

## NEURAL BASIS OF TEETH COORDINATION DURING GASTRIC MILL RHYTHMS IN SPINY LOBSTERS, *PANULIRUS* *INTERRUPTUS*

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### SUMMARY

1. Motoneurons that drive the closing of the lateral teeth during gastric mill rhythms in spiny lobsters start firing before the motoneurons that drive the medial tooth powerstroke. This has the expected behavioural interpretation that the lateral teeth must close on a food particle before the medial tooth is pulled across it.

2. The neural basis of the teeth coordination was examined. Experiments were made during gastric rhythms in *in vitro* preparations comprising the stomatogastric, oesophageal and (paired) commissural ganglia. Identified neurons in the stomatogastric ganglion were polarized to study their functional effects on the phasing and amplitude of bursts in other cells.

3. Evoked firing of the lateral teeth closer motoneurons (especially LC) would evoke a discharge in the medial tooth powerstroke (GM) motoneurons, and suppress the firing of the medial tooth returnstroke (CP) motoneuron. Therefore the coordination pathway starts directly with the lateral teeth closer motoneurons.

4. The CI interneuron was found to be an important link in the coordination pathway. It exerted opposite effects on the medial tooth motoneurons, suppressing firing of the powerstroke GM cells while evoking bursts in the returnstroke CP cell. CI affected other features of the pattern as well.

5. Non-spiking inhibition from the lateral teeth closer motoneurons (LC and GP) to the lateral teeth opener motoneurons (LGs) was found to occur conjointly with spike-mediated IPSPs.

6. Hyperpolarization of the LC, GP or CI neurons could temporarily abolish the gastric rhythm, but bursting in some or all of the other cells would eventually return, although in some cases the phase pattern was altered. It appears that no individual neuron in the gastric network is necessary for rhythm production.

7. The coordination system can be viewed as several 'levels' of synaptic connections, each level being redundant and synergistic with the others.

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## INTRODUCTION

This article continues an analysis of the central pattern generator for the gastric mill rhythm (Russell, 1985). One approach to studying central rhythm generators has been to assemble 'circuit diagrams' of monosynaptic connections among component cells (reviewed in Friesen & Stent, 1978). Connectivity studies on the gastric mill network within the stomatogastric ganglion (STG) have been carried out by Maynard (1972), Mulloney & Selverston (1974*a,b*), Selverston & Mulloney (1974) and Maynard & Walton (1975). These studies were made on isolated preparations of the STG (with the oesophageal ganglion attached in some cases) in which the gastric rhythm was usually absent.

Another way to describe a given cell's role in a network is to alter its firing during on-going rhythms while monitoring effects on the amplitude and phasing of burst production in other cells. This is a 'black box' approach since the functional effects from stimulating a given cell could involve both direct and indirect synaptic connections to the cells affected.

In this article, functional tests were used to study a coordination pathway in the gastric mill generator. A consistent feature of the gastric pattern in semi-intact spiny lobsters (Hartline & Maynard, 1975) as well as *in vitro* preparations is that activity in the lateral teeth motoneurons precedes by a few hundred ms the corresponding activity in medial tooth motoneurons. For example, firing in the lateral teeth closers (LC and GP) precedes that in the medial tooth powerstroke cells (GMs). A behavioural interpretation of the staggered firing is that the lateral teeth must first close to immobilize a food particle before the medial tooth can effectively degrade it. The available behavioural data on the gastric mill (Powers, 1973; Hartline & Maynard, 1975; Fleischer, 1981), and the anatomical organization of the gastric mill teeth (Maynard & Dando, 1974) support this view. The aim of this article is to analyse the neural basis of the teeth coordination.

## METHODS

Methods were as described in the preceding paper (Russell, 1985). Experiments were made during strong, on-going gastric rhythms in isolated *in vitro* 'combined' preparations which included the commissural, oesophageal and stomatogastric ganglia. All the gastric system motoneurons in the STG were bursting. The oesophageal rhythm was monitored. In certain cases, the two superior oesophageal nerves were cut or blocked with isotonic sucrose solution; this blocked descending impulses from gastric neurons in the commissural ganglia such as the E neurons and possibly others (Russell, 1976; Robertson & Moulins, 1981).

Instantaneous firing rates of individual neurons were measured using a spike discriminator and a linear rate meter (Bak Electronics). Firing rates in multi-unit records were displayed using a leaky integrator circuit with a time constant of 2 s. Spikes recorded from two different nerves were in some cases half-wave rectified and displayed as upward and downward half-spikes on a shared baseline. Other displays on a shared baseline may show raw spikes with corresponding firing rates, or rates

from two different units. Pre-triggered averages of synaptic potentials in STG cells were triggered from motor nerve spikes, using an analogue delay (Bak Electronics) and a signal averager.

Terminology follows that of Maynard (1972) and Maynard & Dando (1974); see also Table 1 of Russell (1985).

## RESULTS

### *The lateral-to-medial pathway*

The premise being advanced is that the LC and GP motoneurons (closers for the lateral teeth) evoke the medial tooth powerstroke by evoking a burst in the powerstroke (GM) motoneurons and suppressing activity in the returnstroke (CP) motoneurone (Fig. 1A). This action is demonstrated in Fig. 1B: hyperpolarizing the LC neurone during a gastric rhythm had a reciprocal action on the medial tooth antagonists, releasing continuous firing of CP and depressing activity in the GMs. A sudden onset of LC firing had the opposite effect, evoking a powerful GM discharge.

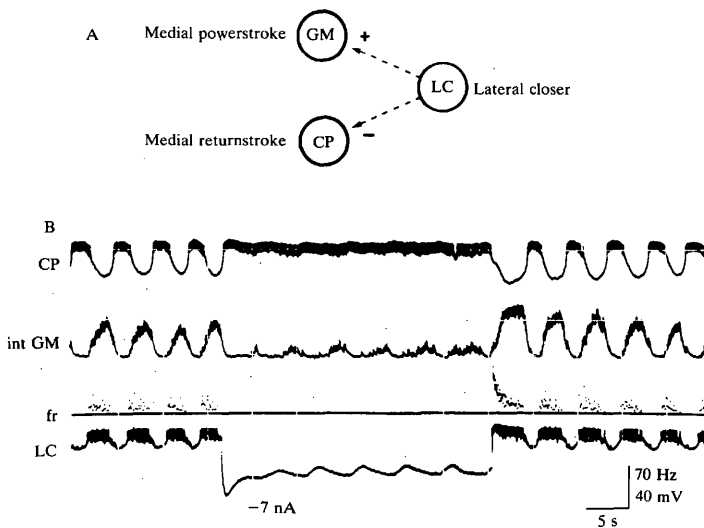


Fig. 1. Lateral-to-medial pathway. (A) The LC motoneurone exerts an overall functional excitatory (+) effect on GM motoneurons, and a functional inhibitory (-) effect on the CP motoneurone of the medial tooth. (B) The LC cell was hyperpolarized, then released. fr: instantaneous firing rate of LC measures its level of excitation. int GM: firing of all four GM motoneurons recorded from the anterior gastric nerve (Maynard & Dando, 1974) was rectified and integrated to better indicate the amount of GM activity. The prolonged depolarization in CP was probably due to its plateau property remaining 'on', since in similar trials the CP could be made to repolarize by brief hyperpolarizing current pulses. Note that LC, GMs and CP all continued to show rhythmic modulation during the time of LC hyperpolarization.

