J. exp. Biol. **114**, 71–98 (1985)
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PATTERN AND RESET ANALYSIS OF THE GASTRIC MILL RHYTHM IN A SPINY LOBSTER, *PANULIRUS INTERRUPTUS*

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Accepted 3 August 1984

SUMMARY

1. The burst pattern of the gastric mill rhythm was studied by varying its cycle period in *in vitro* preparations comprising the stomatogastric (STG), oesophageal and (paired) commissural ganglia.

2. Reset tests using intracellular polarization of identified STG neurones showed that the CI, LC, GP and GM cells can all strongly affect the cycle period, and therefore apparently play a role in generating the gastric rhythm.

3. Variation in the cycle period could be obtained by: (i) cutting certain input nerves; (ii) relative coordination between the gastric and oesophageal rhythms; or (iii) intracellular polarization of identified STG cells, especially the LC motoneurone.

4. Variation in the cycle period by any of these means showed that the gastric pattern (in such preparations) comprises two basic alternating phases: a variable-duration 'powerstroke' and a constant-duration 'returnstroke'.

5. The powerstroke is taken to include bursts in the LC, GP and GM motoneurones (since they evoke closing of the gastric mill teeth and mastication of food), along with the interburst intervals of the other cells. The durations of all these events co-varies over a large range, as a linear function of the cycle period.

6. The activity level of neurones bursting during the powerstroke is directly proportional to their burst length, and hence appears to be a basic parameter affecting the cycle period.

7. The returnstroke is taken to include bursts in the CP, AM and LG motoneurones (since they evoke opening and resetting of the gastric mill teeth), along with the interburst intervals of the powerstroke cells. All these events tended to assume a fixed duration.

8. The two-part gastric mill pattern can be analogized to other two-part rhythms, e.g. for terrestrial locomotion, in which the load-bearing phase has a variable duration and accounts for most of the variation in the cycle period whereas the unloaded phase tends to assume a constant duration.

INTRODUCTION

Rhythmic motor patterns can be classified according to the number of burst phases during the rhythm cycle. In a synchronous rhythm, all the motoneurones

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Key words: Reset, stomatogastric rhythm, bursting.

discharge at about the same time, e.g. in the crustacean neurogenic heartbeat (Watanabe, Obara & Akiyama, 1969; Tazaki & Cooke, 1979). Two-part alternating rhythms are found in terrestrial locomotion, e.g. for the flexor and extensor motoneurones controlling a given joint. A four-phase pattern has been suggested for leech swimming (Friesen, Poon & Stent, 1976).

Basic information about a rhythm generator can come from analysing the variation of the phase pattern when the cycle period is allowed to vary. The discharges in different cell groups can be characterized as having a constant, variable, or constant-phase duration (Kristan, 1980). The utility of pattern analysis is that it places constraints on models for the underlying central pattern generator.

The gastric mill rhythm in spiny lobsters is analysed here. The gastric mill is a region of the crustacean stomach for chewing food. The mill consists of three teeth (one medial and two lateral teeth), supported on ossicles moved by striated muscles. All the motoneurones for the gastric mill muscles are located in the stomatogastric ganglion (STG) (Maynard & Dando, 1974).

Several aspects of the gastric mill have been described, including the functional anatomy (Maynard & Dando, 1974; Meiss & Norman, 1977), the neuromuscular properties (Govind, Atwood & Maynard, 1975; Lingle, 1980), sensory feedback from proprioceptors (Dando & Laverack, 1969) and the behavioural sequence (Powers, 1973; Hartline & Maynard, 1975; Fleischer, 1981).

As seen in semi-intact preparations (Hartline & Maynard, 1975), gastric rhythms have a 5–7 s cycle period and consist of the following sequence (see Table 1 for cell names): firing of the LC and GP motoneurones acts to close the lateral teeth to hold food; the four GM motoneurones fire at about the same time to bring the medial tooth forward in a powerstroke to break up the food. Thereafter the two LG motoneurones fire to separate the lateral teeth and release the food, and at about the same time the CP motoneurone fires to retract the medial tooth and restart the cycle. A tenth motoneurone, AM, controls another region of the stomach but behaves much like the CP cell and so is often considered part of the gastric network.

This study analyses the gastric pattern during on-going rhythms in *in vitro* preparations comprising the commissural, oesophageal and stomatogastric ganglia (Fig. 1A). This type of multi-ganglionic preparation includes about 200–300 neurones, most of which are in the commissural ganglia (Russell, 1977). Besides the gastric rhythm, pattern generators for the pyloric and oesophageal rhythms are also spontaneously active (Selverston, Russell, Miller & King, 1976).

A simplified diagram of synaptic connections among the known gastric elements in *Panulirus* is shown in Fig. 1B (Mulloney & Selverston, 1974*a*,*b*; Selverston & Mulloney, 1974; Selverston *et al.* 1976; see Russell, 1984). The ten motoneurones and one interneurone form a 'gastric network' within the STG, but several neurones in the commissural ganglia such as the 'E' interneurones also participate in the gastric system (Russell, 1976; Selverston *et al.* 1976).

The objective of this study was to analyse the variation in the gastric pattern when the cycle period assumed different values; it is concluded that the pattern has two main alternating phases. Intracellular polarization experiments provided evidence as to which STG neurones generate the rhythm.

METHODS

In vitro 'combined' preparations of the foregut nervous system in Californian spiny lobsters, *Panulirus interruptus*, were made by removing the stomach and dissecting free the stomatogastric, oesophageal and (paired) commissural ganglia and transferring them to a saline-filled dish (Fig. 1A) (Russell, 1976). All four of the inferior and superior oesophageal nerves were intact unless otherwise stated; the stomatogastric nerve was usually not desheathed. Impulse conduction in the stomatogastric or oesophageal nerves could be reversibly blocked by making a circular barrier of petroleum jelly around a nerve and filling it with 750 mmol l^{-1} sucrose solution (Russell, 1977, 1979). Further details on the dissection, electrodes and instrumentation are given in Russell & Hartline (1981, 1984). After desheathing the STG, somata were impaled with separate current and voltage electrodes (3 mol l^{-1} KCl, 35≈60 MΩ, although double-barrel electrodes were used where noted, and single-barrel electrodes were used for the CI interneurone. The configurations of microelectrodes are indicated by diagrams in some illustrations. Somata were identified by time-locking between intracellular spikes and spikes in the motor nerves listed in Table 1. The CI interneurone was identified by antidromic stimulation of the stomatogastric nerve. Nomenclature for neurones, ganglia and nerves follows that of Maynard (1972) and Maynard & Dando (1974).

For some of the illustrations, spikes recorded from two different nerves were half-wave rectified and displayed as upward and downward half-spikes on a shared pen-recorder channel, using reduced-speed playback from tape to increase the pen-recorder bandwidth. Recordings from small motor nerves containing the axons of only one type of motoneurone are labelled as the abbreviated cell name, followed by the letter 'n' (e.g. CPn, GMn, etc.); the formal names of the nerves recorded are given in Table 1.

RESULTS

Gastric rhythms in vitro

Activation

Spontaneous gastric rhythms were usually not seen after isolating the STG: the LG and CI cells fired only tonically while the others fired little or not at all. In contrast, *in vitro* preparations with the paired commissural ganglia left attached to the oesophageal and stomatogastric ganglia usually do produce spontaneous gastric rhythms, at least during the initial 4–6 h after dissection and sometimes longer (Russell, 1976, 1977). The effect of the commissural ganglia is illustrated in Fig. 2E in which large oscillations in the CI and CP cells in a combined preparation (E1) were abolished after isolating the STG by sucrose blockade of the stomatogastric nerve (E2, E3).

Experimental cutting of the different oesophageal nerves indicated that inputs for activating the gastric generator travel in all four of the inferior and superior oesophageal nerves. For example, the rhythm survived with only the *inferior* oesophageal nerves intact (Fig. 2B), whereas cutting them abolished the rhythm

Table 1. *Gastric cells and nerves*

Cell name		No.	Type	Burst timing	Behavioural action	Nerve monitored
GM	(gastric mill)	4	mot	PS	protraction of medial tooth	anterior gastric
LC	(lateral cardiac)	1	mot	PS	closing of lateral teeth	lateral cardiac
GP	(gastro-pyloric)	1	mot	PS	?	gastro-pyloric
E	(excitatory)	2	int	PS		superior oesophageal, stomatogastric
CP	(cardio-pyloric)	1	mot	RS	retraction of medial tooth	cardio-pyloric
AM	(anterior median)	1	mot	RS	constriction of cardiac sac	anterior-median
LG	(lateral gastric)	2	mot	RS	separation of lateral teeth	postero-lateral gastric
CI	(continuous inhibitor)	1	int	RS		stomatogastric

Somata are in the stomatogastric ganglion except for one E cell in each commissural ganglion, which may contain additional gastric neurones.

mot, motoneurone; int, interneurone; PS, powerstroke; RS, returnstroke.

