PHYLOGENETIC PLASTICITY OF CRUSTACEAN STOMATOGASTRIC CIRCUITS

II. EXTRINSIC INPUTS TO THE PYLORIC CIRCUIT OF THE SHRIMP PALAEMON SERRATUS

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Summary

The rhythmic motor patterns produced by the pyloric circuit of the shrimp Palaemon are substantially different from those of large decapods, although the homologous neuronal circuits are very similar (Meyrand & Moulins, 1988). The extrinsic inputs received by the Palaemon pyloric circuit were similar, at least qualitatively, to the extrinsic inputs known to impinge upon the pyloric circuit of large decapods. These include: rhythmic inputs that descend from another oscillator (the commissural pyloric oscillator, CPO) which appears to impose its own rhythm on neurones of the pyloric circuit; and inputs that have long-lasting effects and control the expression of endogenous burst-generating properties of individual neurones within the circuit via modulatory mechanisms.

The AB–PD neurones were not observed to oscillate, and so do not appear to act as pacemakers for the pyloric circuit as they do in large decapods, probably because of differences in the organization of the modulatory extrinsic inputs.

It is our conclusion that differences in the control of expression of intrinsic properties of neurones of homologous (and structurally similar) circuits belonging to related species can explain how such circuits produce widely different patterned motor outputs.

Introduction

Mechanistic descriptions of several 'simple' circuits responsible for rhythmic behaviour are now available (Roberts & Roberts, 1983; Getting, 1988). However, it has become increasingly evident from such studies that, although experimental analysis of these circuits in isolation has been necessary to identify component neurones and determine their synaptic relationships, such an elemental approach does not allow understanding of how the same circuit can produce the range of activity patterns seen in the intact animal. Recent work has begun to address this problem and, in particular, the role played by extrinsic influences in the expression of rhythmic motor circuits (Harris-Warrick, 1988).

Key words: neuronal oscillators, modulatory control, crustacean stomatogastric system.
One of the best documented examples of extrinsic control mechanisms involves the pyloric circuit of large decapods (Nagy & Moulins, 1987). It is known both from bath application of putative neuromediators (Marder, 1987) and from stimulation of identified extrinsic input elements (Nagy & Dickinson, 1983; Dickinson & Nagy, 1983; Cazalets, 1987) that this circuit can express a repertoire of output patterns as a function of the interplay between extrinsic modulatory influences. Moreover, it has been shown that the pyloric circuit, at least in Homarus (Robertson & Moulins, 1981) and Jasus (Nagy, 1981; P. Cardi, F. Nagy & M. Moulins, in preparation), receives phasic inputs from a higher-order ‘pyloric’ oscillator located outside the pyloric circuit, which controls the timing of rhythmic output (Moulins & Nagy, 1985; Nagy & Moulins, 1987).

In the preceding paper (Meyrand & Moulins, 1988) we have shown that the pyloric circuit of the shrimp Palaemon is very similar to the homologous circuit in large decapods in terms of cellular properties and connectivity, but displays patterns of activity which are very different. These differences must arise from external factors, so in the present paper we examine extrinsic inputs to the pyloric circuit of Palaemon and their possible role in the organization of the pyloric output. It is our conclusion that, although extrinsic inputs observed in Palaemon are not qualitatively dissimilar from those described for the pyloric circuits of large decapods, it is differences in the control of the endogenous properties of target neurones by modulatory inputs which underlie the different pyloric patterns in these related species.

Materials and methods

Experiments were performed on female pink shrimp Palaemon serratus (Pennant, 1777) purchased from local fisherman at Royan (France) and maintained in the laboratory in circulating aerated sea water at 15°C. Isolated in vitro preparations of the stomatogastric nervous system were used in which the pyloric chamber was left attached (see Fig. 1) (Meyrand & Moulins, 1986). The saline and recording techniques from muscle fibres, nerves and neurone somata in the desheathed stomatogastric ganglion (STG) were as described in the preceding paper (Meyrand & Moulins, 1988).

Perfusion of an isotonic sucrose solution in a Vaseline pool built up around rostral (commissural and oesophageal) ganglia was used to block impulse conduction, thereby allowing reversible isolation of the STG from extrinsic inputs (Fig. 1). Similarly a Vaseline pool built up around the STG (Fig. 3) was used to isolate rostral ganglia reversibly from STG inputs or to perfuse modified salines containing either dopamine (Sigma) or a muscarinic agonist, oxotremorine (Sigma).