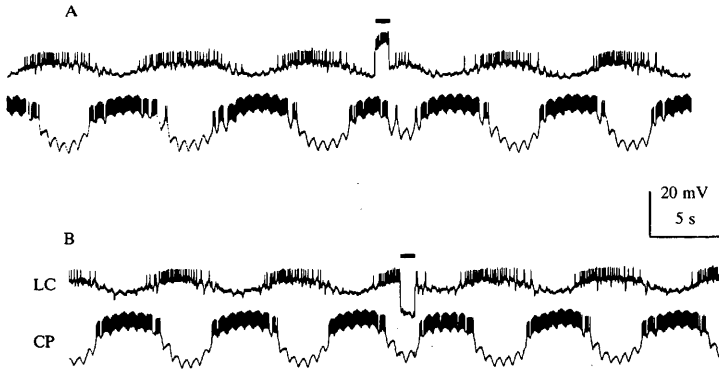


Fig. 2. Simultaneous intracellular records from LC and CP, stimulating LC with 3 nA, 1 s currents (bars). There was a similar effect on the CP bursting from a depolarizing pulse given during the LC interburst interval (panel A) or a hyperpolarizing pulse given during the LC burst (panel B).

The pathway could also be demonstrated by briefly stimulating the LC cell while studying effects on the burst timing of the CP cell (Fig. 2). Evoked LC firing interrupted the CP burst after a delay of several hundred ms (panel A), indicating a functional LC-to-CP 'inhibition'. Similarly, terminating an LC burst with a hyperpolarizing current (panel B) caused the next CP burst to come early, as if the normal interburst interval of CP were due to functional 'inhibition' from LC.

The pathway by which LC 'excites' the GM cells and 'inhibits' the CP cell is traced below, starting with the medial tooth motoneurons.

#### *Transmission from medial tooth motoneurons*

The strengths of synapses from CP were measured by perturbing its firing with injected current during the impulse bursts of a postsynaptic cell (during gastric rhythms; Fig. 3). Synaptic strength was expressed as  $\sigma$  = number of postsynaptic spikes suppressed by one presynaptic impulse (for an inhibitory connection) (see Hartline & Gassie, 1979) (Table 1). The most significant effect was inhibition of the antagonist GM motoneurons. The other connections from CP were discernible but weak. In particular, inhibition of the lateral teeth closer motoneurons (LC and GP) was quite weak and cannot be a credible basis for coordination of the medial and lateral teeth.

The activity and burst phasing of the AM cell resembled that of CP, even though AM innervates a different part of the stomach (the cardiac sac). Like those from CP, connections from AM were weak, as was electrical coupling between the two cells (Fig. 3D, Table 1). The lack of synaptic output is consistent with the inability to reset the gastric rhythm by stimulating CP or AM.

The GM powerstroke motoneurons apparently make no chemical synapses within the STG (Maynard, 1972; Selverston & Mulloney, 1974). Nevertheless they

are able to affect the cycle period, presumably on account of electrical coupling to other cells (Russell, 1985).

#### *Linkage by the CI interneurone*

The CI interneurone (Maynard, 1972; Selverston & Mulloney, 1974) has an important role in the alternate bursting of the medial tooth antagonists, as seen in Fig. 4. A partial reduction of CI firing (Fig. 4A2) was accompanied by a partial demodulation of firing in the GM powerstroke motoneurons, so that GM firing

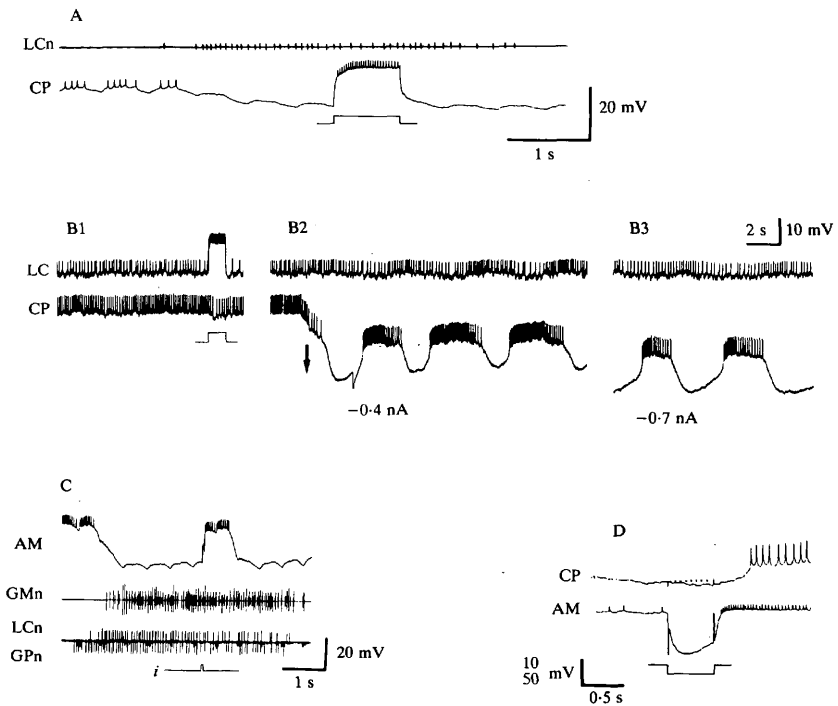


Fig. 3. Strengths of synaptic effects from CP or AM were measured during gastric rhythms. (A) CP was made to fire during a LC burst by a +6 nA current; note that LC firing slowed. (B) For B only, both superior oesophageal nerves were sucrose blocked, resulting in tonic firing of LC and CP. (B1) Pulse indicates +3 nA, 1 s depolarization of LC; the slowing of CP firing may have represented direct LC-to-CP inhibition. (B2, B3) Step hyperpolarization of CP converted its tonic firing into rhythmic bursting, probably due to endogenous mechanisms of CP. Note reduction of LC firing rate during CP bursts, another measure of CP-to-LC inhibition. (C) A plateau potential was triggered in a hyperpolarized AM cell during firing of GM, LC and GP, allowing effects on the firing rates of those cells to be measured. (D) Possible electrical coupling between AM and CP was measured by hyperpolarizing AM by -8 nA during its interburst interval; dotted line indicates the extrapolated control trajectory.

