), allowing the removal of the foregut along with the stomatogastric nervous system. The oesophagus and stomach were then dissected free and removed. The stomatogastric nervous system and the pyloric chamber were pinned out in a Sylgard-lined Petri dish. The stomatogastric ganglion (STG) was desheathed for intracellular recordings from the somata of the pyloric neurones. Extracellular recordings were made using fine platinum wire placed against the appropriate nerve. The small size of the preparation (3–4 cm² compared with 70 cm² for the stomatogastric system of Homarus, for example), required a modified experimental chamber. A large hole was cut in the under-side of the Petri dish allowing access from below for extracellular wire electrodes pushed through the Sylgard. With this approach, sufficient space above the preparation was obtained to record simultaneously from several somata in the STG and from several pyloric muscle fibres with intracellular glass microelectrodes. In each experiment motoneurones were identified by simultaneous recordings from the cell’s soma, its axon in the motor nerve, and the appropriate muscle.

The preparation was continuously perfused with saline (in mmol l⁻¹): 479, NaCl; 12.7, KCl; 20, MgSO₄; 3.9, Na₂SO₄; 14.3, CaCl₂; 5, Hepes buffer; pH 7.45, held at constant temperature (15°C). Glass microelectrodes for recording from neurones or muscle fibres were filled with 2 mol l⁻¹ potassium acetate and had tip resistances of 20–30 MΩ. WPI electrometers were used for intracellular recordings and for current injection, whereas extracellular recordings were made with AC FM122 Tektronix amplifiers. Recordings were displayed on a 5113 Tektronix oscilloscope and stored on an MP 5521 Schlumberger magnetic tape recorder. Permanent recordings on paper were obtained with a Gould ES 1000 electrostatic recorder.

Occasionally a Vaseline pool was built up around the rostral ganglia for perfusion with an isotonic (750 mmol l⁻¹) sucrose solution (adjusted to pH 7.45 with NH₄OH). This solution blocked axonal conduction and was used to isolate the STG reversibly from oesophageal and commissural ganglia.

Results

Anatomy

From studies of the anatomy of the foregut of decapod crustaceans (Mocquart, 1883; Maynard & Dando, 1974; Meiss & Norman, 1977) it appears that large
Fig. 1. Anatomy of the foregut and the stomatogastric nervous system of large decapods (A,C) and Palaemon serratus (B,D). Diagrammatic left lateral view of the stomach of Palinurus (A) and P. serratus (B). Diagrammatic left lateral view of the pyloric chamber of Palinurus (C) and P. serratus (D). Br, brain; CoG, commissural ganglion; CS, cardiac sac; C1, anterior constrictor muscle; C2, posterior constrictor muscle; Dm, dilator muscle; GM, gastric mill; MG, midgut; OE, oesophagus; P, pylorus; SOG, suboesophageal ganglion; STG, stomatogastric ganglion; STO, stomach; cpvl, dorsal cardiopyloric valve muscle; cpv2, ventral cardiopyloric valve muscle; dvn, dorsal ventricular nerve; gm1,2,3, gastric mill muscles; gt, gastric teeth; lvn, lateral ventricular nerve; p1–p14, pyloric intrinsic muscles; stn, stomatogastric nerve.

morphological differences exist between reptantian (large decapods such as lobsters and crabs) and natantian (e.g. shrimp) species.

In the shrimp Palaemon serratus, the foregut consists of a very short oesophagus (OE), an elongated stomach region (STO) and, posteriorly, a pyloric chamber (P) (Fig. 1B). The stomach of this shrimp differs from that of the large decapods (Fig. 1A) in its complete absence of gastric teeth (gt) and associated extrinsic muscles (gm1, gm2, gm3). The pyloric chamber is also modified, although it is still possible to recognize the homologous groups of muscles described for the pyloric chamber of large decapods (see Maynard & Dando, 1974; Meiss & Norman, 1977). The dilator muscles comprise one dorsal (Dm) and one ventral muscle (Fig. 1D) which are equivalent to cpvl and cpv2, respectively, in large decapods (Fig. 1C). There are two groups of constrictor muscles, one anterior (C1) and one posterior (C2). C1 appears to be equivalent to the anterior constrictor muscles of large decapods (p1–p2; Fig. 1C), C2 consists of a group of longitudinal muscle fibres that insert on the posterior region of the pyloric chamber and on this basis can be considered equivalent to muscles p3–p14 of large decapods (Fig. 1C).
The muscles of the foregut are innervated by the stomatogastric nervous system (Figs 1B, 2A). Just as in large decapods (Selverston et al. 1976), the stomatogastric nervous system of Palaemon consists of four ganglia: the stomatogastric ganglion (STG), the oesophageal ganglion (OG) and the two commissural ganglia (CoG). The stomatogastric ganglion lies on the anterior surface of the stomach and, as in other decapods, is connected to the more rostral ganglia (the oesophageal ganglion, OG, and the commissural ganglia, CoG) via a single nerve tract, the stomatogastric nerve (stn). The dorsal ventricular nerve (dvn), which emerges posteriorly from the stomatogastric ganglion, is the main pyloric motor nerve. It divides into two lateral ventricular nerves (lvn) which run along the walls of the stomach to the pyloric chamber. Each lvn then divides into a dorsal branch that innervates the dilator muscles and a ventral branch that innervates the constrictor muscles.