Most nomenclature is from Maynard & Dando (1974). Mulloney & Selverston (1974*b*) used the cell names LG, MG, DG, LPG and Int 1 instead of the cell names LC, GP, CP, LG and CI (respectively).

(Fig. 2D). Inversely, the rhythm continued in another preparation when only the *superior* oesophageal nerves were left intact (Fig. 3E1). In some cases, any single oesophageal nerve (the other three nerves having been cut or blocked) could support continued gastric bursting in the STG cells, as demonstrated in Fig. 2C for a single inferior oesophageal nerve. This is consistent with the isolation of 'gastric command fibres' from the inferior oesophageal nerves which, when stimulated continuously, can activate gastric rhythms in the isolated STG (Russell & Hartline, 1984).

The oesophageal ganglion appears to have little or no role in the gastric activation since the blockade of synapses using a barrier around it filled with $0{\cdot}1 \times Ca^{2+} \cdot 6 \times Mg^{2+}$ saline (see Russell & Hartline, 1981), or the removal of the anterior part (Fig. 3E), did not usually abolish gastric output from the STG. Also, the gastric system was typically inactive in preparations with only the oesophageal ganglion attached to the STG (Fig. 2D).

General features of the pattern

A typical gastric rhythm in a combined preparation is shown in Fig. 2A. Some principal features were as follows. (i) For the medial tooth system, firing of the GM powerstroke cells alternated with firing of the CP retractor cell. The AM cell behaved much like CP. (ii) For the lateral teeth system, firing of the LC and GP closer cells alternated with firing of the LG opener motoneurones. (iii) Activity in the medial tooth system was delayed by 0·1–0·2 cycle relative to the lateral teeth system; e.g. GM firing started and sometimes ended later than GP-LC firing. (iv) The GP cell consistently started firing a few hundred ms before the LC cell even though they have been considered synergists (Mulloney & Selverston, 1974*a*). (v) The bursts in a given set of antagonist motoneurones occupied most of the cycle; e.g. the sum of the CP and GM burst durations was only slightly less than the cycle period, so that 'silent periods' were relatively short. (vi) Bursts in a given set of antagonists did not extensively overlap (except for low-frequency firing of LG cells).

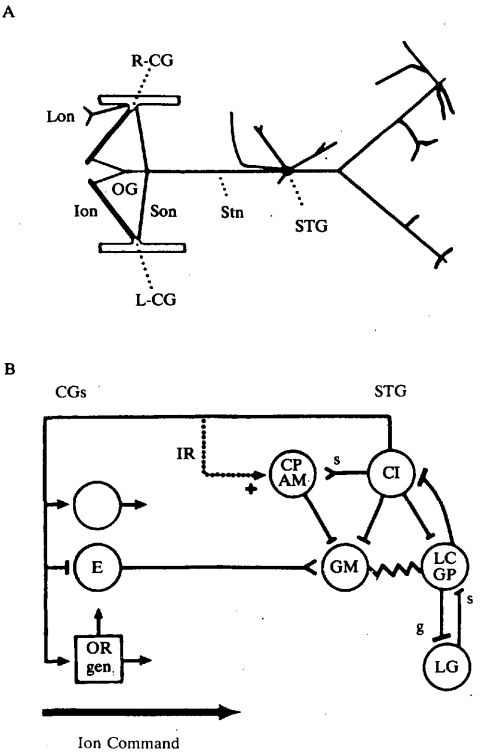

Fig. 1. (A) Diagram of a combined preparation. The paired inferior and superior oesophageal nerves (Ion, Son) connect the paired commissural ganglia (CG) to the oesophageal ganglion (OG; it has an anterior and a posterior region). The stomatogastric nerve (Stn) connects the OG to the stomatogastric ganglion (STG). The paired lateral oesophageal nerves (Lon) were used to record the oesophageal rhythm. (B) Simplified diagram of known synaptic connections in the gastric system. CG, neurones in the commissural ganglia; STG: neurones in the stomatogastric ganglia. See Table 1 for names of neurones. Open circle: possible additional gastric neurones in the commissural ganglia. Bar, fork, resistor symbol: inhibitory, excitatory, electrical synapses. g: conjoint and non-spiking transmission and IPSPs. s: discrete PSPs not visible. Arrow: undefined interaction. +: functional excitation. IR: indirect reflex by which firing of the CI cell gates bursts in the CP cell. OR gen: pattern generator for the oesophageal rhythm. Ion command: fibres in the inferior oesophageal nerves that can activate the gastric network in the STG. Data sources are listed in the text. Larger symbols indicate stronger synapses.

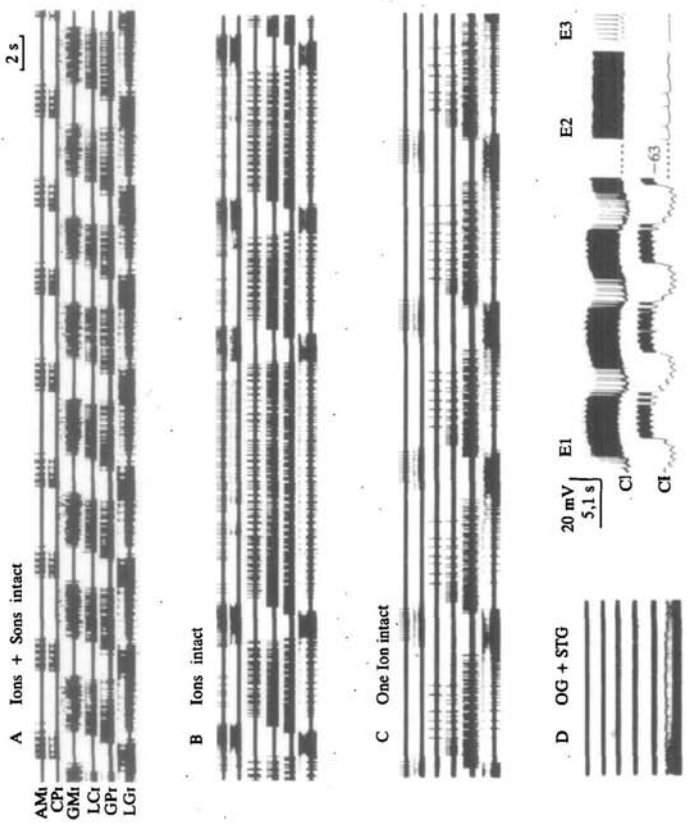

Fig. 2. (A–D) Nerve cutting experiment in a 5-h old combined preparation; spontaneous activity of all the gastric motoneurones is shown. AMn = firing of the AM cell, etc.; Table 1 lists the nerves recorded. (A) All four of the inferior oesophageal nerves (Ion) and superior oesophageal nerves (Son) were intact. (B) Both Sons were cut. (C) Only the right Ion was intact. (D) All four Ions and Sons were cut, leaving the oesophageal ganglion (OG) attached to the stomatogastric ganglion (STG). The large spikes in the LGn traces were from only one of the LG cells. Spike attenuation in the CPn trace (B) and LGn trace (C) was due to the instrumentation. (E) Intracellular activity of the CI and CP cells before (E1) and after (E2, E3) sucrose blockade of the stomatogastric nerve; (E3) is an expanded record.

The instantaneous firing rates during bursts in the different cell types showed consistent relative values and rate profiles. The highest peak rate was in the LG cells (approx. 60 Hz), although the CP and GP cells could also reach relatively high peak rates (approx. 40 and 50 Hz respectively). The peak rate in CI was not more than 15–20 Hz. The lowest peak rate (approx. 20 Hz maximum) and average rate was in the LC cell. The rate profiles in the CP, AM and GP cells typically showed a peak near the start of the burst with a gradual decline afterwards. In contrast, the LG and CI cells showed a ramp-like increase in firing rate during a burst.

The membrane potential and firing rate of the AM, CP, LG and CI cells showed strong 2-Hz modulation due to direct and indirect input from the pyloric rhythm generator (see Fig. 2E1) (Mulloney, 1977; Russell, 1978).

The most labile part of the pattern was the bursting of the lateral teeth closer motoneurones (GP and LC), especially LC. They were usually the first neurones to stop firing as a preparation aged.

