

## A corollary discharge of total foregut motor activity is monitored by a single interneurone in the lobster *Homarus gammarus*\*

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### SUMMARY :

1° In *Homarus*, an identified interneurone (the L cell), which possesses the largest cell body in the commissural ganglion and projects to the brain, exhibits a complex firing pattern (Fig. 2 a).

2° It is shown that the L cell discharges with each of the 4 pattern generators of the stomatogastric nervous system which organize the rhythmic motor activity of the foregut (Fig. 2 b-e).

3° Manipulation of the membrane potential of the L cell does not induce any change in the 4 rhythms (Fig. 3), and it is concluded that the L cell is driven by the 4 pattern generators.

4° The functional meaning of this complex corollary discharge of the total foregut motor activity is discussed.

**Key words :** Corollary discharge. Rhythmic motor program.

### INTRODUCTION

Corollary discharge neurones are interneurons having a pattern of firing that is an approximate copy of the motor activity that they monitor (GYR, 1972). Their definition is purely descriptive and takes no account of the possible role of such discharges. Thus, corollary discharge neurones have been variously described as acting to prevent refference (sensory information arising from an animal's movements) (KENNEDY *et al.*, 1980), or in some way to compensate for this (DELCOMYN and DALEY, 1979), to co-ordinate the different parts of a distributed pattern generator (DAVIS *et al.*, 1973), and to mediate the neuronal processes underlying behavioural choice (KOVAC and DAVIS, 1980). In most cases, the monitored motor pattern is simple, and this, by definition, is reflected in the output of the particular corollary discharge

neurone. We present evidence that the activity of a pair of identified large (L) cells in the commissural ganglia of *Homarus gammarus* is generated by the activity of 4 different motor rhythms, each with its own characteristics, to provide to the brain a complex corollary discharge of total foregut activity. Present knowledge of the L cells is such that reasonable speculation as to their function is possible, and this will certainly be useful in formulating future research strategy.

Movements of the foregut of decapod Crustaceans involve at least 4 different motor rhythms. These are the oesophageal (peristalsis; period 5 s-10 s), cardiac sac (storage; period 20 s-70 s), gastric (trituration; period 10 s-20 s) and pyloric (filtration; period 1 s-3 s) rhythms (SELVERSTON *et al.*, 1976; MOULINS and VEDEL, 1977). The generation of these rhythms is not dependent on sensory input, and they can be recorded in an experimentally isolated nervous system consisting of the commissural, oesophageal and stomatogastric ganglia (Fig. 1). The pattern generators for the oesophageal and cardiac sac rhythms are located in the commissural and oesophageal ganglia, while those of the gastric and pyloric rhythms are contained within the stomato-

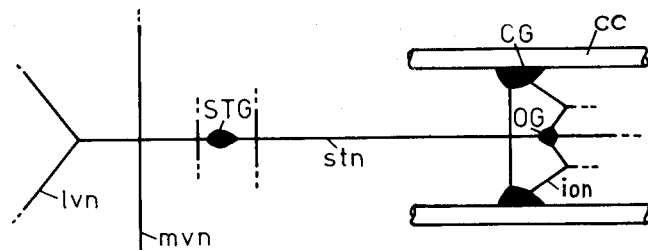


FIG. 1. — The stomatogastric nervous system as used for *in vitro* recordings.

cc, circumoesophageal connective (connecting the brain to the ventral cord); CG, commissural ganglion; ion, inferior oesophageal nerve; lvn, lateral ventricular nerve; mvn, medial ventricular nerve; OG, oesophageal ganglion; STG, stomatogastric ganglion; stn, stomatogastric nerve.

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gastric ganglion. The commissural ganglia are located on the circumoesophageal connectives which connect the cerebral and suboesophageal ganglia, and they each contain the cell bodies of some 700 cells. To date, there is only one cell in each ganglion which can be reliably and quickly identified on morphological grounds due to its very large cell body (100-130  $\mu\text{m}$ ) and prominent position at the anterior end of each ganglion over the exit of the superior oesophageal nerve. These are the L cells (SELVERSTON *et al.*, 1976). The similarity between different species of decapod crustacean is enough to justify the use of results obtained with each. Previous studies have demonstrated that the L cells have axons in only the ipsilateral circumoesophageal connective. These pass first to the brain and then double back to terminate in a peripheral neurohaemal organ, the pericardial organ (COOKE and GOLDSTONE, 1970). L cells are known to be dopaminergic (KUSHNER and MAYNARD, 1977; BARKER *et al.*, 1979) and to receive depolarising input with the oesophageal motor rhythm (SELVERSTON *et al.*, 1976). They, in fact, exhibit a complex spontaneous discharge, and there is now evidence that this complex discharge is a result of being driven also by the cardiac sac, pyloric and gastric rhythms.