Although many foregut muscles (especially gastric teeth muscles) appear to be missing in shrimp, its stomatogastric ganglion contains about the same number of neurones (25) as in the large decapods. This ganglion is typically arthropod in design with a dorsal rind of neurone somata overlying a central neuropilar mass. In Palaemon, however, there are two or three strata of superimposed somata and this considerably limits the possibility of impaling more than a few neurones during the course of one experiment.

### Spontaneous pyloric motor patterns

The pyloric motor pattern was monitored in all experiments with intracellular recordings from the pyloric muscles. As we will see later, all fibres of the same muscle are identically innervated. Thus recording of excitatory junction potentials (EJPs) from only one muscle fibre can be considered as a monitor of activity in the whole muscle and of motoneurones innervating that muscle. These muscle recordings were obtained either from semi-intact preparations, after minimal opening of the carapace giving access to the pyloric chamber, or from in vitro preparations (Fig. 2A) in which the pyloric chamber, with the stomatogastric nervous system, was transferred to a Petri dish. No differences were observed in the results obtained with either preparation. In more than 80 % (73 out of 90) of such experiments, a spontaneous rhythmic activity was recorded in pyloric muscles. Two different patterns were observed.

1. In 60% of these active preparations, the pattern was monophasic, involving only the anterior constrictor muscles (C1) (Fig. 2B). The rhythm was slow and the pyloric cycle period (as measured from the onset of consecutive anterior constrictor bursts) was characterized by a large variability ($X = 4.43 \pm 1.17 \text{s}$; Fig. 2D, open columns).

2. In the remaining 40% of active preparations, the activity pattern was biphasic involving alternation between the two types of constrictor muscles (C1 and C2) (Fig. 2C). In this case, the pyloric period was very short and characteristically constant ($X = 0.45 \pm 0.04 \text{s}$; Fig. 2D, hatched columns).

Thus, in none of our experiments did the pyloric dilator muscle (Dm) exhibit...
any spontaneous rhythmic activity, totally unlike observations in large decapods, where it has been shown both in vivo (Maynard, 1972; Maynard & Selverston, 1975) and in vitro (Rezer & Moulins, 1983) that the pyloric dilator muscles are always spontaneously and rhythmically active. The latter finding is not surprising since it is well known (see Miller, 1987) that the neurones innervating the dilator muscles act as pacemakers along with the AB interneurone for the whole STC network which organizes the triphasic pyloric activity.
Identification of pyloric STG neurones

Identification of pyloric motoneurones in the stomatogastric system of large decapods (Maynard, 1972) involves routine recording from terminal branches of motor nerves. In *Palaemon* these nerves are too short to record motoneuronal activity. However, our *in vitro* preparation (Fig. 2A) does allow identification of pyloric STG motoneurones by simultaneous recordings from muscle fibres, the main motor nerve (lvn) and neuronal somata in the STG. With this approach it was possible to correlate muscle EJPs one-for-one with neuronal spikes recorded extracellularly from the axon and intracellularly from the cell body (e.g. Fig. 3B). In each case, cell identification was confirmed by: (1) the constancy of the latencies between intracellular somata spike, axon spike and muscle EJP (e.g. Fig. 3C); (2) stimulation of the motor nerve evoking an antidromic spike in the cell body and an EJP in the muscle fibre (e.g. Fig. 4E); (3) depolarization of the neuronal cell body inducing spikes which again corresponded one-for-one with subsequent axonal spikes and muscle EJPs.

**Dm motoneurones**

Although the motoneurones innervating the dilator muscles (Dm) were never spontaneously active, they could be made to fire by injecting depolarizing current into the soma or by electrical stimulation of their axons in the lvn. This has revealed (data previously published in Meyrand & Moulins, 1986) that the Dm muscle receives strictly two excitatory motoneurones from the STG and that this dual innervation is shared by all Dm muscle fibres. Because Dm is equivalent to the pyloric dilator muscle of the large decapods, we have followed the convention for Dm neurones by calling them pyloric dilators (PD).