Variation in the cycle period

Since it was essential to be able to vary the gastric cycle period, to study variation in the pattern, this section presents three different methods for doing so. The cycle period in a given preparation tended to remain more or less fixed, although there was wide variation between preparations (see Fig. 3B, ordinate).

(i) *Cutting input nerves*

A different cycle period could be obtained by cutting (or blocking) the oesophageal nerves. It was typically found that cutting both of the superior oesophageal nerves caused an *increased firing rate* during bursts in most cells (except the GMs) accompanied by a *longer cycle period* (compare Fig. 2B and 2A).

(ii) *Relative coordination*

The gastric rhythm showed relative coordination with the oesophageal rhythm. The latter is generated in the commissural and oesophageal ganglia and controls peristaltic contractions of the oesophagus (Selverston *et al.* 1976; Moulins & Vedel, 1977). The gastric period was usually (but not always) longer than the oesophageal period (Fig. 3B,C), in some cases three times as long (Fig. 3C).

Relative coordination was seen in fresh preparations in which the two rhythms slowly shifted in phase (Fig. 3A). There appeared to be a 'forbidden' phase for LC bursts near the end of the oesophageal burst (Fig. 3D). The relative coordination is also seen in Fig. 3E3, with CP bursts tending to start near the end of the oesophageal discharges. The relative coordination was associated with change in the gastric cycle period. In the example of Fig. 3A, the gastric period varied from 4·9 to 6·4 s as the two rhythms shifted in phase.

The mechanisms for the relative coordination might involve input from the oesophageal generator to gastric neurones in the commissural ganglia or the STG (see Fig. 1A, 'OR gen'). However the gastric generator also affects the oesophageal generator since a sudden increase in firing of the CI interneurone (in the STG)

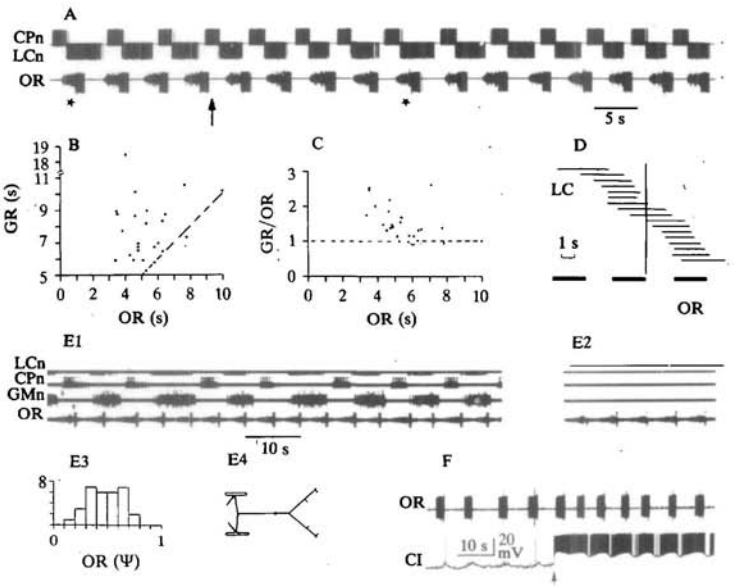

Fig. 3. Relative coordination. (A) Nerve records of the gastric rhythm (GR; spikes from the CP and LC cells are shown on a shared baseline) and the oesophageal rhythm (OR). Asterisks mark the time required for the two rhythms to phase shift until assuming the same relation again. (B) Gastric cycle period (ordinate) and oesophageal cycle period (abscissa) are plotted from 25 preparations that were 3·3–7·4 h old; data points were averages of 8–10 cycles; dashed line indicates equivalence. (C) Data from (B) were replotted as the ratio of the gastric and oesophageal periods (ordinate); it illustrates that the greatest disparity in the periods was associated with a fast oesophageal rhythm. (D) Relative coordination, replotted from (A); thick horizontal bars correspond to oesophageal bursts; vertical line corresponds to the arrow in (A); thin horizontal bars represent a series of LC bursts (from upper to lower). (E) Data from another preparation with the anterior part of the oesophageal ganglion removed (as E4). (E1) Nerve records of the two rhythms; the OR was recorded from a commissural ganglion nerve (Lon, Fig. 1A). (E2) The gastric rhythm stopped when the superior oesophageal nerves were cut, showing that activity in these nerves indeed supported the gastric rhythm in (E1). (E3) Incidence of the start of bursts in the CP gastric cell (see upward arrowhead, E1) at different phases of the oesophageal rhythm (downward arrowhead in E1 shows phase = 0 = 1 in the oesophageal rhythm). (F) Another preparation; firing of the CI interneurone in the STG was suppressed with current, then released at the arrow.

could cause a transient speed-up of the oesophageal rhythm (Fig. 3F). Hence there may be a two-way coupling between the two generators.

(iii) *Cell polarization*

It was found that intracellular polarization of certain STG neurones, but not others, could alter the cycle period.

CP and AM motoneurones. These both acted like 'follower' cells, since strong intracellular stimulation of either had little or no effect on the gastric cycle period

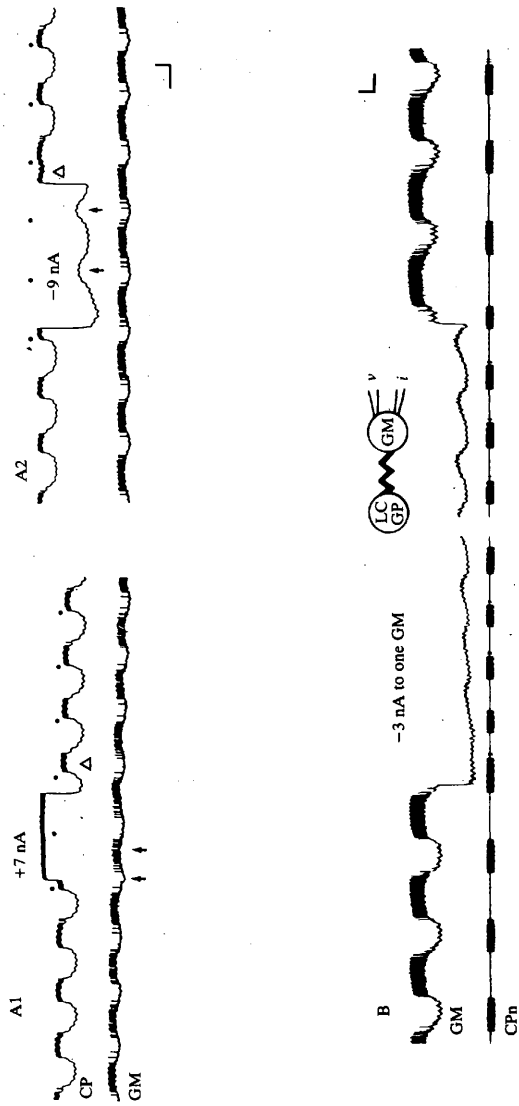

Fig. 4. (A) Intracellular stimulation of CP, either with +7 nA depolarizing current lasting 1·5 cycles (A1) or a −9 nA hyperpolarizing current lasting 2·5 cycles (A2) did not reset the gastric rhythm as monitored intracellularly from a GM cell. Dots mark the expected start of CP bursts, extrapolated from the rhythms before stimulation. The premature onset of the CP burst just after the stimulus in (A2) (triangle) apparently was due to triggering of the CP plateau mechanism; it did not reset the subsequent CP bursting. Arrows in (A1) mark inhibition of the GM cell by CP, causing a hyperpolarization and reduced GM firing. (B) Hyperpolarization of one GM cell by −3 nA caused a shorter cycle period for CP bursting. Note the break in the record. The current was applied in the left part of the record and released in the right part. *v*, *i*: separate voltage recording and current injection electrodes. Calibrations: (A): 20 mV for CP, 10 mV for GM, 2 s; (B): 10 mV, 2 s.

(Fig. 4A for CP). The CP bursts required a few cycles following a long stimulus to return to a normal duration, but then started at the expected time (points, Fig. 4A1 and 4A2). The burst timing of other gastric cells was not significantly disturbed. The *apparent* shift in CP timing just after a stimulus (triangles) may have been due to the regenerative plateau property of CP (Russell & Hartline, 1984); e.g. a depolarizing current might tend to inactivate the plateau mechanism, resulting in briefer bursts just afterwards (Fig. 4A1). A lack of burst resetting of the other gastric neurones was also seen in numerous experiments of triggering or terminating plateau potentials in the CP cell using brief current pulses (Russell & Hartline, 1978; Hartline & Russell, 1984). However, the significant CP-to-GM inhibition (arrows, Fig. 4A1), together with the ability of the GM cells to affect the cycle period (below), suggest that CP might be capable of exerting a measurable resetting effect if stimulated strongly.