Table 1. *Synaptic strengths of AM and CP connections*

Interaction	Sigma	S.D.,	N	Notes
CP-i-GM	-0.24*	0.07,	8	
CP-i-LC	-0.09	0.11,	9	
	-0.12	0.08,	9	a
LC-i-CP	-0.17*	0.05,	4	a
AM-e-CP	cc = 0.02	-	-	b
	+0.04	0.08,	9	c
	+0.001	-	-	d
AM-i-GM	-0.27	0.18,	3	e
AM-i-LC	-0.04	0.07,	3	
AM-i-GP	-0.06*	0.01,	4	
CI-i-E	-1.4*	graph slope		f

For chemical synapses, firing of CP or AM was evoked during the bursts of a presumed postsynaptic cell, during on-going gastric mill rhythms. Mean 'sigma' and its standard deviation were measured over 3-9 trials (N) by counting spikes to detect a decrease (-) or increase (+) in the number of postsynaptic spikes compared to average controls expected normally at corresponding phase in the gastric rhythm. Sigma = (number of postsynaptic spikes per time unit during stimulation - postsynaptic control) ÷ (number of presynaptic spikes per time unit). Asterisks: sigma values which were significantly different from zero at the  $P < 0.02$  level (one-tailed *t*-test). Interactions: CP-i-GM stands for CP cell inhibits the GM cells. e = excites.

Notes: a: both of the superior oesophageal nerves were blocked, giving tonic firing of LC and CP cells. b: 'cc' = coupling coefficient of presumed electrical coupling = postsynaptic voltage deflection ÷ presynaptic hyperpolarization. c: sigma was measured from expanded records of triggering AM bursts near the midpoint of CP bursts, compared to CP bursts without AM firing. d: sigma was measured from comparing 15 CP bursts with normal AM firing to 15 CP bursts with AM firing abolished by steady hyperpolarization. e: sigma was measured by triggering AM plateau potentials during GM bursts and counting all four GM units in nerve record. f: sigma for the CI-E connection was measured by varying the CI firing rate with intracellular current while recording E spikes from the stomatogastric nerve, plotting their respective rates, and dividing the slope value in half since spikes from both E neurones were totalled.

continued even during their 'interburst intervals'. Complete suppression of CI firing caused the GM discharge to become virtually tonic (Fig. 4A3) (Russell, 1976). This indicates that the burst formation in the GM powerstroke motoneurones depends strongly on bursting in the CI interneurone, and that the degree of GM modulation is *proportional* to the CI firing rate. Bursts in the CP retractor motoneurone appear to be driven largely by functional excitation from CI, since interrupting a CI burst similarly interrupted a CP burst (Fig. 4B1), and since suppressing the firing of CI caused the firing of CP to stop for several seconds (Fig. 4B1,B2).

Nevertheless, firing of CI was *not necessary* for burst formation in CP and certain other gastric cells, since 20-60 s after suppressing all CI firing, 'residual' bursting in the CP, GP, LC and LG cells would usually reappear (Fig. 5). The firing rate during residual CP bursts was lower than normal, but firing rates during residual bursts in the LC and GP cells reached normal values (Fig. 5, rate meter traces). These findings show that other mechanisms (besides CI bursting) contribute significantly to burst formation in CP, LC and GP. The residual bursting could assume various phase relationships relative to the oesophageal bursts (Fig. 5, ORn trace), suggesting that it was not simply driven by the oesophageal generator.

An important effect of suppressing the firing of CI was a phase shift whereby CP bursts could become nearly synchronous with LC-GP bursts (Fig. 6A2), in contrast

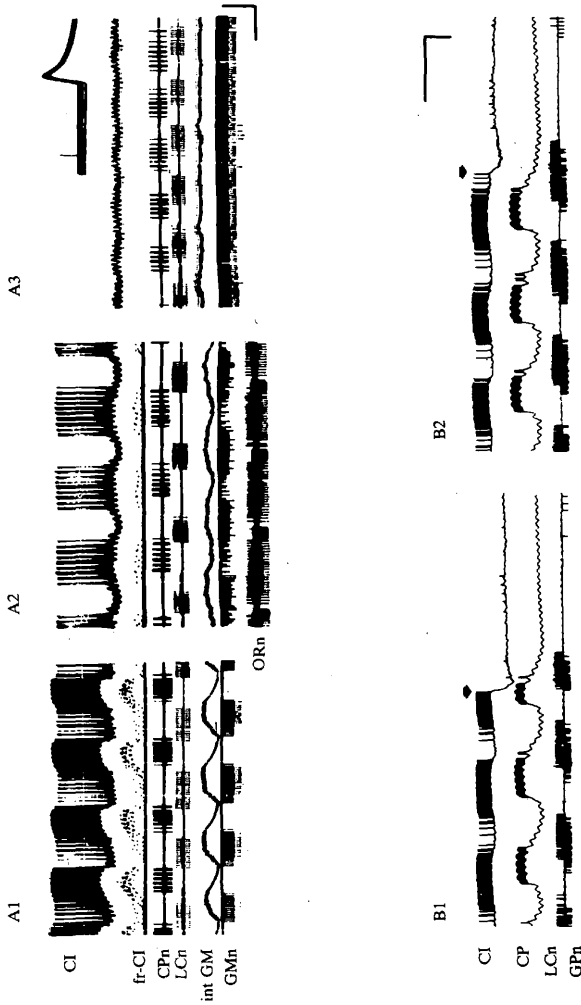


Fig. 4. Functional effects from polarizing CI. (A1) Control activity. Note that the instantaneous firing rate of CI (fr-CI trace) linearly reflected the ramp-like form of the intracellular CI oscillation (CI trace). GMn: half-wave rectified GM spikes (pointing down). int GM: integrated record of rectified GM spikes portrays the GM firing rate. (A2) Firing of CI was reduced by a -1 nA offset. The ORn trace (this panel only) monitored the esophageal rhythm, to which the gastric bursting was locked 1:1. (A3) Complete suppression of CI firing by -3 nA offset. Inset shows identification of CI by antidromic stimulation of the stomatogastric nerve. The fr-CI trace was deleted. (B) Another preparation; CI was hyperpolarized at two different points in the rhythm cycle. Calibrations: (A) 20 mV CI, 30 Hz CI rate, 5 s. (Inset): 20 mV CI, 20 mV CP, 5 s.