Results

In Palaemon, as in large decapods, the pyloric chamber possesses three groups
of muscles: the dilator (Dm), the anterior constrictor (C1) and the posterior constrictor (C2) (Fig. 1). These muscles are innervated either by one (LP for C1) or two electrically coupled (two PD for Dm, two PY for C2) motoneurones that are organized into a central pattern generating circuit in the stomatogastric ganglion (STG) (Meyrand & Moulins, 1988). In addition to these motoneurones, a single interneurone (AB) participates in this network, and its axon projects rostrally in the stomatogastric nerve (stn). The spontaneous patterns displayed by this pyloric circuit could be recorded from in vitro preparations by impaling the three muscles simultaneously and monitoring the spiking activity of each pyloric motoneurone via their excitatory junction potentials (EJPs) in each muscle (Figs 1, 3). Two patterns could be observed: either a biphasic pattern involving C1 and C2 muscles (i.e. LP and PY) (Fig. 1A) or a monophasic pattern involving C1 alone (i.e. LP) (Fig. 3A).

These two patterns are very different from the triphasic pattern observed in large decapods in comparable experimental conditions, although the corresponding pyloric circuits are very similar. For this reason it was our conclusion (Meyrand & Moulins, 1988) that only inputs arising from outside the STG can explain how these different patterns are produced. Our preparation consists of the stomatogastric ganglion (STG), the oesophageal (OG) and commissural (CoG) ganglia connected to the STG via the stomatogastric nerve (stn), the superior oesophageal nerve (son), the inferior oesophageal nerve (ion) and the pyloric chamber (see diagram in Fig. 1). In principle, extrinsic inputs to the pyloric circuit would arise from the rostral ganglia via the stn or from proprioceptive elements associated with the pyloric chamber and projecting to the STG via the lateral ventricular nerve (lvn). However, if such proprioceptive inputs exist, they do not play a major role in the organization of the pyloric pattern since the latter remains unmodified by section of the terminal branches of lvn near the pyloric muscles (Meyrand & Moulins, 1988). For this reason we have considered all inputs in this study to arise from rostral ganglia and to project to the STG via the stomatogastric nerve (stn).

**Modulatory extrinsic inputs and the biphasic pyloric pattern**

To determine whether extrinsic influences impinging on the pyloric network from rostral ganglia play a major role in the organization of the biphasic pyloric pattern described in the preceding paper (Meyrand & Moulins, 1988), our first approach was to test the effects of removing these influences during ongoing production of this pyloric pattern (Fig. 1). In the experiment of Fig. 1A, the pyloric output had the characteristics of a biphasic pattern; the two constrictor muscles C1 and C2 (innervated by the LP and PY motoneurones, respectively) were alternately active while the dilator muscles (Dm) (innervated by the PD motoneurones) remained silent. The cycle period was short, less than 0.7 s. When all extrinsic inputs were suppressed by perfusion of a sucrose solution on the rostral ganglia, the pyloric output was strongly modified and became monophasic in nature. Although the posterior constrictor muscles (C2) were no longer activated, indicating that the PY motoneurones were now silent, the anterior
Fig. 1. Inputs from rostral ganglia in the somatogastric nervous system are necessary for the expression of the biphasic pyloric pattern. (A) The spontaneous biphasic pyloric pattern as recorded intracellularly from muscle fibres of the three different types of pyloric muscles, and extracellularly from the lateral ventricular nerve (lvn). The dilator muscle (Dm) remains silent, while the anterior constrictor C1 and the posterior constrictor C2 muscles show alternating bursts of EJPs that indicate suprathreshold activity in LP and PY neurones, respectively. (B) When inputs to the stomatogastric ganglion (STG) are blocked by perfusion of the rostral ganglia (see diagram) with an isotonic sucrose solution, C2 becomes silent. The LP neurone is still bursting, although its period is considerably increased. (C) After the removal of the sucrose block, the biphasic rhythm returns. CoG, commissural ganglion; OG, oesophageal ganglion; ion, inferior oesophageal nerve; son, superior oesophageal nerve; stn, stomatogastric nerve. Horizontal bars, 0.5 s; vertical bars, 10 mV.
constrictor muscles (Cl) continued to be excited alone (by rhythmic bursts in the LP motoneurone). Moreover, the period of the rhythm became much longer (more than 2 s). When the sucrose block was removed (Fig. 1C), the biphasic pattern returned immediately. We can thus conclude that the stomatogastric ganglion (STG), when isolated from rostral ganglia, is unable to produce rhythmic pyloric activity other than the monophasic pattern in which the LP neurone fires alone. In other words, the STG (and the pyloric network) receives inputs from rostral ganglia that are necessary to generate the biphasic pattern.