The arrows in Fig. 4A2 point to excitatory synaptic waves, apparently the main determinant of the burst timing in CP. They are gated by the CI interneurone (see Russell, 1985; Russell & Hartline, 1984).

GM motoneurones. Since GM cells do not appear to make chemical synapses within the STG (Maynard, 1972; Selverston & Mulloney, 1974) it was interesting to find that polarization of GMs could reset the rhythm timing or alter the period, even for mild stimuli not causing the GM membrane potential to exceed its normal oscillation range. This was better seen if equal currents were given to two or three GM cells simultaneously. Fig. 4B shows that a step hyperpolarization of a GM cell caused a shorter cycle period in another network cell, CP. Electrical coupling of GMs to the important LC and GP cells (Mulloney & Selverston, 1974*b*; Maynard, 1972) may explain these results.

LG motoneurones. Experiments of polarizing the two LG cells, sometimes together, were inconclusive. In some trials they could be strongly hyperpolarized without measurably affecting the period of a brisk gastric rhythm (that was phase-shifting relative to the oesophageal rhythm).

CI interneurone. A reset study using hyperpolarizing pulses is shown in Fig. 5A. Stimulation during the interburst interval of CI was generally ineffective (Fig. 5A1)

Fig. 5. (A) Intracellular stimulation of CI cell with −1 nA, 1 s pulses at different phases, showing intracellular activity of CI and CP and a summed nerve record of LC and GP firing. (B) Phase response curve for data in (A). Measuring method is shown in (A3); a, b, d = estimated start of CI bursts; c = start of stimulus. Ordinate = normalized phase of next CI burst = $cd \div ab$. Abscissa = normalized stimulus phase = $bc \div ab$. ψ indicates 'phase'. The rule was followed of ignoring the first bout of CI firing just after a stimulus. Bars along the axes indicate the normal timing of CI bursts. Horizontal dashed lines indicate the expected start of CI firing if the stimulus had no effect. (C) LC reset, showing the on-going 15-s cycle period (CI) and the effects of a +5 nA, 1 s pulse (bar, C2) or a −3 nA, 1 s pulse (bar, C3). (D) Phase response curve for depolarizing stimuli to LC, from two preparations with cycle periods of 4·4 s (circles) and 15 s (asterisks) respectively. Measurements were made relative to the start of LC bursts (see A3 and B for graph method). Filled symbols indicate the 'next' LC burst; open symbols indicate the subsequent LC burst to confirm that the rhythm was permanently reset. Note that at short cycle periods (circles) the LC burst was delayed by an amount nearly equal to the stimulus delay, whereas at long periods (asterisks) stimuli were effective mainly during the interburst interval of LC. (E) Phase response curve for hyperpolarizing stimuli to LC (cycle period = 15 s). Closed circles = start of 'next' LC burst; open circles = start of subsequent LC burst. Stimuli that terminated an LC burst caused the timing of the next and subsequent LC bursts to be advanced. Stimuli: (circles, D): +10 nA, 0·5 s; (asterisks, D): +5 nA, 1 s; (E): −3 nA, 1 s. Calibrations for (A) and (C) are all 20 mV, 5 s.

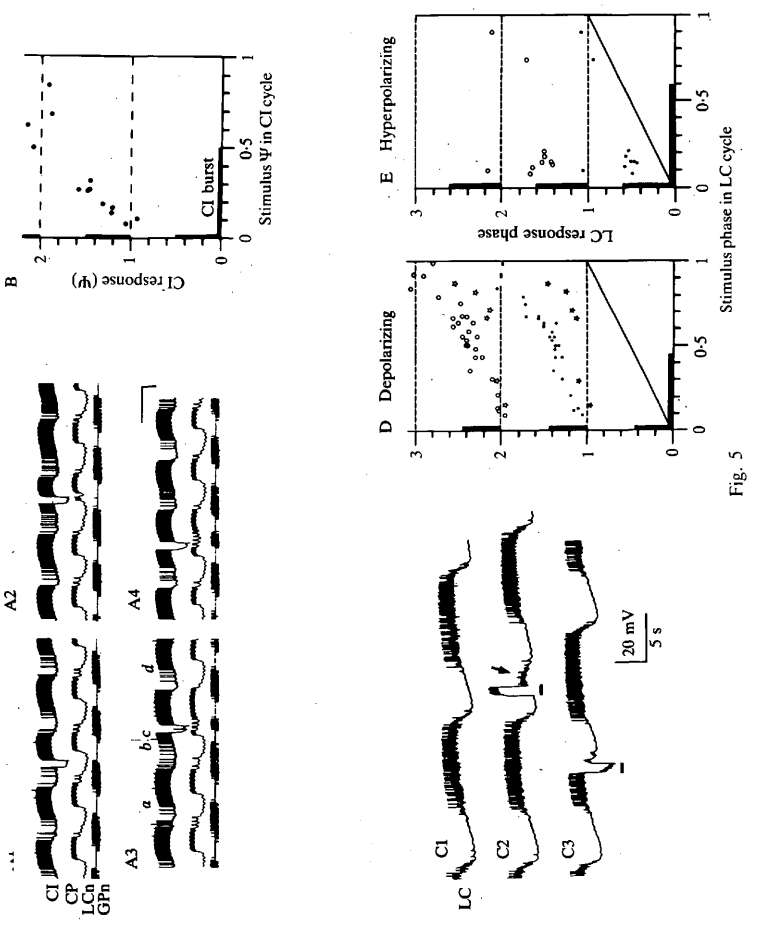
A2
A3
A4
CI
CP
LCn
GPn
a
b
c
d
B
CI response (ψ)
CI burst
0·5
Stimulus Ψ in CI cycle
D Depolarizing
E Hyperpolarizing
LC response phase
0·5
Stimulus phase in LC cycle
C1
C2
C3
LC
20 mV
5 s

Fig. 5

whereas stimulation during a CI burst acted promptly to terminate it and reinstate a new complete burst (Fig. 5A4). As a stimulus was moved to later points in the CI burst, the next CI burst was progressively delayed (Fig. 5B), with concurrent reset of bursting in all the other gastric cells (Fig. 5A).

LC and GP motoneurones. Current pulses to LC could strongly reset the rhythm timing. Fig. 5C shows the main results that a depolarizing pulse given in the interburst interval would delay the subsequent LC burst (C2) associated with a barrage of EPSPs (arrow). A hyperpolarizing pulse given during an LC burst could terminate it and advance the next LC burst (C3). Such effects were consistent in three preparations with cycle period ranging from 4·4 to 15 s.

As a depolarizing pulse was moved to a later phase, the next LC burst (filled symbols, Fig. 5D) as well as the subsequent burst (open symbols) were progressively delayed. Thus there was a permanent resetting of the rhythm. A hyperpolarizing stimulus was effective only if given during an LC burst (Fig. 5E); if the stimulus terminated the LC burst, then the timing of subsequent bursts was advanced.

Effects of prolonged polarization. Polarization of LC with ramp or step currents was an effective way to alter the cycle period. In Fig. 6A, a hyperpolarizing current was gradually released to form a depolarizing ramp, which caused the cycle periods of both the LC and CP cells gradually to lengthen. The application of step currents is seen in Fig. 6B. Both a depolarizing current (B1) or a strong hyperpolarizing current (B3) caused a longer cycle period than control (B2). Thus a plot of cycle period *vs* offset current was U-shaped, reaching a minimum period at mild hyperpolarizing currents (Fig. 8A, GR trace).

In one case the GP cell was seen to affect the cycle period. By blocking the superior oesophageal nerves, a simplified preparation was produced with a period of 20–30 s (Fig. 7; left part of panel A). A step hyperpolarizing current (downward arrow) promptly caused a shorter period, which promptly re-lengthened upon reduction of the offset (upward arrow). With a more extensive series of currents, the change in the cycle period was also immediate upon stepping the current (Fig. 7B).

A simple result emerges from these data: depolarization of the LC or GP cells caused a longer cycle period, while mild hyperpolarizing currents (less than about −3 nA) caused a shorter cycle period. In other words, the cycle period became longer with increasing 'excitation' of the LC-GP cells. This was also seen using a different measure of excitation, the average firing rate during LC bursts ($\bar{f}_b$ = number of interspike intervals ÷ burst duration). There was an approximately linear relationship between the cycle period and $\bar{f}_b$ of the LC cell (Fig. 9A). It did not seem to matter what altered the LC firing rate, since data points from spontaneous variation, depolarizing offsets, post-depolarization depression, or post-hyperpolarization rebound, all fell on the same relationship. Thus the excitation level of the LC and GP cells appears to be an important parameter affecting the gastric cycle period.