## MATERIAL AND METHODS

Male and female *Homarus gammarus* were used. The stomatogastric nervous system (Fig. 1) was dissected free from the foregut and pinned in a Sylgard-lined Petri dish. Extracellular recordings were taken from the nerve trunks with fine platinum wire electrodes insulated from the surrounding medium (artificial sea water) with vaseline. Intracellular recordings were made with fibre-filled glass microelectrodes pulled to a resistance of 20-30 M $\Omega$  and filled with 3 M KCl. Conventional techniques were used to amplify, display and make permanent records of the nervous activity.

In some instances, it was desirable to abolish the effect of a particular pattern generator in order to see better the remaining activity of the L cell, and this was performed by reversibly blocking the connecting links between the ganglia. Small portions of the nerve trunks were desheathed by microdissection and isolated in a chamber fashioned from vaseline. Perfusion of these chambers with isotonic (750 mM) sucrose reversibly blocked nervous conduction in these pathways.

## RESULTS

Intracellular recordings from an L cell in the commissural ganglia reveals a complex spontaneous discharge which is difficult to resolve into different components (Fig. 2 a). However, in favourable preparations, when one or more of the foregut rhythms is silent or when the influence of particular pattern generators is blocked, the discharge of the L cell is seen to result from several different inputs which are co-ordinated with the separate rhythms. Unfortunately, individual postsynaptic potentials could not be distinguished, possibly due to the electrical distance of the inexcitable cell body from the synaptic input sites.

Fig. 2 b confirms (SELVERSTON *et al.*, 1976) the relationship of the L cell discharge with the oesophageal rhythm. In this experiment, the oesophageal motor rhythm is monitored by recording from the inferior oesophageal nerve (*ion*, Fig. 1) which innervates at least some oesophageal constrictor muscles. Each *ion* burst is associated with a depolarisation of the L cell. The pyloric rhythm can be monitored by recording from one of the three endogenous burster neurones of the stomatogastric ganglion which govern the rhythm (PD, Fig. 2 c) or from the main output nerves of the stomatogastric ganglion (*mvn*, *lvn*, Fig. 1) (Fig. 2 d). In each case, simultaneous recording from the L cell body shows that each pyloric cycle is associated with a depolarisation of the L cell. The cardiac sac rhythm is revealed by long bursts of spikes of cardiac dilator neurones in the stomatogastric nerve (*stn*, Fig. 2 d) (MOULINS and VEDEL, 1977). During such bursts, there is a massive depolarisation of the L cell which produces a long burst of high frequency spikes (Fig. 2 d). In this experiment, the L cell was also depolarised with each pyloric cycle (see *mvn* and *lvn* recordings). Finally, the relationship with the gastric rhythm can be revealed by recording from the L cell body and from a gastric motor neurone (GM) which drives the medial teeth of the gastric mill with each burst of spikes in the GM cell (corresponding to a gastric cycle) is associated a short burst of spikes in the L cell (Fig. 2 e). In this

FIG. 2. — The L cell activity (recorded from the cell body in the commissural ganglion) is correlated with the 4 rhythms governing the motor activity of the foregut.

a, complex discharge of a L cell in an experiment in which three of the four motor rhythms of the foregut were cycling.

b, correlation with the oesophageal rhythm: the L cell fires during each oesophageal burst recorded on the inferior oesophageal nerve (*ion*). In this experiment, conduction along the stomatogastric nerve was blocked with an isotonic sucrose solution.

c, correlation with the pyloric rhythm: the L cell fires with each pyloric dilator burst recorded from the cell body of a pyloric dilator neurone (PD) in the stomatogastric ganglion.

d, correlation with the cardiac sac rhythm: the L cell fires a high frequency burst with each cardiac sac dilator burst recorded extracellularly from the stomatogastric nerve. Note that in this experiment the L cell membrane potential is also modulated by the pyloric rhythm recorded extracellularly on the *mvn* and *lvn* (see Fig. 1).

e, correlation with the gastric rhythm: the L cell is activated with each burst of spikes of a motoneurone (GM) of the medial gastric teeth.

Vertical bars, 10 mV; horizontal bars, 5 s.