**PY oscillatory properties and the biphasic pattern**

It is well known that although all pyloric neurones of large decapods have endogenous regenerative properties that contribute to burst formation, these properties are conditional, i.e. they are expressed only in the presence of modulatory 'permissive' inputs coming from rostral ganglia (Nagy & Moulins, 1987; Marder, 1987). On this basis it can be postulated that although PY neurones in *Palaemon* do not at times appear to have regenerative properties (Meyrand & Moulins, 1988), such properties do exist but can be expressed only under the influence of rostral inputs. This notion was tested in the experiment of Fig. 2A. A brief stimulation (400 ms at 20 Hz) of the stomatogastric nerve (stn) in a preparation in which a PY neurone was quiescent provoked a long-lasting (more than 30 s) activation during which the PY neurone began to oscillate and produced rhythmic bursts of spikes. In pyloric neurones of large decapods, two types of regenerative properties promoting bursting have been described (Russell & Hartline, 1982). In the first, termed 'plateau' properties, the neurone displays intrinsic membrane bistability. A diagnostic feature of this property is the ability of a short pulse of intrasomatic depolarizing current to induce a large depolarizing shift in membrane potential which considerably outlasts the duration of the triggering pulse. In the second, termed 'oscillatory' properties, the membrane potential is unstable and oscillates regularly. Although an oscillatory cell may also respond to a brief pulse of depolarizing current with a regenerative shift in membrane potential (and a full oscillation which outlasts the duration of the pulse), an endogenous oscillatory mechanism is generally characterized by the voltage-dependence of its cycle frequency (Frazier *et al.* 1967) revealed by injection of sustained current.

In large decapods, it is known that some cholinergic (muscarinic) agonists such as oxotremorine are able to evoke the regenerative properties of pyloric neurones (Nagy *et al.* 1985). In *Palaemon* also this pharmacological treatment was able to switch PY from a non-oscillatory to an oscillatory state (Fig. 2B,C). Under control conditions, the PY neurone did not express any regenerative properties and responded passively to a brief pulse of depolarizing current (Fig. 2Bi) or to a sustained depolarization (Fig. 2Ci). However, after bath application of $10^{-6}$ mol l$^{-1}$ oxotremorine on the STG, a short pulse of depolarizing current triggered a plateau potential and a burst of spikes (Fig. 2Bii) and sustained depolarization induced rhythmic oscillations, whose frequency was voltage-
Fig. 2. Inducing oscillatory properties of PY neurones either by stimulation of the stomatogastric nerve (stn) or by pharmacological stimulation of the STG with oxotremorine. (A) A brief stimulation of the stn causes long-lasting oscillatory activity in PY. (B,C) In normal saline (control), a short depolarizing pulse of current injected into the soma of PY (I) produces only a passive depolarization of the neurone (Bi). After bath application of oxotremorine \((10^{-6} \text{ mol}1^{-1})\), the same depolarizing pulse triggers a regenerative depolarization and resultant firing (Bii). Although sustained depolarization of PY produces only a passive depolarization in the control (Ci), in oxotremorine, sustained depolarization produces regenerative oscillations, the frequency of which is voltage-dependent (compare C2 and C3). Horizontal bars, 1s in A,C and 50 ms in B; vertical bars, 10 mV or 2 nA.

dependent (Fig. 2Cii,Ciii). From these experiments it can be concluded that PY possesses oscillatory properties, but that expression of these properties depends upon extrinsic influences. Thus, although these conditional properties are not expressed during the monophasic pyloric pattern (Meyrand & Moulins, 1988) it is probable that a biphasic pattern occurs only when PY neurones are in an oscillatory state.

This notion was tested in the experiment shown in Fig. 3 in which we used
oxotremorine to evoke PY oscillatory properties and thereby change the type of pattern expressed by the pyloric network. In control conditions (Fig. 3A), the pyloric network displayed a monophasic pattern; the anterior constrictor muscles (C1) were rhythmically activated (by LP motoneurones) but all the other muscles were quiescent. After bath application of oxotremorine on the STG (Fig. 3B), the pyloric rhythm became biphasic with LP (see C1) and PY (see C2) bursting in...
antiphase, while PD (see Dm) remained silent. Thus we conclude that inducing the oscillatory properties of PY (here obtained with oxotremorine) is essential to obtain the transition from a monophasic to a biphasic pattern. In this artificial experimental situation, the rhythmic pattern appears to be generated by only two oscillatory neurones (LP and PY) coupled by reciprocal inhibition and without requiring any neuronal input from rostral ganglia. This was confirmed by the observation that, in the presence of oxotremorine, the pyloric output remained biphasic (Fig. 3B) and its period was unchanged after cutting the stn (not shown). However, it must be noted that the cycle period of the drug-induced rhythm was 2–3 times greater than that of the spontaneous biphasic rhythm (cf. Fig. 3B and Fig. 1A). This suggests that the spontaneous biphasic rhythm depends not only on the induction of PY oscillatory properties (by some extrinsic modulatory influences) but also on some other extrinsic inputs that are responsible for its higher cycle frequency.