The U-shaped relationship between offset current and cycle period apparently resulted from interactions between the gastric and oesophageal rhythms. That is, the minimum gastric period (obtained by mild hyperpolarization of LC) could be *shorter* than the oesophageal period (Fig. 6B2 and Fig. 8A at −2 nA), whereas strong hyperpolarization could cause the two rhythms to become coupled 1:1 resulting in a longer-than-minimum gastric period (Fig. 6B3 and Fig. 8A at − 7 nA). In

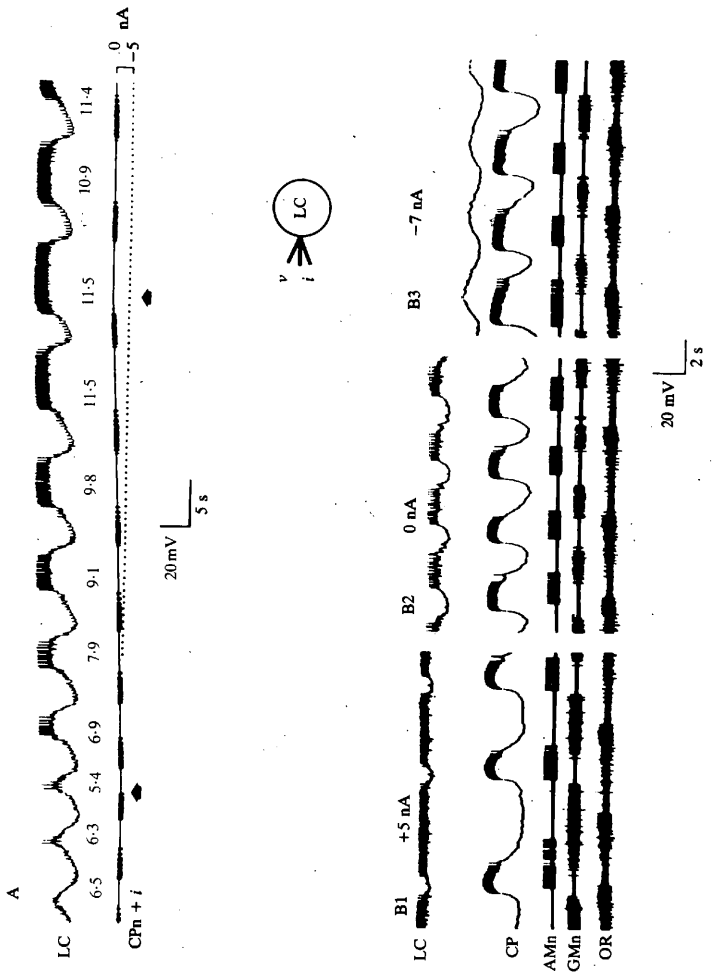

Fig. 6. Polarizing LC affects the cycle period. (A) Arrows mark a positive-going ramp in which a −5·5 nA offset current was gradually released (ending at zero current). The nerve bursts from CP were summed with the bath current monitor. Numbers between the traces give the period for individual cycles. (B) Step currents were applied to the LC cell in panels (B1) and (B3) as noted. Intracellular activity of LC and CP cells is shown with nerve monitors of AM and GM firing, and of the oesophageal rhythm (OR). Note that the gastric period was normally shorter than the oesophageal period in this case (B2). Double-barrel microelectrodes were used in both (A) and (B). *i*, *v*: current voltage electrode barrels.

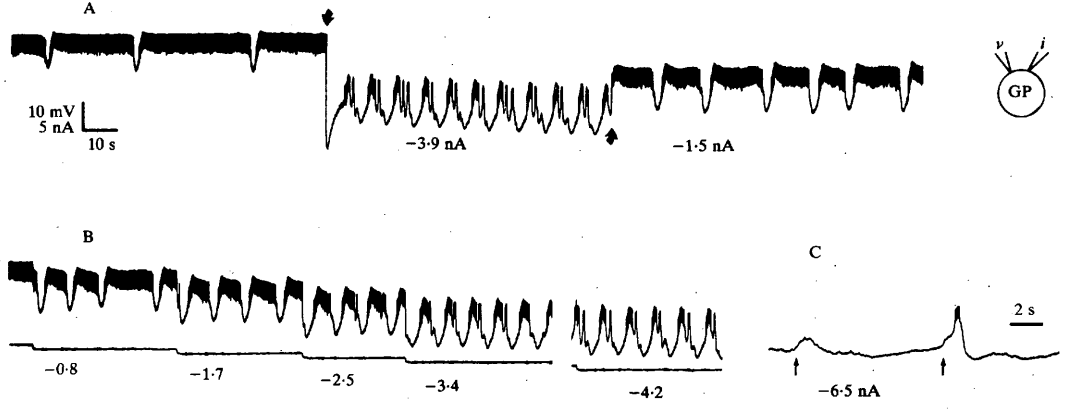

Fig. 7. Polarization of the GP motoneurone, using separate current (i) and voltage (v) electrodes. Both superior oesophageal nerves had previously been blocked with sucrose. (A) Arrows mark the application and partial release of hyperpolarizing current. (B) Numbers give total magnitude of offset current, which was progressively augmented. (C) Near the current intensity at which GP firing stopped, subthreshold waves (arrows) apparently were due to synaptic input. The short burst was due to the regenerative plateau property of the GP cell, since similar bursts could be triggered and terminated using current pulses in other records.

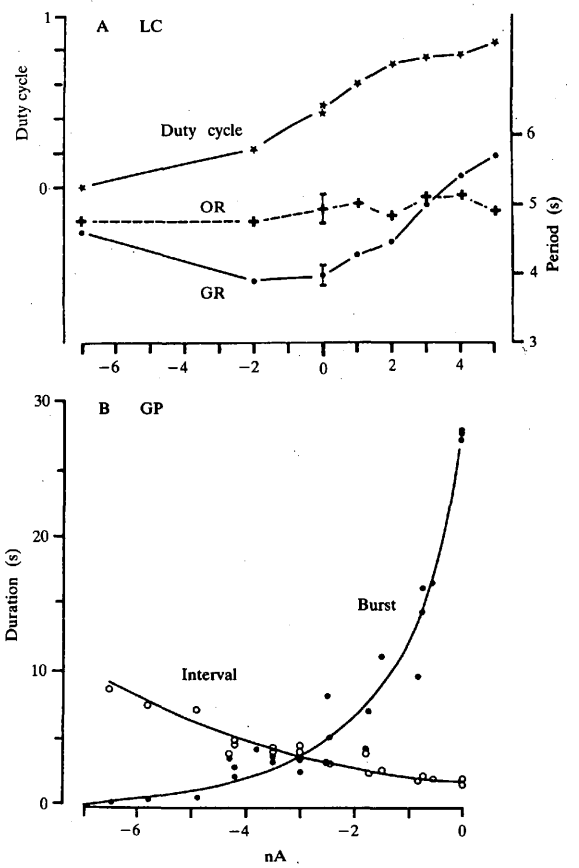

Fig. 8. Relationship between polarization intensity and the bursting of LC and GP cells. (A) Step polarization of LC. Abscissa: offset current to LC. Right-side ordinate: cycle period of the gastric rhythm (closed circles, GR) and of the oesophageal rhythm (plus signs, OR). Left-side ordinate: duty cycle of LC bursts (= burst duration ÷ cycle period) (asterisks) increased almost linearly. (B) Data from step hyperpolarization of the GP cell, with both superior oesophageal nerves blocked (from Fig. 7). Abscissa: hyperpolarizing offset current to GP. Ordinate: durations of GP bursts (closed circles) and intervals (open circles). Values were averaged over 8–10 cycles for spontaneous cycling, or over the initial three cycles after a step hyperpolarization. Burst length was measured from the first to last spike, ignoring gaps.