**Cl motoneurone**

The anterior constrictor muscle almost always exhibited spontaneously rhythmic bursts of EJPs. Each EJP was associated with a neuronal spike recorded extracellularly from the lvn and intracellularly in the cell body of one STG neurone (Fig. 3B). As shown in Fig. 3C the latencies between the cell body spike, axonal spike in the lvn and EJP in the muscle fibre were strictly constant. Several arguments suggest that Cl is innervated by only one motoneurone. First, all EJPs recorded in any Cl fibre were correlated with only a single unit in the lvn. Second, it never proved possible during the same experiment to penetrate more than one STG neurone which projected to Cl. Third, single electrical shocks delivered to the lvn provoked only a single-amplitude EJP, whatever the strength of stimulation (Fig. 3D). In other words, it never proved possible to recruit more than one motoneurone innervating Cl by stimulation of the motor nerve. It is therefore our conclusion that Cl receives only one excitatory motoneurone. Because Cl seems to be homologous to the p1–p2 muscles of the large decapods this neurone has been called the lateral pyloric (LP).
Fig. 3. Identification of Cl motoneurone (LP). (A) Diagram of the motor innervation to the left and right Cl and the recording/stimulating conditions for B,C. (B) During spontaneous activity each burst of EJPs in a muscle fibre of Cl is correlated with a burst of extracellular spikes in the motor nerve (lvn) and a burst of spikes recorded in the cell body of the LP motoneurones in the STG. (C) Superimposed oscilloscope sweeps (5) triggered by spontaneous cell body spikes in LP. Each soma spike is followed at constant latency by an extracellularly recorded impulse in the motor nerve (lvn) and an EJP in a Cl fibre. (D) Stimulation of lvn provokes an EJP, at constant latency, in Cl. Only one amplitude of EJP is obtained at all stimulus strengths. Horizontal bars, 500 ms in B; 5 ms in C,D; vertical bars, 20 mV.

C2 motoneurones

When the posterior constrictor muscles were active, they showed rhythmic EJP bursts which could be correlated with bursts of spikes in the motor nerve (lvn) and in the cell body of a STG neurone (Fig. 4B). Each spike recorded from the impaled cell body was followed at constant latency by an extracellular axonal spike in the motor nerve (lvn) and an EJP in the C2 muscle fibre (Fig. 4C). That all these
Fig. 4. Identification of C2 motoneurones (PY). (A) Diagram of the motor innervation to the left and right C2. (B) Spontaneous rhythmic activity of the posterior constrictor neuromuscular system as recorded from the cell body of a PY neurone, the lvn motor nerve and from a C2 muscle fibre. (C) Superimposed oscilloscope sweeps (3) triggered by spontaneous intracellular soma spikes in the PY neurone: each spike is followed at constant latency by a spike in the motor nerve (lvn) and an EJP in a C2 muscle fibre. (D) Individual EJPs in a C2 fibre are associated with spontaneous spikes in one or other of two PY neurones simultaneously impaled in the STG. The second and third muscle depolarizations correspond to summation of EJPs due to simultaneous firing in the two PYs. (Ei) Extracellular stimulation of lvn (four sweeps) provokes an antidromic spike at constant latency in the soma of PY, and an EJP in C2. (Eii) Changing the strength of the stimulus (S) reveals only two populations of EJPs. The larger EJP (single arrows) is correlated with an antidromic spike in the penetrated PY, but the smaller one (double arrow) is not. Ei and Eii are from the same experiment. Stimulus frequencies were always less than 0.2 Hz to avoid facilitating or defacilitating effects on EJP amplitude. Horizontal bars, 10 ms in C, E1, E2 and 200 ms in B,D; vertical bars, 10 mV.
events arose in the same neurone innervating C2 was confirmed by electrical stimulation of the motor nerve (lvn); this always evoked an antidromic spike in the penetrated cell body (top trace in Fig. 4Ei) and also caused a constant-latency EJP in the C2 muscle fibre (bottom trace in Fig. 4Ei). However, C2 was innervated by more than one motoneurone: in the recording of Fig. 4B the muscle showed EJPs which did not correspond to spikes in the impaled cell body. In some experiments, moreover, it was possible to penetrate two cells simultaneously in the STG that clearly innervated C2 (Fig. 4D). In these cases, all spontaneous EJPs could be correlated with a soma spike in either of these motoneurones. That only two motoneurones seem to innervate C2 is further demonstrated by the experiment in Fig. 4Ei. In this experiment the lvn was stimulated repetitively at low frequencies to eliminate facilitatory or defacilitatory effects on EJP amplitudes. The strength of the electrical stimulus was then progressively increased to recruit progressively any different axons innervating the muscle. At a certain stimulus threshold an EJP appeared in the posterior constrictor muscle (double arrows in Fig. 4Eii, lower trace) which was not associated with an antidromic spike in the penetrated C2 motoneurone (Fig. 4Eii, top trace). However, with further increase in stimulus strength, a larger EJP (single arrow in the lower trace) appeared which corresponded one-for-one to an antidromic spike in the penetrated motoneurone. Further increase in the strength of the stimulus never produced a further increase in EJP amplitude. The ability to distinguish two EJP amplitudes (and only two) strongly suggests that C2 is innervated by two motoneurones only. Further relevant observations are that, during the same experiment, we have never penetrated more than two motoneurones in the STG that innervate C2 and each time that these two motoneurones were recorded simultaneously, all EJPs in C2 could be correlated with spikes in either of the motoneurones. It is evident that all fibres of the C2 muscle are innervated by these two motoneurones. Since the homologous muscles (p3–p14) of large decapods are innervated by neurones named PY, the same nomenclature has been used for C2 motoneurones of shrimp.