**Rhythmic extrinsic inputs: the commissural pyloric oscillator**

In contrast to the spontaneous monophasic pyloric pattern, the biphasic pattern is characterized by rhythmic depolarizations and spike bursts in the dilator AB interneurone and the constrictor motoneurones, while the dilator PD motoneurone, although rhythmically depolarized with AB, again does not fire (Meyrand & Moulins, 1988). This pattern was always associated with bursting activity in units (other than AB) recorded in the stomatogastric nerve (stn) (Fig. 4A) and time-locked to the rhythmic activity of the pyloric network. These second pyloric bursts in stn commenced with the depolarizing phase of AB and PD (see also Fig. 6A) but ceased when AB started to fire (Fig. 4B). That this extra bursting activity originates in the commissural ganglion is shown in Fig. 4C, where the oesophageal ganglion and inferior oesophageal nerves (ions) were removed; simultaneous recordings from the superior oesophageal nerve (son) and the stn showed that each spike in the latter was correlated one-for-one and preceded by an impulse in the former.

In principle, this rhythmic activity descending from the commissural ganglion could be generated either *via* a long loop from the pyloric network itself in the STG or by a discrete oscillator located in the commissural ganglion. To distinguish between these two possibilities we have performed experiments in which the commissural ganglia were reversibly isolated from ascending rhythmic inputs from the pyloric network of the STG (Fig. 5). This was achieved by application of an isotonic sucrose solution in a Vaseline pool built around the STG. Before sucrose block (Fig. 5A), a biphasic pyloric pattern was recorded on the main motor nerve (lnv) and rhythmic pyloric-related activity arising from the commissural ganglia was recorded in the left and right superior oesophageal nerves (son₁ and son₂). After sucrose block (Fig. 5B), pyloric motor output stopped (see lvn) but, although altered, the bursting activity occurring in the pyloric phase of the rhythm on the oesophageal nerves in Fig. 5A continued. When the sucrose block was removed, the original pyloric motor rhythm returned and the rhythmic activity
Fig. 4. The STG receives pyloric rhythmic input from rostral ganglia. (A) During a pyloric biphasic pattern (see C1 and C2 muscle recordings) a burst of spikes time-locked with motor output (see also recordings from somata of AB interneurone and PD motoneurone) occurs in the stn. (B) This burst is composed of large spikes which do not belong to the AB neurone, whose axonal activity can be seen intrasomatically and as small spikes in the stn. (C) These spikes arise from the commissural ganglion (CoG) as shown by their timing in simultaneous recordings from the son and stn. Horizontal bars, 250 ms in A,B and 10 ms in C; vertical bars, 5 mV.

recorded on the sons recovered its control period and phase relationship with the pyloric motoneuronal activity (Fig. 5C). Since commissural ganglia, when isolated, are still able to produce a rhythmic output, the conclusion is that they contain an oscillator which projects to the STG.

The alterations which occurred in the rhythmic activity recorded on the sons after sucrose block were two-fold. First, two separate rhythmic inputs (labelled
Fig. 5. The commissural ganglia contain a pyloric oscillator. (A) Control: rhythmic activity time-locked with the pyloric biphasic motor output (lvn) recorded in the right (r) and left (l) sons. (B) The STG is bathed with isotonic sucrose solution to block impulse conduction and thereby suppress pyloric motor output (see lvn) and any rhythmic pyloric input to the commissural ganglia. The rhythmic bursting activity in the two sons slows down but does not disappear, indicating that the commissural ganglion (CoG) contains an independent pyloric oscillator. This activity becomes separated into two distinct bursts of spikes (open and filled arrowheads), suggesting that each CoG contains a pyloric oscillator that operates independently from its contralateral partner in the absence of STG inputs to the rostral ganglia. (C) After washing the STG with saline, the biphasic pyloric motor output returns (see lvn) and the two commissural oscillators again produce synchronized bursts (arrowheads) in the sons. Horizontal bar, 0.5 s.
with open and filled arrowheads in Fig. 5B) became apparent. Although difficult to distinguish in low-speed recordings, these two units were also present in the control recording (Fig. 5A) but their rhythmic bursts were synchronized. Our interpretation is that each unit arises from one or other of the two commissural ganglia and travels to the STG via the stn and to the contralateral CoG via the contralateral son. As long as the STG pyloric circuit is active, these two units fire in synchrony due to common (bilateral) feedback from the circuit (see below); otherwise they fire independently at their inherent cycle frequency. The second alteration which occurred after sucrose block was that the periods of these two commissural oscillators were considerably increased, although remaining very stable (note that the intrinsic period of the oscillator marked with open arrowheads is considerably shorter than that of its counterpart). From these observations we conclude that the two oscillators receive STG inputs which, during biphasic pyloric output at least, synchronize and increase the frequencies of their respective activity. Because these oscillators (when connected to the STG) are bursting in time with pyloric output, they have been called commissural pyloric oscillators (CPO). They are directly comparable to the CPO described in Homarus (Robertson & Moulins, 1981).