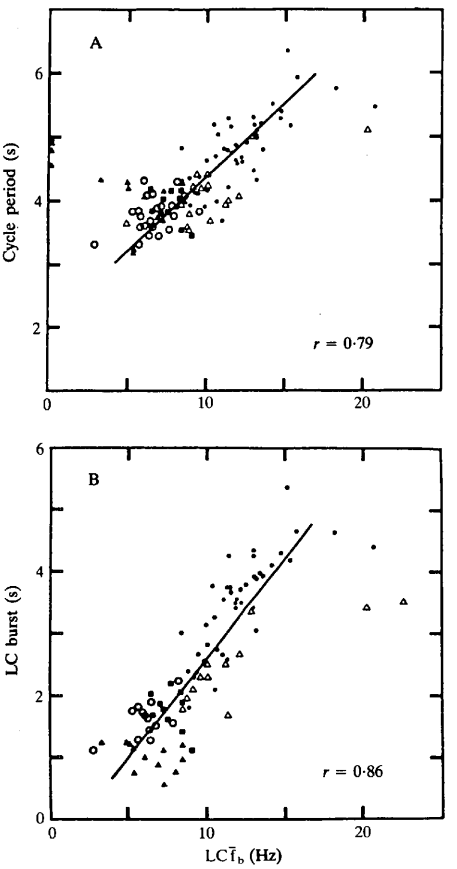

Fig. 9. Relationship of the cycle period (A) or the duration of LC bursts (B) to the average firing rate during LC bursts = $\bar{f}_b$. Each point indicates one burst. Closed squares: spontaneous rhythm. Closed circle: during depolarizing step current to LC. Open circle: depression after releasing depolarizing current. Open triangle: rebound after releasing hyperpolarizing current. To fit the regression lines and calculate the correlation coefficient (r), the points at $\bar{f}_b = 0$ were excluded, as were the right-most three points in (B).

general, it appears that strong hyperpolarization of LC reduces its ability to affect the gastric generator, which may then be 'paced' by the oesophageal generator. Such results are not further considered.

Variable and constant phases of the gastric cycle

Since the gastric cycle period could be altered, it was possible to study how the burst duration and interval duration in the different cell types varied as a function of the cycle period. The main conclusion was that the gastric pattern could be divided into two basic alternating phases: a variable-duration 'powerstroke' and an approximately constant-duration 'returnstroke'.

Powerstroke events

Inspection of Figs 2, 3, 5, 6 and other data shows that bursts in the LC, GP, GM and E neurones were approximately synchronous, had comparable durations, and corresponded to the interburst intervals in the other cells. All these events will be termed the 'powerstroke'. An example of their co-variation is seen in Fig. 6B: a depolarizing current to the LC cell caused its bursts to lengthen but also caused longer bursts in the GM motoneurones and longer intervals between bursts in the CP and AM motoneurones. Neurones bursting during the powerstroke can be termed 'powerstroke neurones'; motoneurones in this group evoke closing of the lateral teeth and the masticatory protraction of the medial tooth (Hartline & Maynard, 1975).

Returnstroke events

Bursts in the CP, AM, LG and CI cells were approximately synchronous with each other and with the interburst intervals in the powerstroke neurones. All these events will be termed the 'returnstroke'. They all showed a similar but not identical duration. For example, a comparison of Figs 6B1 and B3 reveals co-variation of the durations of CP bursts and GM intervals in this case. Neurones bursting during the returnstroke will be termed 'returnstroke neurones'; motoneurones in this group evoke separation of the lateral teeth, release of food and resetting of the medial tooth.

Variable powerstroke, fixed returnstroke

All three methods of varying the cycle period showed that powerstroke events had a more variable duration than did returnstroke events.

(i) The nerve cutting experiment of Fig. 2B showed that the longer cycle period (compared to Fig. 2A) was associated with longer bursts in the powerstroke cells (LC, GP, GMs). In contrast, bursts in the returnstroke cells (CP, AM, LGs) retained approximately the same duration as in Fig. 2A, as did the *interburst intervals* of the powerstroke cells.

(ii) Change in the cycle period due to relative coordination gave a similar result. Fig. 3A shows that the LC burst duration ranged from 2·4 to 4·3 s whereas the CP burst duration varied much less, from 1·6 to 1·8 s.

(iii) Polarization of GP revealed a much wider variation of its burst duration than its interval duration (Fig. 8B). With polarization of the LC cell, the durations of powerstroke events varied over a three-fold range (Fig. 10A), whereas the durations of returnstroke events remained clustered (Fig. 10B).

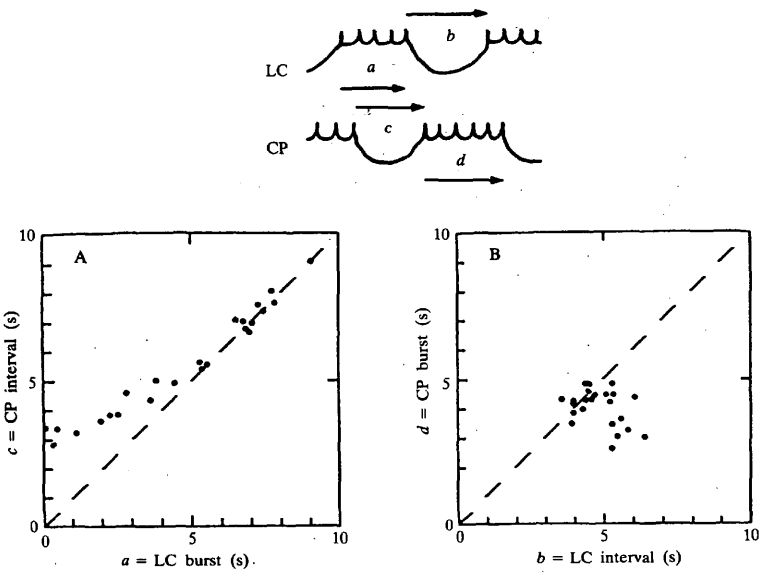

Fig. 10. Comparison of powerstroke and returnstroke range. Schema shows the quantities measured. Dashed line shows x = y. (A) Powerstroke events. Abscissa: burst duration of LC. Ordinate: simultaneous interburst interval of CP. (B) Returnstroke events. Abscissa: interburst interval of LC. Ordinate: simultaneous burst duration of CP.

All these data are consistent with the idea that change in the cycle period was mainly associated with change in the powerstroke duration.

Variation with cycle period

An informative way to analyse a pattern is to plot the durations of bursts or intervals as functions of the cycle period. This is shown in Fig. 11 for the activity of a powerstroke neurone (LC, panel A) and a returnstroke neurone (CP, panel B). In general, the duration of LC bursts increased *linearly* over the mid-to-high range of cycle periods; correlation coefficients were 0·99 and 0·98 in two cases.

As expected, the interburst intervals of the CP cell lengthened in linear proportion to the cycle period whereas its bursts retained an approximately constant duration (Fig. 11B). Significant departures from this rule could occur at short cycle periods since the CP burst duration progressively lengthened with the cycle period (Fig. 11B, for periods of 5·5–8 s).

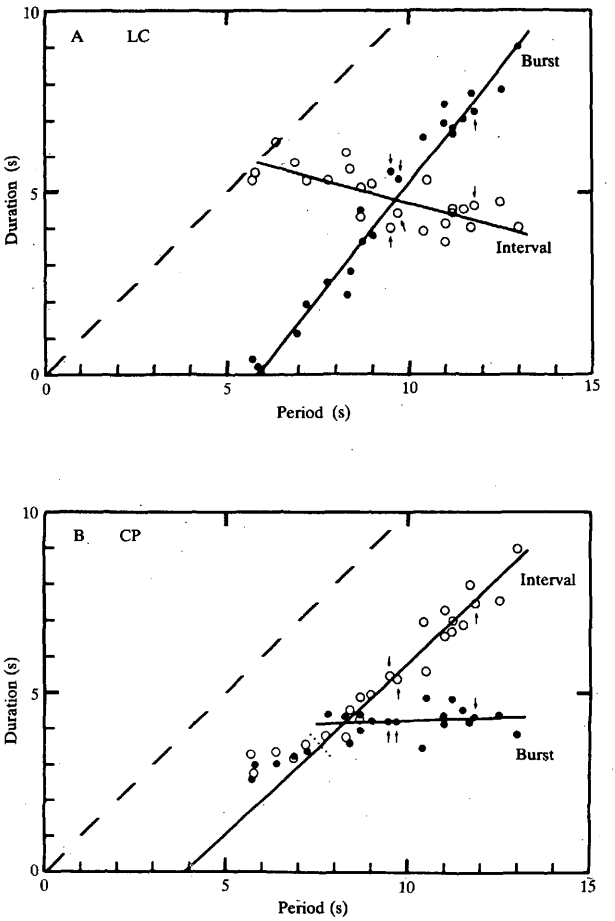

Fig. 11. Burst and interval durations as functions of cycle period. The period varied spontaneously (arrows), or was altered by depolarizing or hyperpolarizing the LC cell. (A) LC data. Regression analysis gave the following line equations (units = seconds): LC burst duration = 1·3 (period) −7·3, $r = 0{\cdot}98$, $N = 24$. LC interval duration = −0·26 (period) +7·3, $r = -0{\cdot}73$, $N = 24$. (B) CP data. CP burst duration = 0·035 (period) +3·9, $r = 0{\cdot}14$, $N = 19$. CP interval duration = 0·95 (period) −3·7, $r = 0{\cdot}96$, $N = 19$. Points to the left of the dotted line in (B) were excluded from the CP regression analysis.