In summary, five motoneurones innervate the pyloric muscles of the shrimp Palaemon: the dorsal dilator muscle (Dm) receives two PD neurones, the anterior constrictor muscle (C1) receives one LP neurone and the posterior constrictor muscle (C2) two PY neurones. In the shrimp, as in the large decapods, all motoneurones to muscles of the pyloric chamber appear to be purely excitatory since no inhibitory junction potentials have been observed occurring either spontaneously in muscle fibre recordings or in response to lvn stimulation.

**STG pyloric interneurone**

We have also identified one neurone in the STG which could be either silent (during the monophasic pyloric pattern, see Fig. 11) or rhythmically active, producing bursts of spikes in time with the biphasic pyloric motor rhythm (Fig. 5B). This neurone does not belong to the group of pyloric motoneurones as indicated by the data in Fig. 5C, which show that it is impossible to correlate any spike recorded on the two motor nerves (lvn) with the cell body spike of this
Fig. 5. Identification of the STG pyloric interneurone (AB). (A) Diagram summarizing the axonal geometry of the interneurone which projects to the commissural ganglia via the left and right superior oesophageal nerves (son_l, son_r). (B) The cyclic variations in membrane potential (and rhythmic bursts of spikes) of AB are in phase with the pyloric motor output monitored by recordings from C1 and C2 muscle fibres. (C–D) Superimposed oscilloscope sweeps (10) triggered by AB soma spikes show that AB does not project in the left and right lvn, and D demonstrates that the AB axon projects to the commissural ganglia via the left and right son. (E) Electrical stimulation of the son evokes an antidromic spike, at constant latency, in the cell body of AB (five sweeps). Horizontal bars, 500 ms in B and 10 ms in C,D,E; vertical bars, 10 mV.

neurone. Rather, this cell is an interneurone that projects rostrally to the commissural ganglia (CoG), since its cell body spike could always be associated with a spike recorded in the two superior oesophageal nerves (son) (Fig. 5D) that connect the STG to the CoG. Moreover, electrical stimulation of the son always evoked an antidromic spike in the cell body (Fig. 5E). An equivalent STG pyloric interneurone, the anterior burster neurone (AB), has been identified in the stomatogastric system of large Crustacea. We have also named this neurone AB in the shrimp.
Synaptic relationships between STG pyloric neurones

The above demonstrates how individual pyloric neurones penetrated in the STG can be identified without ambiguity in each experiment. To understand how the observed pyloric patterns are organized, however, requires analysis of the synaptic relationships between these neurones. Questions then arise as to the nature of the wiring diagram and how it compares with the equivalent cellular network already described in large decapods. To achieve this ideally requires paired recordings, pre- and postsynaptically, from identified neurones. However, for technical reasons, this is difficult to achieve from this preparation. In some cases, therefore, the firing activity of a presynaptic neurone (Fig. 7A,B), or even of the postsynaptic neurone (Fig. 8B,D), has been monitored by recording only extracellularly from its axon in the motor nerve or intracellularly from its target muscle.

Despite these experimental limitations, however, we have sufficient data to construct a wiring diagram for the pyloric CPG of the shrimp (Fig. 13). This network is characterized by two principal features: (1) pyloric neurones innervating the same muscles (PDs for Dm; PYs for C2) are electrically coupled and (2) reciprocal inhibitory connections generally occur between pyloric neurones innervating different muscles.

Electrical synapses

In a preceding paper (Meyrand & Moulins, 1986), we have shown that the two PD motoneurones are electrically coupled.

The PD neurones are also electrically coupled to the single AB interneurone (Fig. 6A). Injection of depolarizing current into the cell body of the interneurone caused depolarization of PD (Fig. 6Ai) whereas injection of the same depolarizing pulse into PD caused depolarization of AB (Fig. 6Aii). In contrast, a hyperpolarizing pulse injected into AB (Fig. 6Aiii), or into PD (Fig. 6Aiv), caused hyperpolarization of the other neurone. No other synaptic relationships were evident between the two PD motoneurones and the AB interneurone, suggesting that the three cells tend to operate in synchrony.

Using the same experimental method, we have demonstrated that the two PY motoneurones are also electrically coupled. Depolarization or hyperpolarization of one PY caused equivalent depolarization or hyperpolarization of the other. No other synaptic relationships evidently exist between these neurones.