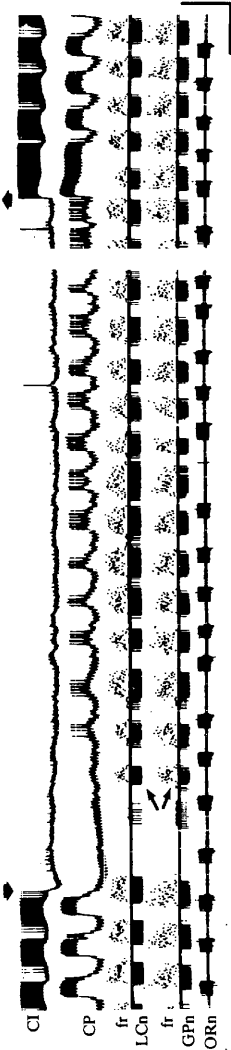


Fig. 5. The CI cell was hyperpolarized by  $-3$  nA at the downward arrow and released at the upward arrow. The gap in the record was 65 s. The CI and CP traces show intracellular records. The LCn and GPn traces show half-wave rectified spikes and instantaneous firing rates on shared baselines. Arrows (left) point to LC and CP bursts in the absence of any CP firing, hence CP was not involved in forming their bursts. Calibrations: 160 mV CI, 40 mV CP, 60 Hz LC and GP rate, 10 s.

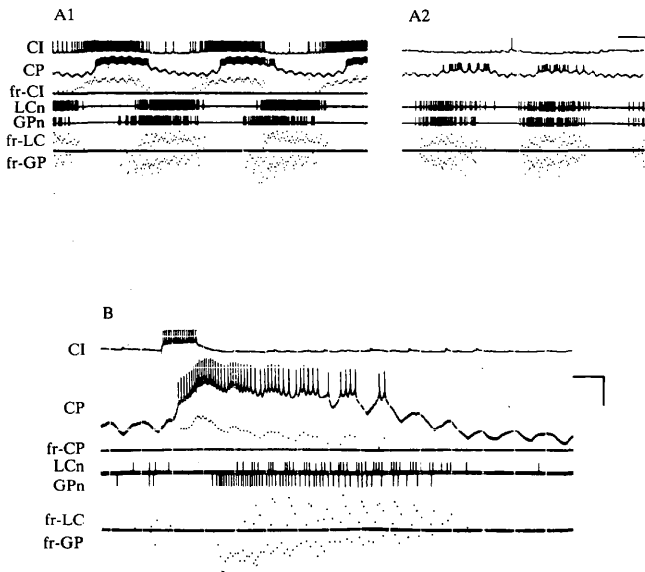


Fig. 6. Control of GP-LC phase shift by CI. (A1) Control pattern, with firing rates for CI, LC and GP; the latter two are shown on a shared time base. (A2) CI firing was suppressed by  $-3$  nA offset. Note synchrony of LC, GP and CP bursts. (B) Effects of a brief CI discharge. CI firing was suppressed with hyperpolarizing current, then released for 0.5 s to fire at 35 Hz. Calibrations: (A) 80 mV CI, 40 mV CP, 30 Hz, 2 s. (B) 80 mV CI, 10 mV CP, 30 Hz, 0.5 s.

to the near-alternation seen when CI was bursting (Fig. 6A1). The overlap of CP and LC bursts was in fact inversely *proportional* to the CI firing rate (Fig. 4A). These data suggest the existence of a separate burst generator for the returnstroke and the powerstroke (see Discussion) and indicate that CI firing may be necessary for their alternation.

Another effect of CI concerned the delay whereby LC bursts normally started a few hundred ms later than GP bursts (Fig. 6A1). This delay was abolished when CI was silent (Fig. 6A2), even immediately after silencing CI (Fig. 4B1), but reappeared when CI was stimulated (below, Fig. 6B). Hence the delay depends on CI firing.

Complementary results came from briefly stimulating the CI cell shortly after suppressing its firing, when the gastric system was non-rhythmic (as Fig. 4B). A CI discharge evoked, after a delay of several hundred ms, a large depolarization in the CP cell that greatly outlasted the CI stimulus (Fig. 6B). This supports the proposal that CI essentially 'drives' the burst in CP (directly or indirectly). Brief firing of CI also evoked single bursts in the LC and GP powerstroke cells having normal firing rates and phase delays (Fig. 6B). This was surprising since CI is usually considered

to 'inhibit' LC and GP. Such results may involve indirect pathways and additional gastric interneurons, not presently characterized, whose transmission to gastric motoneurons may be 'gated' by firing of CI (Russell & Hartline, 1984).

Fig. 7 shows an attempt to assess the role of monosynaptic CI-to-GM and CI-to-GP inhibition (Maynard, 1972; Selverston & Mulloney, 1974) in forming the interburst intervals in these cells, by asking whether the membrane potential was inversely proportional to the CI firing rate. This was approximately true for the GM cell since its membrane potential reached the most negative point when the CI firing rate was maximal. In contrast, the most negative point of the GP interval (arrow) occurred well in advance of the peak in CI firing rate, and in fact the GP cell started depolarizing while the CI rate was still accelerating. This was not due to anti-facilitation ('fatigue') of the CI-to-GP IPSPs since their mean amplitude was constant. Monosynaptic inhibition from CI may therefore have a significant role in forming the bursts in GM cells but cannot account for the membrane potential trajectory of the GP cell.

#### *Synaptic potentials in CI*

Given its importance, the different types of synaptic potentials visible in intracellular recordings from the CI soma were studied during on-going gastric rhythms (Fig. 8). (i) The membrane potential and firing rate of CI were modulated in time with the pyloric rhythm (PDn, A1), due in part to trains of small EPSPs

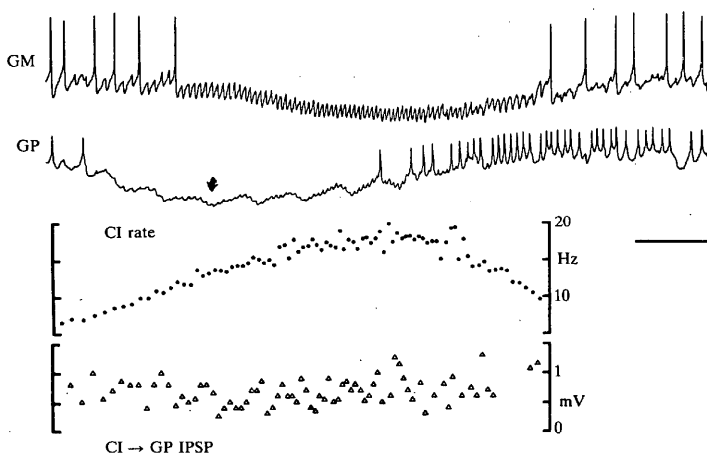


Fig. 7. Intracellular activity of GM and GP cells during on-going rhythm, after sucrose blocking both superior oesophageal nerves. On the same timebase are shown measurements of the CI firing rate and the amplitude of CI-to-GP IPSPs, measured from expanded records replayed from tape (not shown). The CI firing rate was taken from the intervals between large IPSPs in the GM record, synchronous and 1:1 with IPSPs in GP. The slow waves in the GP record were synchronized with the pyloric rhythm. Calibrations: 8 mV GP, 10 mV GM, 1 s.