Such plots allowed a test of a model which assumes that the cycle period is equal to the sum of the powerstroke and returnstroke durations. The model worked well over the normal-to-high range of periods. Thus in Fig. 11 there is a 4–5 s difference between the LC burst duration and the cycle period, which corresponds to the 4–5 s duration of CP bursts over this range of periods.

The model was less satisfactory at shorter cycle periods. If the powerstroke duration is assumed to be zero, then the returnstroke should presumably occupy the entire cycle. Fig. 11A shows a residual cycle period of 5·8 s (at zero LC burst duration) which is substantially longer than the 3 s duration of CP bursts at the same cycle period. Similar results came from two other preparations in which LC was hyperpolarized: the CP bursts were too short to account for the residual cycle period when the LC burst duration was extrapolated to zero.

Role of LC excitation

Bursts in the LC cell became longer when it was depolarized, and shorter when hyperpolarized (Fig. 6). An alternative measure of LC excitation, $\bar{f}_b$, also correlated directly (sometimes linearly) with its burst length (Fig. 9B). This 'proportionality' explains why the cycle period was also proportional to the LC excitation level (above, Fig. 9A), since the returnstroke duration tended to be fixed. Another consequence was that the powerstroke occupied a progressively larger fraction of the cycle period as the LC excitation increased, as seen in duty cycle plots for its bursts (Fig. 8A; duty cycle = duration ÷ cycle period). The GP data in Figs 7 and 8B also gave a direct linear relation between offset current and burst duty cycle ($r = 0{\cdot}96$).

As the excitation level of the LC cell increased, its interburst intervals became *shorter* (open circles, Fig. 11A, at long cycle periods). This may be significant in relation to models for the gastric generator invoking a reciprocal inhibitory network as a basis for rhythmicity.

Lack of constant-phase behaviour

A strict 'constant-phase' system is one in which a given event always occupies a constant fraction of the cycle period (see Kristan, 1980). This could be conveniently studied with plots of duty cycle *vs* period (Fig. 12A). A horizontal relationship would be expected for constant-phase behaviour.

Bursts in CP and AM showed signs of constant-phase behaviour at short cycle periods (open circles, left side of Fig. 12A), since they lengthened somewhat with the period. This changed to constant-duration behaviour over the normal-to-high range of periods, giving a declining duty cycle.

Powerstroke bursts did not show constant-phase behaviour, since their duty cycle period steadily increased with the cycle period (Fig. 12A, closed circles). This resulted from the large residual cycle period when the LC burst duration was extrapolated to zero (see Fig. 11A).

Thus the gastric generator does not appear to be a constant-phase system, except possibly for the returnstroke duration at short cycle periods.

Summary

The different behaviour of powerstroke and returnstroke neurones is portrayed in Fig. 12B by plotting burst duration *vs* interval duration. The relationship for powerstroke neurones (closed circles = LC data) was vertically orientated because their burst duration had a larger range than their interburst interval. The relation had a negative slope because the intervals grew shorter at longer bursts. In other cases the relationship for LC assumed a hyperbolic form, tending more rapidly towards longer intervals at shorter bursts.

The relationship for returnstroke neurones (open circles = CP data) was horizontally orientated because the interburst intervals had the wider range. The relationship had a positive slope because the bursts and intervals lengthened together.

DISCUSSION

This article analyses the pattern of gastric mill rhythms in *in vitro* 'combined' preparations, and presents reset data to try to determine which cells generate the rhythm.

Gastric rhythms in the multiganglionic preparation

The principal advantage of the 'combined' *in vitro* preparation is that the gastric rhythm is spontaneously active. The disadvantage is the substantial number of neurones involved and the complexity of the preparation. The relative coordination of the gastric and oesophageal rhythms was especially a problem; e.g. the latter might drive the gastric system in aged preparations, which typically show 1:1 coupling.

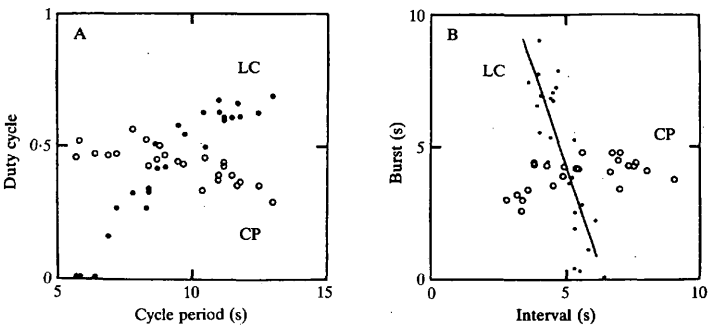

Fig. 12. Comparison of timing relations for LC motoneurone (closed circles) and CP motoneurone (open circles). The schema in Fig. 10 shows the quantities measured. (A) The ordinate is duty cycle of bursts; the abscissa is cycle period. (B) Burst duration (ordinate) *vs* interburst interval duration in the same cell and the same cycle (abscissa). The cycle timing was varied by polarizing the LC cell.

The rhythm activation involves modulation of the cellular properties of STG neurones by descending inputs that synaptically induce the ability of certain STG cells (including CP, AM, LC, GP and CI) to produce slow regenerative depolarizations (Russell & Hartline, 1978, 1984). Such regenerativeness is seen during active rhythms but not in the non-rhythmic isolated STG.

Neurones generating the gastric rhythm

The gastric network contained within the STG is *sufficient* to generate a gastric rhythm, since the isolated STG can occasionally produce spontaneous gastric rhythms (Selverston *et al.* 1976), and since stimulation of command inputs can activate gastric rhythms after isolation of the STG (Russell & Hartline, 1984).

Intracellular polarization of the CI, LC, GP or GM cells could promptly reset the gastric rhythm timing or alter its cycle period. According to standard criteria (see Kristan, 1977), these cells either participate in the gastric generator or synaptically affect cells which do so. A problem with the reset tests was that the coupling of the gastric and oesophageal rhythms could tend to limit change in gastric period, e.g. when coupled 1:1, and hence give false negative results. For example, polarization of GM cells strongly affected the gastric period in a fresh preparation (showing different gastric and oesophageal periods), but identical GM stimulation was ineffective a few hours later when the two rhythms were coupled 1:1 (not illustrated).

Ayers & Selverston (1984) have studied perturbation of the gastric system by stimulation of a certain nerve, but their results do not permit identification of generator neurones.

None of the 'generator' neurones gave phase response curves resembling those reported for endogenously bursting neurones (Hartline, 1976; Pinsker, 1977; Benson, 1980; Hartline, Gassie & Sirchia, 1981). The only major point of similarity was the ability to terminate bursts in LC or CI with hyperpolarizing pulses; this may be related to the slow regenerative property of these cells. The longer cycle period resulting from depolarization of the powerstroke cells (with offset current) is opposite to the conventional rule for endogenous bursters (Chalazonitis, Takeuchi & Arvanitaki, 1967; Watanabe, Obara & Akiyama, 1967). Paradoxically, the CP neurone (normally a 'follower' cell) can burst endogenously under certain conditions when the rest of the gastric network is not active (Hartline & Russell, 1984).

Winfree (1980) has classified different bursting systems as 'Type 0' or 'Type 1' according to the shape of the phase response curve when plotted in a certain way. When the data for depolarizing stimuli to the LC cell (closed circles, Fig. 5D) were replotted in that way, the response curve had a 'Type 0' shape (not illustrated), due in part to the ineffectiveness of stimuli given just after the LC burst.

Gastric patterns

The gastric mill pattern reported here resembled that seen in semi-intact *Panulirus* (Hartline & Maynard, 1975). Of course the results here came from *in vitro* preparations, whereas sensory feedback and interactions with the sub-

oesophageal and other higher-level ganglia may affect the operation of the system *in vivo* (Powers, 1973; Fleischer, 1981).