Finally, it has been possible to show that the dilator group (PD–AB) is also electrically coupled to the posterior constrictor group (PY) (Fig. 6B). Experimental depolarization of a PD neurone (Fig. 6Bi) or of a PY neurone (Fig. 6Bii) induced depolarization of the other. Conversely, experimental hyperpolarization of a PD neurone (Fig. 6Biii) or of a PY neurone (Fig. 6Biv) caused hyperpolarization of the first neurone.

Chemical inhibitory synapses

LP inhibits the two other groups of pyloric neurones. In most experiments LP
Fig. 6. Electrical coupling between AB and PD neurones (A) and between PD and PY neurones (B). Depolarization (i) or hyperpolarization (iii) of AB by current injection (I) causes depolarization or hyperpolarization of PD. A similar result is obtained for AB by manipulating the membrane potential of PD (ii, iv). (B) A depolarizing (i) or a hyperpolarizing (iii) pulse of current injected into PD causes depolarization or hyperpolarization of PY. Similarly, depolarization (ii) or hyperpolarization (iv) of PY evokes depolarization or hyperpolarization of PD. Horizontal bars, 200 ms in A and 1 s in B; vertical bars, 10 mV or 5 nA.
was spontaneously active and produced rhythmic bursts of action potentials. Each LP burst was associated with hyperpolarization of the PY neurones (Fig. 7A) and of PD neurones (Fig. 7B). In each case this hyperpolarization resulted from the summation of inhibitory postsynaptic potentials (IPSPs) which, in some exper-
ments, were sufficiently discrete to be associated one-for-one with LP action potentials (Fig. 7A,B). Generally, however, these IPSPs defacilitated very quickly and so during a presynaptic (LP) burst only the first IPSPs could be of sufficient amplitude to be recognized individually and correlated with presynaptic spikes (see Fig. 7C). That LP inhibits the two other groups of pyloric neurones was also evident from experimental depolarization of the LP neurone. Again this evoked hyperpolarization of the postsynaptic neurones as shown for PD in Fig. 7D.

The PD–AB group inhibits the two groups of constrictor neurones. In our experimental conditions, the PD neurones were always silent. However, induction of firing by depolarizing current injection always produced hyperpolarization and/or cessation of firing in the PY (Fig. 8A) and LP (Fig. 8C) neurones. Examination of the records from PY and LP did not allow recognition of individual IPSPs during PD stimulation and for this reason we have no direct evidence for the possible monosynaptic nature of these synaptic relationships. However, the same results could be obtained when the STG was isolated from rostral (oesophageal and commissural) ganglia by superfusing the latter with an isotonic sucrose solution (see Materials and methods) (Fig. 8C). Thus the LP and PY inhibition, at least, cannot be due to an intercalated neurone located outside the STG.

Depolarization of the AB interneurone, which is electrically coupled to the PD neurones, was also able to produce inhibition of PY and LP as is shown indirectly in Fig. 8B,D. In this experiment, PY and LP firing was monitored by intracellular recordings from C2 and C1 muscle fibres, respectively. A strong depolarization of AB, to increase and maintain its firing, caused concomitant cessation of EJP activity in both C2 (Fig. 8B) and C1 (Fig. 8D). This indicates that the AB depolarization (and firing) inhibited and prevented spontaneous firing in the motoneurones PY (Fig. 8B) and LP (Fig. 8D) innervating these muscles.

The PY group inhibits LP. During spontaneous rhythmic activity, each burst of spikes in PY neurones was associated with periodic hyperpolarization of LP (Fig. 9A). This hyperpolarization was relatively smooth; unitary IPSPs could not be recognized and so could not be correlated with any presynaptic spikes. That PY participated, directly or indirectly, in this inhibition, however, is evident in Fig. 9B. In this experiment a PY neurone, which was spontaneously silent, was activated by injection of depolarizing current; this in turn provoked inhibition of LP which stopped firing. These effects could also be obtained when the STG was isolated from rostral ganglia, indicating that PY inhibition of LP cannot be mediated by an intercalated neurone located outside the STG. Moreover, in this experiment the activity of the PD and AB neurones was also monitored. Both cells remained silent during the PY depolarization. Thus, it is not immediately evident that the electrical coupling between PY and the PD–AB group is indirectly responsible for the inhibition of LP by PY depolarization (Fig. 4B), although the possibility of non-spike-mediated release of transmitter (Graubard et al. 1983) cannot be excluded.