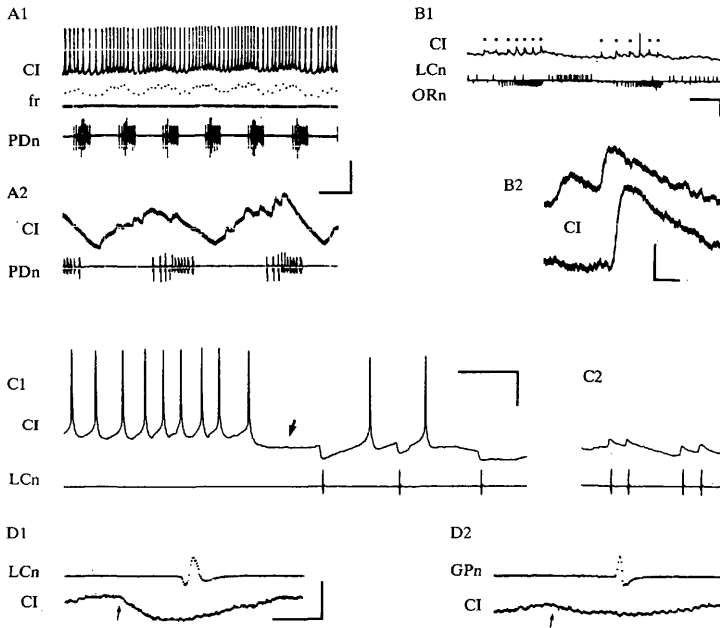


Fig. 8. Synaptic potentials in CI. (A1) Simultaneous records of CI intracellular activity, CI firing rate (fr), and nerve record of bursts from the PD motoneurons of the pyloric rhythm generator. The record was taken during the middle of a CI burst, when LC and GP were not firing. (A2) CI was hyperpolarized by  $-3$  nA; note trains of EPSPs in time with the pyloric cycle, probably from interneurons in the commissural ganglia. (B1) CI was hyperpolarized by  $-3$  nA to show trains of large EPSPs (points). Nerve trace monitors the gastric rhythm (LCn) and the oesophageal rhythm (ORn) on a shared timebase. (B2) Expanded records of EPSP; upper trace suggests two unitary EPSPs. (C1) The end of a CI burst; note that CI firing stopped (arrow) well before the first IPSP from LC. (C2) IPSPs from LC were inverted by a  $-3$  nA current to CI. (D) Pre-triggered averaged ( $N = 16$ ) records of IPSPs from LC (D1) and GP (D2), taken from the same records near the end of CI bursts for valid comparison of IPSP amplitude. The signal averager was triggered by motor nerve spikes from LC or GP. Arrows indicate start of IPSPs. Calibrations: (A1) 20 mV CI, 30 Hz CI, 0.5 s. (A2) 4 mV CI, 0.2 s. (B1) 20 mV CI, 2 s. (B2) 5 mV CI, 20 ms. (C) 10 mV CI, 0.2 s. (D) 1 mV CI, 10 ms.

(A2). (ii) Trains of large EPSPs (B1, B2) were typically seen at intervals of 5–10 s, although their source is unknown. (iii) The LC and GP motoneurons have been reported to inhibit CI (Mulloney & Selverston, 1974b). During on-going rhythms, the IPSPs from LC could be several mV in amplitude (C1). They were readily inverted by hyperpolarization (C2) and so were probably typical conductance-increase IPSPs. It was also possible to record IPSPs in CI (during on-going rhythms) that were time-locked to spikes in the GP motoneurone, although the

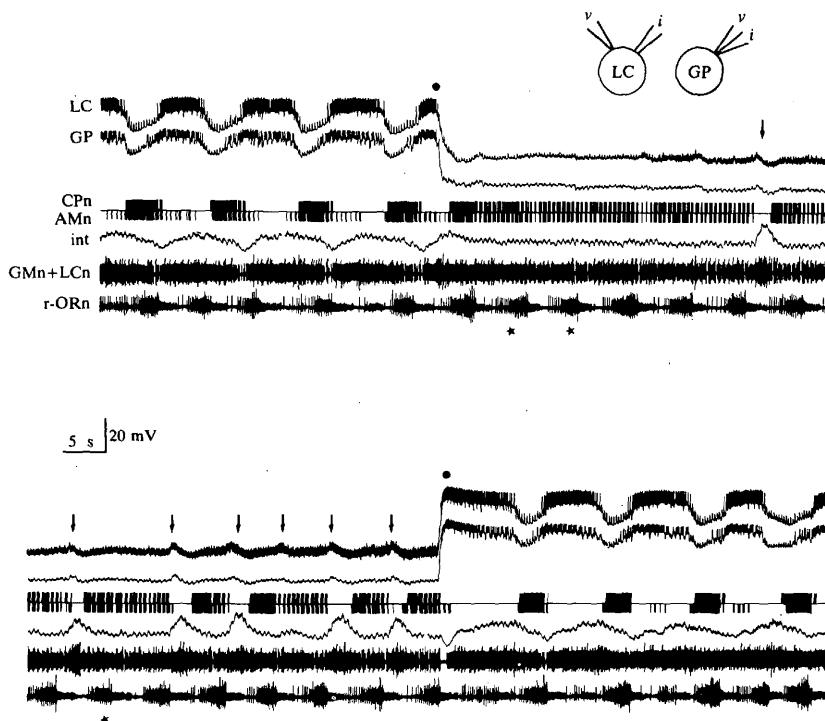


Fig. 9. Simultaneous intracellular records from LC and GP cells, hyperpolarized between dots. Upper and lower panels are continuous. CPn, AMn: nerve records of CP and AM firing on a shared baseline. GMn+LGN: activity in a dorsal-lateral ventricular nerve, with spikes from GM and LG cells. int: same, after rectification and integration with an effective time constant of 0.5 s. r-ORn: the oesophagus rhythm was recorded from a motor nerve from the right-side commissural ganglion. Thickening of LC trace during hyperpolarization was an instrumentation artifact. Currents to LC and GP were in the range of  $-8$  to  $-10$  nA each.

IPSPs were smaller than those from the LC cell (D1, D2). This inhibitory input to the CI interneurone from the LC (and possibly GP) motoneurone is an important connection in the lateral-to-medial pathway.

#### *Effects of firing LC-GP*

It is known that strong hyperpolarization of the LC and GP cells can abolish bursting in the other gastric cells for tens of seconds (Selverston, Russell, Miller & King, 1976). It causes the CP and LG neurones to fire tonically, whereas the GM and E neurones stop firing or fire tonically at low frequency. However, bursting in

the other cells does eventually recover (Fig. 9), with small biphasic deflections in LC and GP (presumably due to synaptic input; arrows) being accompanied by pauses in CP and AM firing and by increases in GM firing (integrated trace). Such events were not locked 1:1 to the oesophagus rhythm (asterisks). Note that separate current and voltage electrodes were placed in both the LC and GP cells, so that it can be said that both cells were hyperpolarized about 20 mV below the normal trough  $V_m$  level, enough to abolish even non-spiking synaptic transmission from other STG cells (Graubard, Raper & Hartline, 1980). Hence Fig. 9 indicates that alternate bursting of the medial tooth antagonist motoneurons can continue despite abolishing chemical synaptic transmission from the LC and GP cells.