We have not been able to penetrate CPO neurones and thus have no direct evidence for their effects on the STG neurones. However, the CPO appeared to excite (probably monosynaptically) most, if not all, neurones of the STG pyloric circuit. In each pyloric cycle AB and PD exhibited a burst of summated excitatory postsynaptic potentials (EPSPs) during the CPO burst (Fig. 6A). The resulting depolarization was slow and AB started to fire only towards the end of the CPO burst. The PY neurones were also slowly depolarized by a burst of EPSPs in synchrony with the EPSPs in AB-PD, but were able to fire only after the CPO burst had terminated (Fig. 6B). For LP we do not have direct evidence that it also receives an excitatory drive from the CPO burst. However, LP was the first neurone to start firing in each pyloric cycle and this occurred during the second half of the CPO burst (Fig. 6C), suggesting that it is the cell most sensitive to CPO excitation.

Together the present results suggest that the biphasic pyloric pattern depends on (1) the induction of oscillatory properties in PY neurones, and (2) the simultaneous phasic activation of all STG pyloric neurones by the CPO. In such a perspective, however, the role played by the dilator (AB and PD) neurones remains unclear.

**Dilator neurone oscillatory properties**

In large decapods, the dilator group of neurones (PD-AB) plays a major role in the generation and organization of the pyloric pattern: they act as pacemakers in the pyloric circuit (Maynard, 1972) and impose their endogenous rhythm on the constrictor neurones via strong inhibitory synaptic influences (Miller, 1987). However, it is difficult to imagine that they have a comparable role in Palaemon, since in this species they never display endogenous oscillatory capability.
Although the AB neurone produces rhythmic bursts during the spontaneous pyloric biphasic pattern, the underlying depolarizations are not due to any endogenous oscillatory ability (Meyrand & Moulins, 1988) but appear to be synaptically produced, resulting from strong phasic excitatory input from the CPO. The PD neurones also displayed membrane potential oscillations during the biphasic pattern, but here again these oscillations were not due to some active intrinsic property as indicated by the following experiments. First, the frequency of PD oscillation did not display voltage-dependence as is characteristic of endogenous oscillatory neurones: injection of increasing levels of steady depolar-

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**Fig. 6.** Pyloric neurones in the STG are rhythmically activated by the commissural pyloric oscillator (CPO). During the biphasic pyloric pattern, each pyloric neurone (see AB and PD in A, PY and PD in B) starts to depolarize with the CPO burst of spikes recorded in the stn. This is also probably true for LP which starts to fire (as monitored by Cl EJPs) during the CPO burst (C). Horizontal bars, 0.5 s; vertical bars, 15 mV.
Fig. 7. PD does not express endogenous oscillatory properties during the biphasic pyloric pattern. (A) The frequency of spontaneous membrane potential oscillation in PD (third trace) is not increased by injection of increasing levels of sustained depolarizing current (1 nA, second trace; 2 nA, first trace). (B) A brief pulse of current injected into PD soma (I) produces a passive depolarization only. Horizontal bars, 0-5 s; vertical bars, 10 mV or 2 nA.

izing current into a PD neurone eventually caused spikes to appear on the crest of each oscillation, but had no effect on the frequency of oscillation (Fig. 7A). Second, this rhythmic behaviour in PD did not involve regenerative plateau properties, since injection of a brief depolarizing pulse into the cell body of PD produced passive depolarization only and never a maintained plateau (with spiking) (Fig. 7B; see Fig. 2B for a similar test on PY).