In summary, the six pyloric neurones are associated in a network (see Fig. 13) in
Fig. 8. The dilator neurones (PD–AB) inhibit the constrictor neurones (LP and PY). (A,C) Injection of depolarizing current into a PD cell body causes overall hyperpolarization and cessation of firing in a PY neurone (A) or LP (C). C was obtained from a preparation in which the STG was isolated from rostral ganglia by sucrose block. (B,D) Injection of depolarizing current into the soma of AB to increase its firing suppresses the bursts of EJPs recorded from a C2 muscle fibre (innervated by the PY neurones) (B), and from a C1 muscle fibre (innervated by the LP neurone) (D). Horizontal bars, 500 ms; vertical bars, 10 mV.

which: (1) the two PD motoneurones (innervating Dm muscles) and the two PY motoneurones (innervating C2 muscles) are electrically coupled; (2) the AB interneurone is electrically coupled to the PD motoneurones; (3) the dilator group (PD–AB) is electrically coupled to the PY neurones; (4) the LP motoneurone (innervating C1 muscle) inhibits the dilator group (AB–PD) and the PY neurones; (5) the dilator group inhibits (directly?) the LP and PY neurones; (6) the PY neurones inhibit (directly?) the LP neurones.
Fig. 9. The PY neurones inhibit LP. (A) During spontaneous activity, each burst of spikes in a PY neurone is phase-locked to hyperpolarization in LP. (B) Injection of suprathreshold depolarizing current (I) into a previously silent PY neurone hyperpolarizes and stops the firing of a previously active LP neurone. B was obtained from a preparation in which the STG was isolated from rostral ganglia by sucrose block. Horizontal bars, 500 ms; vertical bars, 15 mV, 5 nA.

Intrinsic properties of the STG pyloric neurones

It is now well established that the output pattern of rhythm-generating neuronal networks depends both on the synaptic connections within the network and on the individual properties of the constituent neurones (Selverston & Moulins, 1985). In large decapods, all the pyloric neurones can express intrinsic membrane properties which promote their bursting discharges (Russell & Hartline, 1978; Miller, 1987; T. Bal, F. Nagy & M. Moulins, in preparation) and which play a major role in the organization of the pyloric output. In this context, it is important in the present study to know if the shrimp pyloric neurones also possess intrinsic regenerative properties.

It is possible to demonstrate such regenerative properties using simple tests of current injection. In cells which display 'plateau potentials', a brief pulse of depolarizing current of sufficient amplitude provokes a jump in membrane potential whose duration considerably outlasts that of the trigger pulse and during which the neurone produces a burst of action potentials (Russell & Hartline, 1978, 1982; Tazaki & Cooke, 1979). Moreover, in this type of cell a brief pulse of hyperpolarizing current delivered during the discharge of the neurone can prematurely and definitively terminate this discharge. In our experiments with Palaemon, only one neurone, LP, consistently displayed such bistable behaviour. A depolarizing current pulse of 100 ms injected into the soma was sufficient to
Fig. 10. Intrinsic regenerative properties of pyloric neurones. (A) The LP neurone always expresses active plateau potential properties. A brief pulse of depolarizing current (I) injected into the soma of LP provokes a long duration discharge (Ai), but a brief pulse of hyperpolarizing current prematurely terminates an ongoing spontaneous burst of spikes (Aii). (B,C) During the monophasic pyloric pattern neither PY nor PD neurones express regenerative properties. Brief pulses of depolarizing current (to test for plateau properties) fail to induce a long-duration discharge in PY (Bi) or PD (Ci); a sustained depolarization by current injection (I) (to test oscillatory properties) does not provoke oscillatory activity in either PY (Bii) or PD (Cii). (D) AB is not an endogenous oscillatory cell. Injection of sustained depolarizing current (second and first traces) or hyperpolarizing current (fourth trace) do not modify the frequency of spontaneous rhythmic depolarizations in AB. This lack of voltage-dependence of the frequency indicates that the AB oscillatory behaviour is synaptically driven and not of intrinsic origin. Horizontal bars, 1s in Bi, Cii; 500 ms in A,D and 100 ms in Bi,Ci; vertical bars, 15 mV in Bi, Cii, D; 10 mV in A, Bi, Ci; 5 nA in Bi, Cii and 2 nA in A, Bi, Ci.

induce a regenerative depolarization (and discharge) of more than 2-5 s (Fig. 10Ai). Conversely, a brief hyperpolarizing pulse could switch off an ongoing plateau discharge by triggering repolarization to the baseline (Fig. 10Aii). In contrast, the PD (Fig. 10Ci) and AB neurones never produced such maintained...
Fig. 11. Lack of activity in the PD–AB neurone group during the spontaneous monophasic pyloric pattern. PY neurones (see C2) are silent and only LP produces rhythmic bursts of spikes (see lvn) which rhythmically excite the Cl muscle. Note the apparent lack of inhibition of PD and AB by LP. Horizontal bar, 1 s; vertical bars, 5 mV.

depolarization in response to a brief pulse of depolarizing current. This was also true for the PY neurones when tested during the monophasic pyloric pattern (Fig. 10Bi) (see Meyrand & Moulins, 1988).