The lateral-to-medial pathway could be well demonstrated under the non-rhythmic conditions obtained just after hyperpolarizing the LC and GP cells (Fig. 10). A brief discharge of LC (in the absence of GP firing) evoked a burst in the E interneurons and GM motoneurons, and a pause in the firing of CP. Note that firing of the LC, E and GM cells began in that sequence, although the GM firing began about 160 ms after the first LC spike, somewhat earlier than the 280 ms delay seen during normal rhythms in this preparation. Thus the LC discharge evoked an isolated burst in the medial tooth powerstroke system while suppressing the returnstroke output, consistent with the lateral-to-medial pathway being proposed.

#### *Non-spiking inhibition of LG cells*

The two LG motoneurons act to separate the lateral teeth (Hartline & Maynard, 1975). They are inhibited by the LC and GP motoneurons (Maynard, 1972; Mulloney & Selverston, 1974a) and this inhibition is important in creating the alternate opening and closing of the lateral teeth.

It appears that both non-spiking transmission as well as IPSPs subserve the inhibition. One sign of non-spiking inhibition was that a hyperpolarizing current applied to the LC cell (under conditions when it was not firing) caused a depolarization and faster spiking in the LGs (Fig. 11A, trace 1). This indicates a continuous release of inhibitory transmitter even when the presynaptic (LC) cell is at rest (see Graubard *et al.* 1980). Depolarization of LC caused a smooth inhibition of LG (traces 2, 3), which became progressively larger at larger stimulus currents (Fig. 11B). It was only the strongest currents that evoked a few LC spikes and accompanying IPSPs in the LG cell (Fig. 11A, trace 4). Hence very strong non-spiking inhibition of the LGs can be demonstrated by polarizing the LC cell.

During rhythms, LG bursts could end with a smooth hyperpolarization about 0.2 s in advance of the initial GP spike (arrowheads, Fig. 12A). This may have been due to non-spiking inhibition evoked by the positive-going (but still subthreshold) membrane potential trajectories in the GP and LC cells.

The LG cells reciprocally inhibit the LC-GP cells (Mulloney & Selverston, 1974a), although discrete IPSPs are not seen and it is not clear if the connection is monosynaptic. Polarizing the LG cells did affect the bursting activity of the GP cell. While the  $V_m$  level of the GP burst depolarization was unchanged, the depth of the interburst 'trough' in GP became shallower (asterisks, Fig. 12B), so that the amplitude of its oscillations (see double-headed arrow for measurement method) was reduced from  $14.9 \pm 1.3$  mV normally to  $10.8 \pm 1.4$  mV (mean  $\pm$  s.d.) with the LGs

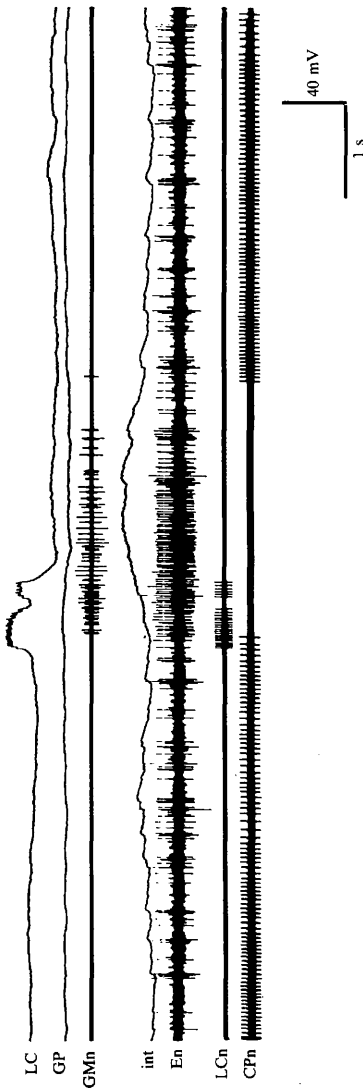


Fig. 10. The gastric rhythm had been stopped a few seconds prior to this record by hyperpolarizing the LC and GP cells (intracellular records). En: activity in the stomatogastric nerve; the largest units were from the E neurones since they fired in bursts synchronized with GM-cell bursts when the rhythm was present (not illustrated). int: rectified and integrated record of activity in the stomatogastric nerve, primarily reflecting change in E neurone firing rate.

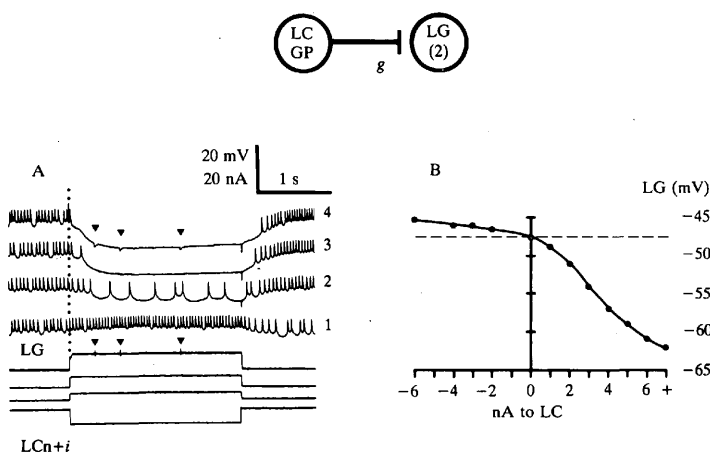


Fig. 11. Graded inhibition of LG cells. (A) Isolated STG preparation, injecting current into LC while recording from one of the LG cells (which are normally firing tonically). Lower traces show the bath current monitor, summed with a nerve record of LC spikes (triangles).  $g$  = graded or non-spiking transmission. The onset of LG inhibition became much faster with stronger LC depolarization (vertical dotted line), while the recovery time to resumption of LG firing after a pulse became longer (traces 2–4). In trace 1, the LG firing rate increased from 14 Hz beforehand to 19 Hz near the end of the hyperpolarizing stimulus to LC. There were negative after-effects, including rebound for traces 2–4 and refractoriness for trace 1. (B) Transfer function plotted from records like (A). The absolute membrane potential of LG was measured at the point of maximum voltage deflection. Horizontal dashed line shows the normal level during tonic LG firing.

hyperpolarized, a significant reduction ( $P < 0.002$ ,  $t$ -test,  $N = 20$ ). Also, the duty cycle of GP bursts expanded. Thus 'inhibition' from LGs helps form the GP bursts. Their access to the important LC-GP cells suggests that the LGs probably should be able to affect the timing of the gastric rhythm, although such was not the case in Fig. 12B.