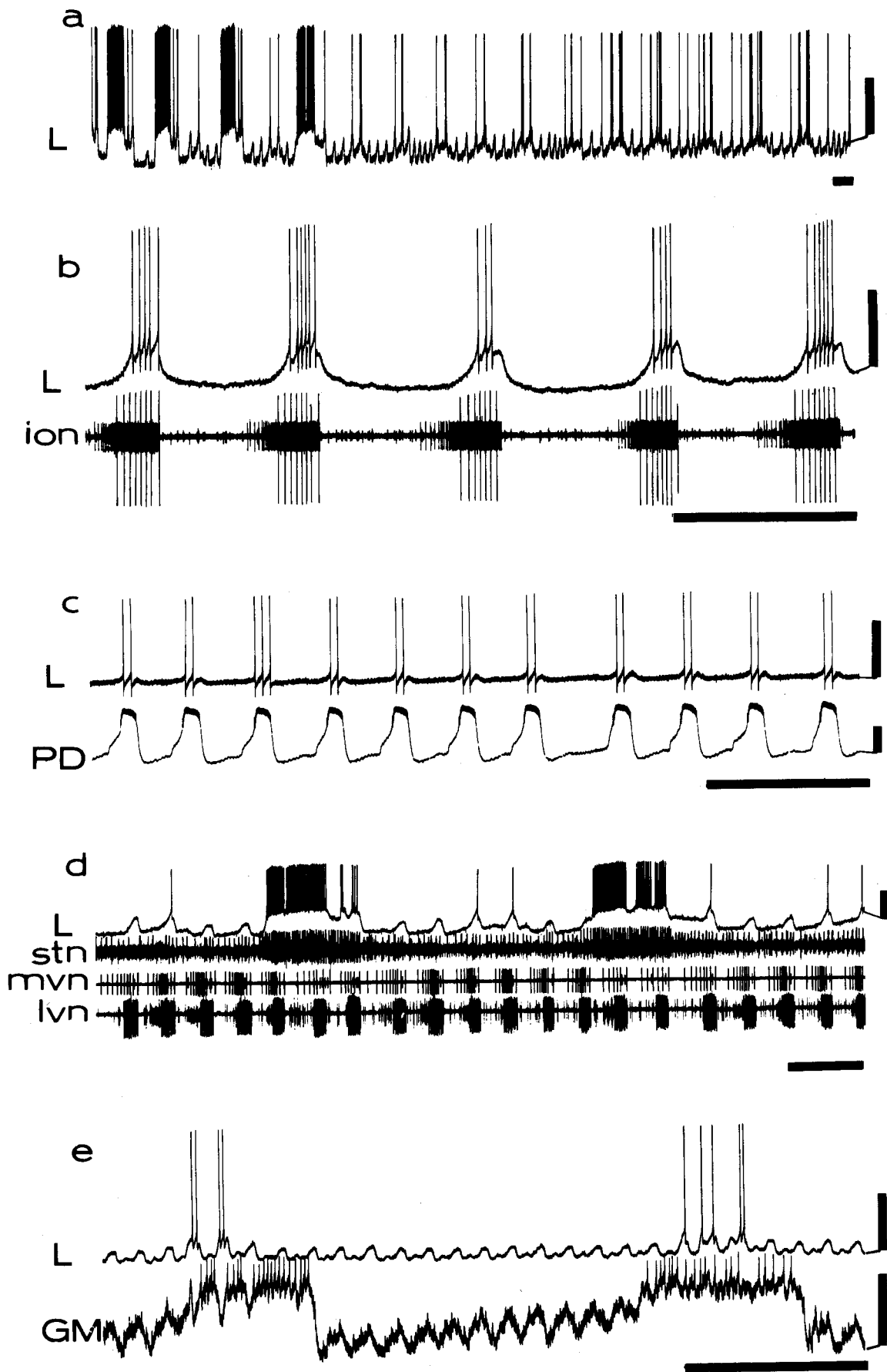


FIG. 2.

experiment, the small cyclic depolarisations of the L cell (and of the GM cell) are correlated with the pyloric rhythm. It can be concluded that the L cell activity is correlated with the 4 foregut motor rhythms.

The L cell does not participate in the generation of any of these 4 motor rhythms, but is driven by the 4 pattern generators. This can be shown by manipulation of the membrane potential of the L cell (*i.e.*, experimental depolarisation or hyperpolarisation by current injection in the cell body) which never induces any modification in any one of the 4 motor outputs. In the experiment of Fig. 3, for example, the oesophageal output is exactly the same when the L cell is silent and when the L cell is firing after experimental depolarisation.