Endogenous oscillatory properties can be tested using sustained experimental depolarization (or hyperpolarization). This manipulation can either induce oscillatory behaviour, if the cell’s membrane potential lies outside the voltage range in which it can oscillate, or modify the rate of oscillation in a voltage-dependent manner if the cell was previously oscillating (Frazier et al. 1967). Sustained depolarization of a PD cell (Fig. 10Cii) or of a PY cell during expression of the monophasic pyloric pattern (Fig. 10Bii) provoked tonic discharge only and no oscillatory activity. During the biphasic pyloric pattern the AB neurone displayed cyclic variations in membrane potential and it discharged in bursts. However, as shown in Fig. 10D, the origin of this behaviour is probably not intrinsic to the interneurone itself: injection of depolarizing (second and first traces of Fig. 10D) or hyperpolarizing (fourth trace of Fig. 10D) current had no effect on the frequency of the cell’s oscillations. Only the amplitude of the membrane potential variations was modified, increasing with hyperpolarization and decreasing with depolarization, suggesting that AB’s cyclic membrane fluctuations were due to a rhythmic excitatory input.

**Spontaneous activity of the pyloric dilator group**

We have shown previously that the two pyloric patterns observed in *Palaemon*, in our experimental conditions, never involve activation of the dilator muscles (Dm) (Fig. 2). Direct intracellular recording from the PD motoneurones also showed that they were silent during both the monophasic (Fig. 11) and biphasic
patterns (Fig. 12A), although in the second case they received cyclic synaptic excitation that was phase-locked to the pyloric rhythm. The AB interneurone, which is electrically coupled to the PD motoneurones, was also silent during the monophasic pattern (Fig. 11) but participated in the pyloric rhythm during expression of the biphasic pattern (Fig. 12A). In this case AB displayed rhythmic depolarizations on which bursts of spikes occurred. However, as shown in Fig. 10D (see above), these depolarizations are not likely to result from the expression of some endogenous oscillatory property but, rather, result from a
cyclic excitatory input. A final comparative point is that in each pyloric cycle, the AB spike burst was seen to be virtually in phase with the constrictor bursts in LP and PY (Fig. 12A,B). This appears to constitute a major difference from the pyloric pattern of large Crustacea in which AB (and PD) always burst in antiphase with constrictor discharges (see Fig. 12C,D).

Discussion

Many studies have been made comparing homologous neurones of related species in a large variety of animals, including leeches (Keyser & Lent, 1977), opisthobranch molluscs (Dickinson, 1980), crabs (Paul, 1981), flies (King & Valentino, 1983) and other insects (Wilson et al. 1982; Arbasi, 1983a,b). The present work was undertaken with a different perspective: its goal was to compare homologous networks of identified neurones belonging to related species and to consider both the patterns of output produced by these networks, and the characteristics of these networks (number of the neurones, properties of the neurones, synaptic relationships between the neurones) in an effort to distinguish those evolutionary features that remain conserved from those that are plastic.

The pyloric patterns and networks of large decapod crustaceans have been studied extensively in the lobster Panulirus interruptus (Maynard, 1972; Maynard & Selverston, 1975) and also in Palinurus vulgaris (Moulins & Vedel, 1977), Jasus lalandii (Nagy, 1981), Homarus gammarus (Robertson & Moulins, 1981; Cazalets, 1987) and Cancer borealis (Hooper et al. 1986). In all these species, which we call 'large decapods', the pyloric patterns of activity are almost indistinguishable and only minor differences have been observed in the properties of the neurones and their synaptic relationships. It is with these data that the results obtained in this paper require comparison. One difference to be noted initially is that in large decapods there is a cardiopyloric valve located in front of the pyloric chamber. Movements of the valve are governed by a dilator and a constrictor muscle. These muscles are innervated by the VD and IC neurones, respectively (Maynard, 1972), which are also participants in the pyloric pattern. These muscles have not been identified in shrimps (Meiss & Norman, 1977) and we have not identified any corresponding motoneurones in Palaemon.

Pyloric patterns

In large decapods, both in vitro and in vivo recordings (Rezer & Moulins, 1983) show that pyloric output is normally a triphasic rhythmic pattern in which the dilator muscles (innervated by PD), the anterior constrictor muscles (innervated by LP) and the posterior constrictor muscles (innervated by PY) are sequentially active. In Palaemon, in the two types of preparations we have developed, the pyloric pattern can be either monophasic, with only the anterior constrictor muscles rhythmically active, or biphasic, with participation of the anterior and the posterior constrictor muscles (i.e. of the LP and PY motoneurones) which are alternately active. That the shrimp pyloric pattern is never triphasic is due to the
fact that the PD motoneurones are never spontaneously rhythmically active. Moreover, there is a large difference in the behaviour of the single interneurone AB in large decapods and *Palaemon*. In the former, AB is always rhythmically active in antiphase with the constrictor motoneurones (LP and PY), whereas when active in shrimp (i.e. during biphasic pattern), AB fires in phase with the constrictor neurones.

In *Palaemon* our recordings were obtained from preparations in which some sensory feedback may occur from proprioceptors associated with the pyloric chamber. However, it appears that this sensory feedback is not responsible for the main characteristics of the pyloric pattern observed in the present study. This is based on the observation that neither the monophasic nor the biphasic patterns are modified after complete isolation of the stomatogastric nervous system from the pyloric chamber. Thus a first step towards understanding how such different output patterns occur requires comparison between *Palaemon* and large decapods as to the nature of the neuronal circuits that generate these pyloric patterns.