Since there are antagonist motoneurones for the medial and lateral teeth, the gastric rhythm has previously been assumed to have four phases (Mulloney & Selverston, 1974*b*; Hartline & Maynard, 1975; see Friesen & Stent, 1978). It is true that the delay of medial tooth activity relative to lateral teeth activity suggests four phases when a rhythm with a fixed cycle period is inspected (e.g. Fig. 2A). However, when the cycle period varies over a wide range, such delays appear relatively minor compared to the huge variation in the burst length of LC, GP and GM cells (the powerstroke) and the striking constancy of the burst length in cells such as CP (the returnstroke). Consistent findings came from all three methods of varying the cycle period. It therefore appears that in combined *in vitro* preparations *the gastric rhythm has basically two alternating phases: a variable-duration powerstroke and a constant-duration returnstroke.*

There were some problems with classifying different features of the pattern as belonging to the 'powerstroke' or 'returnstroke'. (i) Bursts in LGs slightly preceded bursts in CP, which in turn slightly preceded bursts in CI, yet all are classified as 'returnstroke' events. (ii) Low-frequency firing of LGs continued throughout most of the powerstroke, but nevertheless was distinct from their high-frequency returnstroke burst. CP and AM could sometimes behave similarly (see Fig. 2B). (iii) Fig. 11 shows that at long cycle periods the LC intervals shortened while the CP bursts kept a constant duration; both are classified in the returnstroke despite their opposite behaviour. (iv) Activity of CI and E cells was not directly recorded while varying the period. In the available records, their bursts were approximately synchronous with the CP and GM bursts respectively, and so they are assigned to the returnstroke and powerstroke respectively. (v) The GP cell is classified with the powerstroke group based mainly on its burst timing, since questions remain concerning the functions of the muscles it innervates (Hartline & Maynard, 1975).

A variable-duration returnstroke with a fixed-duration powerstroke was seen in a small minority of 90 experiments (e.g. Fig. 7 of Russell & Hartline, 1984); that is, they behaved in a manner opposite to the above. The cause of the spontaneous variation in the cycle period (which is unusual) was not identified. These results could signify momentary *perturbation* of the system, rather than reflecting organizational properties of the gastric mill system *per se*, since other neural systems are active in the commissural and oesophageal ganglia. My conclusions stated above derive from three different types of well-defined data, including experimental manipulations. These disparate findings are reminiscent of conflicting studies on the patterning of the returnstroke and powerstroke in lobster stepping (see Clarac, 1982).

Variable and constant phases

Since a linear relation between the powerstroke duration and cycle period was consistently observed, at least over the middle-to-high range of cycle periods, its behaviour can be described by a linear equation:

$$P = M \cdot D_{pw} + K_1, \quad (1)$$

where P = cycle period, D_{pw} = powerstroke duration, M = slope constant ($\simeq 1$), and K_1 = residual cycle period when $D_{pw} = 0$. That is, the powerstroke duration can vary over a wide range but does not behave as a constant-phase system which would instead predict that:

$$D = \omega P, \tag{2}$$

where ω = the phase angle.

The returnstroke duration (D_{re}) stayed almost constant over the mid-to-high range of periods:

$$D_{re} = K_2. \tag{3}$$

Over this range of periods it was approximately true that M (from equation 1)$\simeq 1$, while $K_2 \simeq K_1$, supporting the model that:

$$P = D_{pw} + D_{re}. \tag{4}$$

That is, the cycle period approximately acted as the sum of a variable-duration powerstroke and a constant-duration returnstroke.

Since the powerstroke duration and cycle period correlated well with the firing rate of the LC and GP powerstroke cells, a linear equation can be proposed for the overall behaviour of the cycle period:

$$P = S \cdot E_{pw} + K_3, \tag{5}$$

where S = positive slope constant, E_{pw} = excitation level of the LC cell and K_3 = lumped constant including the returnstroke duration.

Functional implications

The term 'powerstroke' was adopted because the powerstroke motoneurones evoke closing of the lateral teeth upon a food particle and protraction of the medial tooth to degrade it (Hartline & Maynard, 1975). Apparently the pattern generator is organized to *permit* this *load-bearing* phase to assume a variable duration, commensurate with the variable loads encountered during the powerstroke mastication. Proprioceptors of the gastric mill (Dando & Laverack, 1969) probably have a role in matching the powerstroke discharges to the load conditions. In contrast, the pattern generator is apparently organized to *command* more consistent motions of the teeth during the 'returnstroke', commensurate with the teeth being *unloaded* when they open and retract.

Selective depolarization of the LC cell by EPSPs or plateau induction, etc., would be expected to cause a longer cycle period *in vivo* (assuming other parameters remain unchanged). The possibility that plateau induction may help regulate the gastric period is plausible since the LC and GP cells have been shown to possess a regenerative 'plateau' property whose expression is regulated by modulatory synap-

tic inputs from the CNS (Russell & Hartline, 1978, 1984). The effect of GM excitation on the period is less clear since, although they could affect the period (Fig. 4B), in some situations the period could be quite long despite only a low firing rate in the GM cells (see Fig. 2B).

The variable burst duration of the powerstroke neurones necessarily implies that they do not express strong cellular mechanisms for autotermination of bursts (at least under these conditions). Cells with strong repolarization conductances activated by depolarization would instead be expected to produce 'unit' bursts of relatively consistent duration and waveform, like an action potential or the bursts in endogenous pacemaker neurones (see Russell & Hartline, 1981). Perhaps the consistent duration of the returnstroke may be due to endogenous burst properties of unspecified returnstroke neurones (not necessarily in the STG). That is, the returnstroke might be self-terminating.

Fig. 13 shows the proposed functional organization of the gastric generator based on the results here. The powerstroke neurones form a 'proportional' element whose duration is set by their excitation level, whereas the returnstroke neurones form a 'fixed-duration' element. While the returnstroke events run a consistent time course once started, the *intervals* between them are strongly affected by the powerstroke burst (e.g. Fig. 10A). Inversely, the intervals between powerstroke bursts always corresponded to the burst in returnstroke neurones, and was sensitive to stimulation of at least one returnstroke cell, CI (Fig. 5A). Hence reciprocal negative interactions between the 'proportional' and 'fixed-duration' elements are proposed.

Comparison with other systems

Patterns with two alternating phases, one with a wide range of durations, the other with a constant or less-variable duration, have been reported for several systems

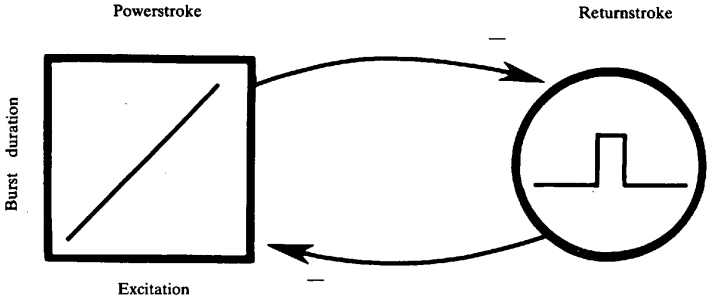

Fig. 13. Functional organization of the gastric generator. A linear relation between burst duration and excitation is shown for the powerstroke cells, but only to indicate proportionality. The square wave within the circle indicate the fixed-duration of returnstroke bursts. Arrows indicate functional inhibitory links between the powerstroke and returnstroke elements.

including dog stepping (Arshavsky *et al.* 1965), cat respiration (Clark & von Euler, 1972), cockroach walking (Pearson, 1972), lobster stepping (Clarac, 1982) and leech swimming (Kristan, Stent & Ort, 1974). Terrestrial locomotion is especially analogous to the gastric system since the loaded 'stance' phase has a variable duration that is proportional to the cycle period, whereas the unloaded 'swing' phase has a relatively constant duration.

An unusual feature of the gastric pattern is the inverse relationship between the durations of bursts and intervals in the powerstroke neurones. In most pattern-generating networks the durations of bursts and intervals in a given cell-type are positively correlated, as in the systems cited above and also the lobster heart rhythm (Mayeri, 1973).

Another distinguishing feature is that the gastric cycle period *lengthens* in relation to increasing excitation of the powerstroke neurones; in many other rhythmic motor systems an increase in excitation leads to a *shorter* cycle period, as found in the systems cited above and also the lobster pyloric rhythm (Hartline & Maynard, 1975), lobster swimmeret rhythm (Davis, 1969), locust flight (e.g. Wilson, 1968), etc. However, some endogenous pacemaker neurones can show longer bursts and periods and larger burst duty cycles when their regenerative 'plateau' mechanism is selectively enhanced by modulatory synaptic input (Watanabe *et al.* 1969; Kononenko, 1979; Russell & Hartline, 1981), like the gastric powerstroke neurones.

Supported by NIH Grants NS09322-04, NS13138 and NS16235. The participation in experiments, suggestions and manuscript comments of D. K. Hartline are gratefully acknowledged, as are manuscript comments by R. E. Sullivan.

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