## DISCUSSION

The results show that the L cell monitors the activity of all the described foregut rhythms and provides to the brain an overall picture of foregut activity. There are two L cells, one in each commissural ganglion, and simultaneous recordings from the right and left L cells show that unpaired pattern generators (*i.e.*, the pyloric, gastric and cardiac sac patterns generators) project on each L cell. In contrast, the oesophageal rhythm is organised by a paired pattern generator. It consists of bilateral oscillators (one in each commissural ganglion) which can function independently of each other (MOULINS and NAGY, 1981). Simultaneous recordings in the left and right commissural ganglia show that each L cell monitors only the activity of the ipsilateral oesophageal oscillator. Thus, the combined output of the two L cells is sufficient to deliver to the brain an accurate indication

of the functional state of the foregut at any time. We are unaware of any description of a similar complex corollary discharge in other invertebrate animals.

It has recently been shown that the gastric and pyloric pattern generators of the stomatogastric ganglion can be driven by independent premotor « control » oscillators located in the commissural ganglia (ROBERTSON and MOULINS, 1981). Nevertheless, when conduction along the stomatogastric nerve, which connects the stomatogastric ganglion to the commissural ganglion, is blocked, the L cell is not activated with the pyloric and gastric rhythms (see Fig. 2 b). This means that the L cell is not driven by the commissural « control » oscillators (premotor level), but by the pattern generators themselves (motor level). In other words, the L cell provides to the brain a discharge which is a picture of the motor output and which takes no account of the activity of the premotor level.

The function of this discharge is still unknown, and the present *in vitro* preparation (minus the terminals of the axons of L cells) precludes such an investigation. However, it is useful to speculate on this based on the known pathways of the L cells. The axon of each L cell passes first towards the brain, and then doubles back on itself, passes its commissural ganglion, and finally terminates in the pericardial organ (COOKE and GOLDSTONE, 1970). It is intriguing to think that the L cells may serve the same function as the postulated corollary discharge neurones of the feeding rhythm of *Pleurobranchaea* (DAVIS *et al.*, 1973). These neurones mediate a behavioural choice in that, during feeding, the response of withdrawal to a stimulus is lessened. The fact that the L cell axons carry an accurate picture of overall foregut activity to the brain indicates that a similar function of co-

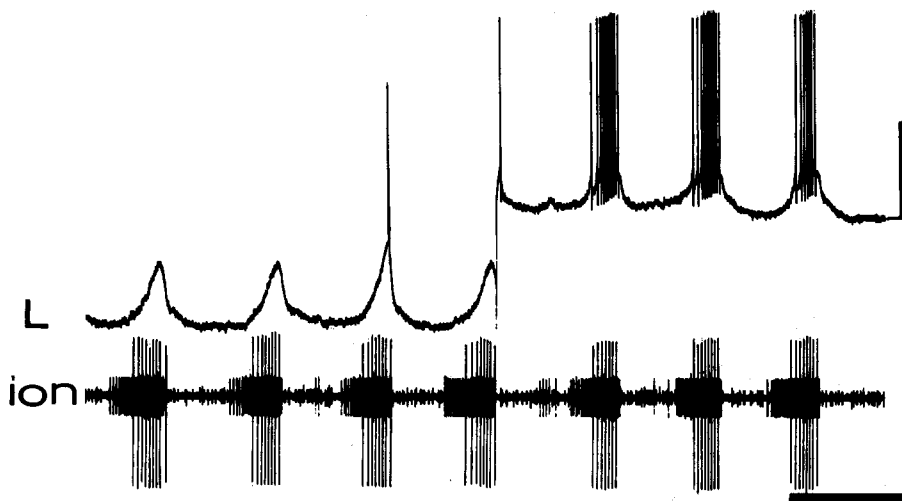


FIG. 3. — The L cell does not participate in the generation of the oesophageal motor rhythm.

In this recording, the L cell is depolarised by current injection, and the oesophageal motor rhythm, recorded on the *ion* (see Fig. 2 b), is not modified. Vertical bar, 10 mV; horizontal bar, 5 s.

ordinating other behaviours (e.g., food searching) with the state of the foregut is feasible.

The terminals of the L cells in a neurohaemal organ, the pericardial organ, could serve a completely different function not necessarily mutually exclusive with their function in the brain. The pericardial organ releases a heart excitatory peptide into the vascular system, and it is documented that monoamines do not have any effects on the release of this substance (COOKE and GOLDSTONE, 1970). However, any dopamine released by an L cell into the pericardial organ, and subsequently into the blood, will eventually reach the stomatogastric nervous system, and thus complete a long and indirect feedback loop with the pattern generators. This is particularly true for the pattern generators of the stomatogastric ganglion, which is located within the ophthalmic artery (MAYNARD and DANDO, 1974), an eminently suitable position to be affected by blood-borne factors. Supporting evidence for indirect feedback to the pattern generators via the L cell lies in the fact that bath-applied dopamine has powerful effects on at least the pyloric pattern generator (ANDERSON and BARKER, 1977 ; and unpublished observations).

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