#### DISCUSSION

This article analyses the neural basis of the teeth coordination in the gastric mill system. The firing of identified cells was altered during rhythms to study their functional effects on the phasing and firing rates of other cells. The functional effects can be related to the reported monosynaptic connectivity (see Selverston *et al.* 1976), and place constraints on models for the gastric generator. Of course these results came from an *in vitro* preparation, whereas sensory feedback and interactions with higher-level ganglia may affect the operation of the gastric system *in vivo*.

#### Coordination of the gastric pattern

The coordination pattern of the medial tooth antagonists apparently derives from several synergistic 'levels' of synaptic organization (Fig. 13). (i) The simplest

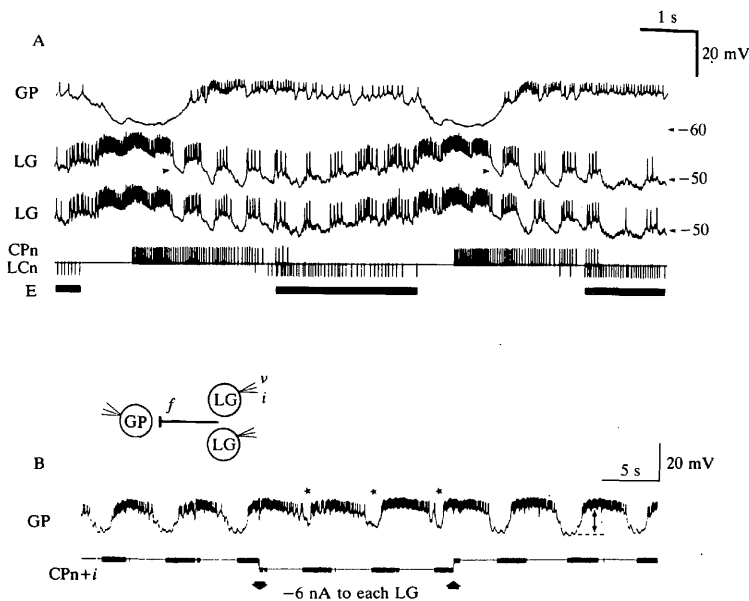


Fig. 12. Activity and polarization of LG motoneurons. (A) Simultaneous intracellular records of spontaneous bursting in GP and both LG cells, with summed nerve records of CP and LC firing. Bars show estimated time of E neurone bursts, from other records. Arrowheads: smooth repolarization of LG cells preceded first spike in GP or LC, and is postulated to arise from non-spiking inhibition. (B) Same preparation. Offset of  $-6$  nA was applied to each LG cell at the downward arrow and released at the upward arrow. Intracellular record of GP. Nerve record of CP bursts was summed with the bath current monitor.  $f$ : functional effect (i.e. not necessarily monosynaptic).  $v$ ,  $i$ : voltage recording and current injection electrode barrels.

mechanism for the alternate firing of the CP and GM cells is the direct CP-to-GM inhibition (Fig. 11A), although it is relatively weak (Table 1). (ii) Firing of CI will tend to create a medial tooth alternation due to its opposite monosynaptic effects of inhibiting the GMs and weakly exciting CP (Fig. 13B; Mulloney & Edwards, 1980). (iii) Interneuronal pathways apparently reinforce the direct effects of CI (Fig. 13C). The CI-E-GM pathway results in a net 'de-excitation' of GM cells when CI fires (Russell, 1976). The functional 'excitation' of CP and AM cells by the CI interneurone during rhythms in 'combined' preparations (see Fig. 6B) probably involves uncharacterized interneuronal pathways ('IR', Fig. 13C) since it is much stronger than the direct CI-to-CP excitation seen in the isolated STG (Russell & Hartline, 1984). These three levels of organization are redundant and synergistic, all tending to make bursts in GMs and CP occur alternately.

Coordination between the lateral and medial teeth appears to originate directly with the LC-GP motoneurons (rather than a possible pre-motor interneurone), since altering their firing strongly affected the coordination pattern. Since direct

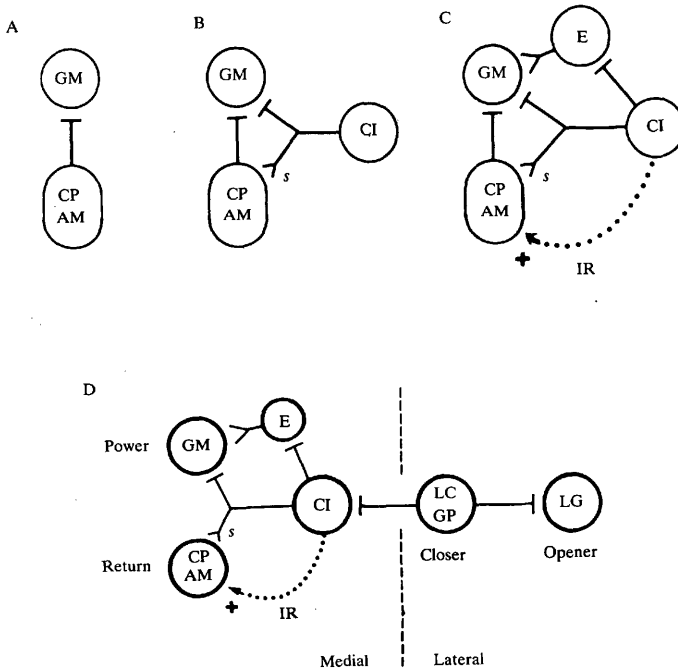


Fig. 13. (A–C) Three redundant synergistic levels of organization contribute to coordinating the medial tooth alternation. (D) The motoneurons closing the lateral teeth, LC and GP, appear central to the coordinating mechanism. fork: monosynaptic EPSP, *s*: slow but direct synaptic effect without discrete PSPs. IR: indirect reflex by which CI strongly 'excites' CP.

inhibitory synapses between the medial tooth cells and the LC-GP cells were weak (Table 1, Fig. 3), it appears that an indirect pathway, *via* the CI interneurone, subserves the normal coordination of the medial and lateral teeth (Fig. 13D). According to this model, inhibition of CI by the LC-GP cells is an important connection in the coordination pathway, consistent with the lack of other visible synaptic potentials in CI synchronized to the gastric rhythm (Fig. 8). It will be interesting to see if the inhibition of CI by LC-GP has a non-spiking component (arrow, Fig. 8C1); if so, then bursting in the medial tooth motoneurons might still be controlled by LC-GP even if the latter cells are not firing spikes (as often seen in aged preparations).