It remains surprising that the pyloric dilator neurones in shrimp never exhibit any regenerative properties under experimental conditions where the homologous neurones in large decapods are particularly noted for expressing a strong endogenous oscillatory capability. This probably does not imply a fundamental difference in membrane properties which has appeared during the course of evolution, because although PD never oscillated spontaneously in *Palaemon*, it could be induced to do so by experimental manipulation (Fig. 8). First, the PD neurones could occasionally be switched from rest to oscillation by stimulation of the stn. In the experiment shown in Fig. 8A, a brief train of electrical shocks to the
Fig. 8. PD is able to oscillate. (A) A brief stimulation of the stn causes a long-lasting switch of the PD neurone from a quiescent state to an oscillatory state. (B) Bath application of dopamine has the same effect. (Bi) Control: the PD neurone receives rhythmic subthreshold synaptic excitation from the CPO and the Dm muscle is silent. (Bii) After bath application of dopamine (10^{-5} \text{ mol l}^{-1}) on the STG, PD expresses rhythmic large-amplitude depolarizations underlying bursts of spikes which, in turn, cause EJP bursts in the Dm muscle fibre. (Biii) Dopamine effects disappear immediately after washing in normal saline. Horizontal bars, 1 s; vertical bars, 10 mV.

stn induced strong oscillations for more than 20 s in a previously quiescent PD neurone. This strongly suggests that although the PD neurone has the potential for endogenous oscillation and that modulatory inputs are able to induce this intrinsic behaviour, these inputs were never spontaneously active in our experimental preparations. Second, it was possible to obtain similar activation of PD by bath application of dopamine to the STG. Intracellular activity of a PD motoneurone and a fibre of the dilator muscle Dm were recorded simultaneously (Fig. 8B). In control conditions (Fig. 8Bi), PD was silent and the cyclic variations in its
membrane potential are assumed to be due only to phasic synaptic excitation arising from the CPO. After bath application of $10^{-5}\text{mol} \text{l}^{-1}$ dopamine, however, PD produced large membrane oscillations with superimposed bursts of spikes that, in turn, rhythmically excited the dilator muscle fibre (Fig. 8Bii). These oscillations in the presence of dopamine, which were completely reversible (Fig. 8Biii), were markedly different in amplitude, shape and frequency from those due to the synaptic subthreshold drive of the CPO, and are probably of endogenous origin.

**AB activity and frequency of the biphasic pattern**

Although, unlike their action in large decapods, the dilator neurones do not act as pacemakers in the STG pyloric network of *Palaemon*, the AB interneurone appears to play an important role in the temporal organization of the biphasic pyloric pattern. In contrast to the PD neurones, AB always bursts during this pattern as a consequence of the excitatory synaptic drive it receives from the CPO. Importantly, moreover, AB itself also seems to exert direct control on the activity of the CPO by virtue of its axonal projections to the commissural ganglion. This feedback loop was evident where CPO burst activity (recorded on a superior oesophageal nerve, son) occurred during the depolarizing phase of AB oscillation in a preparation showing the biphasic activity (Fig. 9). Injection of sustained hyperpolarizing current (left-hand panel) into the soma of AB to prevent firing resulted in an increase in the CPO cycle period similar to that occurring when STG inputs to the commissural ganglia were suppressed by sucrose block (see Fig. 5). Moreover, injection of sustained depolarizing current (right-hand panel) into AB's soma to induce high-frequency tonic firing, caused complete cessation of CPO bursting in the son. These effects suggest that AB has access to the CPO, providing phasic feedback control of the latter's cycle frequency. Moreover, the temporal relationships between AB's bursts and CPO's axonal discharge in the son (control), and the silencing effect of maintained AB firing (AB depolarized), indicate that the connection from AB to CPO is inhibitory.

**Discussion**

In the preceding paper (Meyrand & Moulins, 1988) we concluded that despite production of dramatically different rhythmic motor patterns, the pyloric circuits of the shrimp *Palaemon* and of large decapods are very similar in terms of neuronal composition and synaptic relationships. However, it was also our conclusion that: (1) the intrinsic properties expressed by some homologous neurones in the two circuits may be different; (2) extrinsic inputs projecting to the circuit of *Palaemon* must be invoked to explain how this circuit is able to produce the so-called 'biphasic' pyloric pattern.

The present paper shows that in *Palaemon* all pyloric neurones have, potentially at least, properties that are equivalent to the endogenous properties described for the pyloric neurones of large decapods. Moreover, we have shown that, like the pyloric circuit of large decapods, the *Palaemon* circuit is linked via a feedback loop
to another oscillator (the commissural pyloric oscillator, CPO) located in the commissural ganglion. It is our conclusion that the failure of a single group of pyloric neurones (the dilators) in *Palaemon* to express intrinsic oscillatory properties is the principle reason why this circuit produces a patterned output which is very different from that of large decapods.