**Number of pyloric neurones**

Careful identification of pyloric neurones has shown that the network in *Palaemon* contains only five motoneurones and one interneurone. In most large decapods, the pyloric network consists of 11 motoneurones and one interneurone, excluding the two motoneurones of the cardiopyloric valve (see above). This numerical difference is due largely to the number of PY neurones, eight in most large decapods (Maynard, 1972; Moulins & Vedel, 1977; Robertson & Moulins, 1981) and only two in *Palaemon*. However, only five or six PY neurones have been found in the crab (Hermann, 1979a, b; Hooper et al. 1986) and this species variation in numbers of pyloric elements in large decapods does not appear to be associated with any qualitative differences in the pyloric pattern observed in each species. This is not too surprising because, in each case, the neurones are electrically coupled and function essentially as a single unit. On this basis, therefore, the presence of only two PY neurones in *Palaemon* seems unlikely to explain the observed differences in pyloric output from large decapods.

**Wiring diagram**

As mentioned in the Results, we have not been able to demonstrate conclusively the monosynaptic nature of several synaptic connections in the pyloric circuit of *Palaemon*. However, in all cases where direct evidence has not been obtained, polysynaptic relationships via intercalated neurones with axonal projections outside the STG have been eliminated by observing continued synaptic connectivity between cell pairs after isolation of this ganglion from rostral ganglia.

The main feature of the proposed wiring diagram for *Palaemon* (Fig. 13B) is its structural similarity with that described for large decapods (Fig. 13A). Qualitatively, the only difference that exists is the unique electrical coupling between the PD–AB group and the PY group in *Palaemon*. Such a coupling, although probably weaker, has also been described in the crab *Cancer pagurus* (Hermann, 1979a, b)
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Fig. 13. Comparison of the pyloric networks of large crustaceans and the shrimp (*Palaemon serratus*). Black dots, inhibitory synapses; resistor, electrical synapse; ∫, endogenous regenerative properties.

and the lobster *Jasus lalandii* (Nagy, 1981). However, it is difficult to imagine that this coupling is responsible for the peculiarity of the *Palaemon* pyloric patterns since it can only participate in the phase-locking of bursts in AB with those of the PY pyloric constrictor neurones. From these results we conclude that the two networks belonging to related species, although responsible for completely different patterns of output, are nevertheless characterized by similar wiring diagrams.

**Properties of the neurones**

It is now well established that the output of rhythmic neuronal networks is a product of two general mechanisms: the synaptic relationships within the network (represented by the wiring diagram) and the inherent properties of the neurones themselves (Selverston & Moulins, 1985; Getting, 1988). In large decapods, all the pyloric neurones possess regenerative properties (Miller, 1987; T. Bal, F. Nagy & M. Moulins, in preparation). However, it is the dilator group (PD–AB), via its endogenous oscillatory properties, which plays the role of pacemaker for the network, imposing its own frequency on all other neurones via inhibitory synaptic relationships. This appears to be one crucial difference from the *Palaemon* pyloric network where it is impossible to demonstrate any endogenous oscillatory capability in AB or PD during expression of either of the two pyloric patterns. In *Palaemon*, LP is the only neurone which invariably displays strong regenerative properties, in turn allowing an understanding of how the monophasic pyloric pattern is produced. During this pattern, LP oscillates, perhaps in a free-run mode, and probably because all the other pyloric neurones do not exhibit regenerative properties, the strength of their phasic inhibition by LP is sufficiently weak to be unable to drive them to any rhythmic bursting activity.

In contrast, the results reported here do not allow an understanding of how the *Palaemon* network can produce the biphasic pyloric pattern. How do the PY neurones become able to produce bursts of spikes when during the monophasic pattern they remain silent? Why does the AB neurone produce bursts of spikes in
phase with LP–PY bursts? In relation to the first question, it is important to note that, in large decapods, the regenerative properties of the pyloric neurones are strictly conditional (Russell & Hartline, 1978; Miller & Selverston, 1982; Moulins & Cournil, 1982) and can be expressed only when the neurones are submitted to extrinsic modulatory influences (Nagy & Moulins, 1987; Marder, 1987). Similarly, we can postulate that the biphasic pattern in *Palaemon* occurs only when extrinsic modulatory inputs have evoked regenerative properties in PY. Concerning the second question, the wiring diagram alone cannot explain how AB, which has reciprocal inhibitory synaptic relationships with LP, is able to fire in phase with this neurone (and also PY). Again the explanation for this must reside outside the network itself. The subsequent paper (Meyrand & Moulins, 1988) tries to answer these questions and demonstrate how, under extrinsic input control, similar neuronal networks can produce significantly different patterns of output.

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