#### *Burst generation vs coordination*

The synaptic model in Fig. 13D might explain how the bursts in some cells are *generated*. For example, it suggests that burst formation in the GM cells derives

(directly and indirectly) from bursting in CI, conforming to the results in Figs 4A and 7. Alternatively, the synapses might just *coordinate* the bursts in some cells, generated by other means (below). If the LC-GP cells are assumed to fire in regular bursts, the model could reproduce several principal features of the gastric pattern: alternation of CP and GM bursts; alternation of LC-GP and LG bursts; a two-part sequence corresponding to the LC interval (= returnstroke) and the LC burst (= powerstroke); approximate synchrony of bursts in LC, GP and GM; bursts in GP-LC starting somewhat before the GM bursts; approximate synchrony of CP, LG and CI firing; bursts in CI phased somewhat before bursts in CP, etc. The model does not predict constant-duration behaviour for the returnstroke (Russell, 1985) unless the LC interval is so constrained. The model predicts that properly phased depolarizing or hyperpolarizing stimuli to the LC cell can have equivalent effects on bursts in the CP cell, as in Fig. 2B.

#### *Kinetic properties*

It is striking that some events occurring in the gastric network have a rapid time course, while others are slow. An example of a relatively rapid event is the termination of CI bursts (Fig. 4A), possible due to the large fast-rise IPSPs from LC.

Slow events include the following. (i) The net excitation of CP by CI is delayed and builds up slowly; e.g. there was a 700 ms delay between the start of CI firing and the peak depolarization of CP in Fig. 6B. (ii) The firing rate of CI slowly increases during its bursts (Fig. 4A1). Both (i) and (ii) presumably contribute to the phase lag of CP bursts after those of CI (Fig. 6A1). (iii) The firing rate of the E neurones tends to build up slowly (Fig. 10); e.g. the E firing rate requires 2 s or more to reach maximum when they are disinhibited by hyperpolarizing CI (Russell, 1976).

The performance of the model in Fig. 13D would depend in part on these kinetic parameters. It is interesting to ask whether some of the slow events [especially (ii) and (iii), above] might derive from intrinsic properties of the cells.

#### *Rhythm generation*

The GM, LC, GP and CI cells can affect the cycle period, and so have access to the gastric generator (Russell, 1985). However, strong hyperpolarization of any cell type (e.g. 3 GMs, LC+GP, CI alone) abolishes the rhythm only temporarily. Assuming that strong hyperpolarization is effective in abolishing synaptic output, it would appear that no individual STG cell type is necessary for rhythmic burst production in some or all the other gastric cells. The same was true for gastric neurones in the commissural ganglia with axons descending in the superior oesophageal nerves, since cutting these nerves need not abolish gastric bursting in STG cells. The ability of different models for the gastric system to explain these findings is reviewed below.

#### *Network model*

The gastric network within the STG is sufficient to generate gastric rhythms having a normal pattern, e.g. when activated by command-fibre inputs (Russell & Hartline, 1978, 1984). The rhythm generation has been attributed to an 'emergent

property' of the synaptic connectivity (Mulloney & Selverston, 1974*b*), as shown by computer modelling studies (Warshaw & Hartline, 1976; Friesen & Stent, 1978; Thompson, 1982). The observation that CP and LC bursting continued when CI was silent (Fig. 5) would appear to raise difficulties for these network models, since they assign a key role to the CI interneurone and to the reciprocal inhibition between CI and LC.

It was also significant that alternate bursting of the medial tooth antagonists, CP as well as the GMs, continued when the LC and GP cells were strongly hyperpolarized (Fig. 9). This might be explainable by endogenous bursting of CP (Hartline & Russell, 1984) and CP-to-GM inhibition. However the biphasic wave recorded in LC-GP suggests that the E and CI interneurons may have been bursting as well (they respectively excite and inhibit LC-GP; Russell, 1976; Mulloney & Selverston, 1974*a*), which would not be predicted by the network hypothesis. Of course the network concept was proposed (and may still be valid) for the cells in the isolated STG, whereas the present results came from a more complex preparation.

#### *Pacemaker model*

Robertson & Moulins (1981) suggested that certain neurones in the commissural ganglia of *Homarus* (with axons descending in the superior oesophageal nerves) act as pacemakers to drive the bursting of GM motoneurons in the STG. *Panulirus* may be different since cutting the superior oesophageal nerves need not abolish gastric bursting in the STG cells (see Fig. 2 in Russell, 1985), and since the burst formation in GMs depends strongly on the bursting in CI (Fig. 4A; Russell, 1976).

#### *Two-generator model*

Figs 4–6 show that the degree of burst overlap in the CP and LC cells is inversely proportional to the firing rate of CI. I interpret this finding to mean that bursts in the CP cell might be generated by one mechanism, whereas bursts in the LC and GP cells might be generated by a different mechanism. According to this 'two-generator' model, the function of the CI cell and the lateral-to-medial pathway would be to make the two burst generators work out of phase (besides synaptically forming the bursts in the GM and LG cells).

The burst mechanism for CP has at least three components: endogenous burst properties of CP itself (Russell & Hartline, 1978; Hartline & Russell, 1984), monosynaptic excitation of CP by CI (Selverston & Mulloney, 1974), and a powerful indirect excitatory pathway gated by CI (Russell & Hartline, 1984). The involvement of endogenous-burst properties at one or more levels may explain the tendency of CP bursts to assume a constant duration despite variation in the cycle period (Russell, 1985).

The origin of the continued bursting in LC and GP when CI was not firing (Figs 4A3 and 6A2) is not clear, but was a consistent finding. Evidently another mechanism besides the monosynaptic inhibition from CI (Mulloney & Selverston, 1974*b*) must contribute to forming the interburst intervals of LC and GP. The residual bursting does not depend on input from the GM cells (*via* electrical

coupling) since the bursting of LC and GP could continue despite tonic firing of the GM cells (Fig. 4A3).

Nevertheless, the CI cell does strongly interact with LC and GP. A net 'inhibitory' interaction is suggested by Fig. 4B1, since suppressing CI firing at mid-burst caused the next LC-GP burst to start early, as if disinhibited. On the other hand, a net 'excitatory' interaction is suggested by Fig. 4B2, since an absence of CI firing caused the next LC-GP burst to be much delayed and weak. Excitation is also suggested by the ability of a brief CI discharge to evoke a burst in LC and GP (Fig. 6B). It is debatable whether the monosynaptic connections within the STG could explain these responses; they may instead be mediated by indirect pathways, especially since the CI interneurone is known to synapse on neurones in the oesophageal and commissural ganglia *via* an ascending axon (Russell, 1976).

A 'two-generator' model would be consistent with the pattern analysis (Russell, 1985) showing that the gastric rhythm cycle is basically two-part. One could speak of a 'returnstroke' generator (e.g. for the CP burst) and a 'powerstroke' generator (e.g. for the LC-GP burst). Once again, the main evidence for two distinct generators is that their burst overlap is a graded function of the CI firing rate. This would appear to exclude simpler models for two-part rhythms such as proposed for terrestrial locomotion (Pearson, 1976), in which the extensor system is proposed to discharge tonically except when 'inhibited' by a constant-duration flexor-burst generator.

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