**Extrinsic modulatory inputs and endogenous properties of pyloric neurones**

Although the neuronal properties that concern us here are mainly attributable to voltage-dependent membrane conductances, it is now well established that such conductances are also 'chemical-dependent', i.e. their activation (or inactivation) can be modified by neuromediators (see Kaczmarek & Levitan, 1987). This means that such properties of a neurone are not invariant but must be considered in a dynamic perspective. This concept has been well documented for some endogenous oscillatory neurones in molluscs (Barker & Gainer, 1974) and for the pyloric neurones of large decapods (Harris-Warrick & Flamm, 1986; Nagy & Moulins, 1987). The oscillatory properties of these neurones, although endogenous, are

![Diagram of AB controls the CPO rhythm. The top panel shows spontaneous bursting in AB (intrasomatic) and CPO bursts recorded in a son (CPO activity underlined with bars). When AB is hyperpolarized to stop its firing (left panel) the CPO period is considerably increased. When AB is strongly depolarized it becomes tonically active, and CPO bursting ceases (right panel). Horizontal bar, 1 s; vertical bar, 10 mV.](image-url)
normally under the control of modulatory inputs which can either have 'permissive' (Nagy et al. 1985) or 'suppressive' influences (Cazalets, 1987) on their expression. These influences operate largely via stn fibres which project to the STG; activation of these fibres (Nagy & Dickinson, 1983; Dickinson & Nagy, 1983; Cazalets, 1987) or direct bath application of their putative neuromediators onto the STG (Marder, 1987) are able to switch, in a long-lasting manner, pyloric neurones into (permissive effect) or out of (suppressive effect) an oscillatory state. This means that the properties of such neurones cannot be considered per se but only in the context of the influences they receive at a given time. In the present work on the Palaemon pyloric circuit we have shown that, besides the LP neurone which always appears to be in an oscillatory state (Meyrand & Moulins, 1988), all other neurones can express regenerative properties promoting bursting during stn electrical stimulation or under pharmacological stimulation (oxotremorine for PY and dopamine for PD). Thus, in terms of the dynamic capability of pyloric neurones, there is again no apparent difference between the pyloric circuits of large decapods and Palaemon.

However, there are differences in the conditions that are required to obtain the expression of the regenerative properties of a given neurone. For example, in Palaemon, oxotremorine induces oscillatory behaviour in PY neurones but never in PD neurones, whereas in large decapods, this muscarinic agonist induces regenerative properties in all pyloric neurones and especially the PD neurones (Dickinson & Nagy, 1983). Differences also occur in the effects of dopamine which induces PD oscillatory behaviour in Palaemon but diminishes the oscillatory capability of PD neurones in large decapods (Marder & Eisen, 1984). Thus, although the properties of the neurones are the same in different species, the way in which they are controlled by extrinsic inputs is evidently different. Moreover, with regard to the spontaneous behaviour of pyloric neurones in Palaemon and large decapods under the same experimental conditions (i.e. when the STG is connected to the rostral ganglia), it appears that modulatory inputs do not have the same efficacy in each case. This is particularly evident for the PD-AB neurones which have never been observed, in our experimental conditions, to manifest spontaneous endogenous oscillation in Palaemon as they always do in large decapods.

It has not been possible to determine if such differences are due to species variation in the organization of the neuromodulatory systems projecting to the STG or to inherent differences in sensitivity of homologous neurones to the same modulatory input. Whatever the underlying mechanism(s), however, it is clear that these differences play a crucial role in the phylogenetic plasticity of pyloric output. It is probably because the dilator neurones do not express any oscillatory properties under our experimental conditions (although they have the potential to do so) that the Palaemon pyloric circuit does not display the triphasic pattern that characterizes pyloric activity of large decapods. In the same way it is only when the PY neurones are in an oscillatory state that the Palaemon pyloric circuit can display a biphasic pattern.
Rhythmic inputs and pyloric output

Although the pyloric circuits of large decapods do not need any rhythmic extrinsic input to produce a rhythmic output, it has been shown in *Homarus* that the STG circuit is subject to phasic excitatory drive by another oscillator, the commissural pyloric oscillator (CPO) (Robertson & Moulins, 1981). Identification of one of its constituent elements, the CP neurone, has allowed the demonstration that the CPO entrains the rhythmic activity of neurones of the pyloric circuit in the STG (Nagy, 1981; Moulins & Nagy, 1983). The present work has shown that an equivalent oscillator, whose activity is phase-locked to pyloric output, also exists in the commissural ganglion of *Palaemon*. Thus, the similarity in organization of the pyloric systems in large decapods and *Palaemon* extends to all extrinsic inputs so far described. Moreover, the relationships existing between the CPO and the STG pyloric circuit also appear to be similar: in *Palaemon*, as in *Homarus*, the CPO receives strong inhibitory feedback from the STG pyloric circuit via the AB neurone. However, this feedback appears to be much more powerful in *Palaemon* than in *Homarus*. In the latter, the frequency of CPO oscillation is not modified when this feedback from the pyloric network is suppressed (Robertson & Moulins, 1981; Moulins & Nagy, 1983) whereas in *Palaemon*, a similar decoupling (see Figs 5, 9) causes a considerable decrease in CPO frequency. In this context the role of the AB neurone in large decapods and *Palaemon* appears to be completely different. In the former, AB always oscillates and it plays a major role in the organization of the output of the pyloric circuit by acting as the main pacemaker neurone. Its rostral connection has only a weak regularizing effect on the ongoing activity of the CPO (Nagy & Moulins, 1987). In *Palaemon*, however, AB is never (at least in our experimental conditions) in an endogenous oscillatory state and produces bursts of spikes only by virtue of the excitatory synaptic drive from the CPO. Although it never plays a direct pacemaker role in the pyloric circuit, by virtue of its projection onto the CPO it has a strong, albeit indirect, effect on the pyloric rhythm. Thus, from a functional point of view, the role of AB in *Palaemon* seems to be more that of an efference copy coordinating neurone for the pyloric CPO activity, whereas in large decapods its role is direct, acting as the pacemaker for the pyloric circuit itself.

*Organization of the Palaemon biphasic pyloric pattern*

As mentioned in the conclusion of the preceding paper (Meyrand & Moulins, 1988), an understanding of the pyloric circuit alone is not sufficient to explain how the biphasic pattern is organized in *Palaemon*. However, in combination with the present results on the nature of extrinsic inputs projecting to the circuit, a working model can be proposed as follows.

First, the CPO imposes its rhythm (by repetitive bursts) on the pyloric network. CPO excites all pyloric neurones which therefore start to depolarize simultaneously with the onset of the CPO burst. However, according to their individual intrinsic properties, the follower pyloric neurones will start to fire at different
times. In LP, which has strong regenerative properties, the first few spikes of the CPO burst will immediately provoke a large-amplitude regenerative depolarization so that the neurone fires immediately (see Fig. 4A, for example). Conversely, for AB, which does not have regenerative properties, the time to reach spike threshold will depend on a more gradual summation of excitatory postsynaptic potentials and firing thus may occur almost at the end of the CPO burst. In other words, it is because LP possesses regenerative properties whereas AB does not, that they fire at different times in response to the same excitatory synaptic drive. Moreover, while PY also starts to be depolarized by the first spikes in each CPO burst, the strong inhibitory input it receives from the already active LP slows PY depolarization and prevents PY from firing until LP itself eventually falls silent. At this time AB is depolarized (and fires) and thus may also assist PY to reach firing threshold via their electrotonic junction. Finally, it can be assumed that PY repolarizes according to its endogenous oscillatory property although, again, repolarization of AB (and PD) could contribute to this via their electrotonic connection.

In this scheme, inhibition from AB-PD onto LP and PY in *Palaemon* is apparently unable to maintain the constrictor neurones silent when AB-PD neurones are depolarized, as is the case in large decapods. This is readily understandable since depolarization of the dilators, which is due only to the excitatory drive of the CPO, is much less strong than that observed in large decapods where these neurones always express endogenous oscillatory properties. Thus, it appears that in all pyloric circuits, the CPO exerts simultaneous excitation onto all pyloric neurones, but that the pattern which finally emerges is a direct function of the properties displayed by each neurone at that time and the nature of the synaptic relationships existing within the circuit. The only difference between the pyloric circuits of large decapods and of the shrimp is the existence in the latter of an electrotonic junction between PD/AB and PY neurones (although this connection also exists in some large Crustacea; see Meyrand & Moulins, 1988). As shown above, this connection is not instrumental in determining the species differences in output patterns but may serve only to assist endogenous oscillatory activity in PY and to regulate its phase of firing in the biphasic pattern.

In conclusion, the pyloric circuit we have described (Meyrand & Moulins, 1988) has the potential to produce either a monophasic, a biphasic (as in *Palaemon* itself) or even a triphasic pattern as in large Crustacea (Fig. 10). Although a spontaneous triphasic pattern has never been observed in the shrimp in our experiments; it can be assumed that it could occur under certain conditions which remain to be determined. The particular pattern that the circuit displays at a given time is really only a question of which individual neurones express endogenous regenerative properties at that time. This selection is achieved via modulatory control (open arrows in Fig. 10) and may result in the production of a monophasic pattern (when only LP is in an oscillatory state), a biphasic pattern (when LP and PY are in an oscillatory state) and finally in a triphasic pattern (when all the neurones are in an oscillatory state). However, there are indications that differences do exist between
species in the organization of the modulatory inputs which exert this control; this suggests that phylogenetic plasticity in motor pattern production by the stomato-gastric nervous system of Crustacea does not derive from structural changes in the corresponding central neuronal circuits themselves, but in the modulatory system controlling these circuits.

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References

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