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CONTROL OF RHYTHMIC BEHAVIOUR BY A HIERARCHY OF LINKED OSCILLATORS IN CRUSTACEA

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In Homarus, the central pattern generators for the rhythmic motor activities of the gastric teeth and the pyloric chamber are located in the stomatogastric ganglion. It is shown that independent gastric and pyloric oscillators are also contained in higher nervous centres (the commissural ganglia) and provide a phasic rhythmic input to the stomatogastric pattern generators. This demonstrates that rhythmic behaviour can be organized by a hierarchy of linked oscillators each capable of producing the rhythm.

It is generally accepted in invertebrates, and in vertebrates as well, that most neurogenic rhythmical motor activities, such as locomotion [3, 10], respiration [12] and feeding [5], are controlled by central pattern generators which, while under modulatory influence from the periphery, can function in isolation. In all cases where the timing mechanism has been characterized it has been ascribed to the endogenous membrane properties of a single cell (or a group of electrotonically coupled cells) or to the emergent properties of an interconnected network of neurones none of which possesses the capability for endogenous burst generation [4]. In certain instances, notably where control is exerted over serially and/or bilaterally repeated structures (e.g. segmental limbs), the pattern generator may be distributed within the central nervous system and composed of local control centres linked by interneuronal coordinating fibres [10]. Central action on pattern generators via command fibres serves primarily to 'trigger' or 'gate' the appropriate behaviour [2, 10].

Movements of the foregut of decapod crustaceans are controlled by four documented rhythms: the oesophageal (peristalsis), cardiac sac (storage) [6], gastrc (trituration) and pyloric (filtration) rhythms [5, 9]. For the last two of these the pattern generators have largely been elucidated, and have been shown to reside almost completely within the stomatogastric ganglion (STG, Fig. 1a) (approximately 30 cells). Timing of the pyloric rhythm is controlled by the endogenous membrane properties of a group of electrotonically coupled bursting neurones (the PD (pyloric dilator)/AB (anterior burster) group), while the gastric rhythm can be generated by the interactions within a network of interconnected neurones [9]. However, although elements which are mainly motoneurones wholly within the stomatogastric ganglion are sufficient to produce the two rhythms, it is

now known that their pattern generators are distributed to include interneurones found in a higher nervous centre, the commissural ganglia (CG) (distant, but linked to the stomatogastric ganglion by the single stomatogastric nerve, stn, Fig. 1a) [7, 9]. These commissural ganglia neurones, P neurones for the pyloric rhythm and E neurones for the gastric rhythm, are active in phase with the relevant rhythms. However, it must be stressed that although P and E neurones give phasic input to the stomatogastric ganglion, their rhythmical activity arises as a result of a feedback loop from pyloric and gastric neurones within the stomatogastric ganglion [7, 9]. They are described as being inherently tonically active, a property which becomes apparent when their connection with the stomatogastric ganglion is interrupted. Thus, there has until now been no direct evidence for a phasic (transmitting timing information) modulatory input to a pattern generator where the phasic input could originate from higher nervous centres and be independent of the activity of the pattern generator. In this paper we provide evidence for the existence of independent interneuronal oscillators located in higher nervous centres and capable of driving the pattern generators for the pyloric and gastric rhythms in the lobster *Homarus gammarus*.

The preparation used was an in vitro stomatogastric nervous system of *Homarus gammarus*, consisting of the commissural, oesophageal and stomatogastric ganglia and their connecting and main output nerves (Fig. 1a). Intracellular searches of the commissural ganglia confirm for Homarus the existence of P neurones. In addition to these can be found neurones which burst with the pyloric rhythm (Fig. 1b). They cannot be shown to have an axon in the stomatogastric nerve (stn) and experimental modification of their membrane potentials to alter their firing rate has no effect on pyloric output. However they receive phasic input, as revealed by bursts of EPSPs (excitatory postsynaptic potentials) occurring in phase with the pyloric rhythm (Fig. 1b). The AB neurone in the stomatogastric ganglion exhibits similar EPSPs and these occur with a fixed latency after the EPSPs in the commissural ganglion neurone (Fig. 1c). It is probable that AB and the commissural ganglion neurones are followers of the same bursting neurone. Thus the follower (F) in the commissural ganglion can be used as an indicator of the activity of a neurone which is bursting and providing an excitatory input to at least one (AB) of the pacemaker neurones of the pyloric rhythm.

When the stn is blocked the pyloric rhythm stops while the F neurone in the commissural ganglion continues to receive bursts of EPSPs with very little, if any, change in the burst parameters (frequency, duration) (Fig. 1d–f). The fact that the pyloric rhythm stops (Fig. 1e) is not significant in itself because there are other, separate inputs which act to sustain pyloric cycling [8], and these would also be blocked. However, two conclusions can be made about the bursting neurone providing EPSPs to AB and F: firstly its activity does not originate from the stomatogastric ganglion, and secondly it does not rely on stomatogastric pattern generator neurones for the generation of its burst.

The results for neurones in the commissural ganglia involved with the gastric rhythm are similar (Fig. 2). Each commissural ganglion contains an interneurone which will be designated CGD (commissural gastric driver) for the following

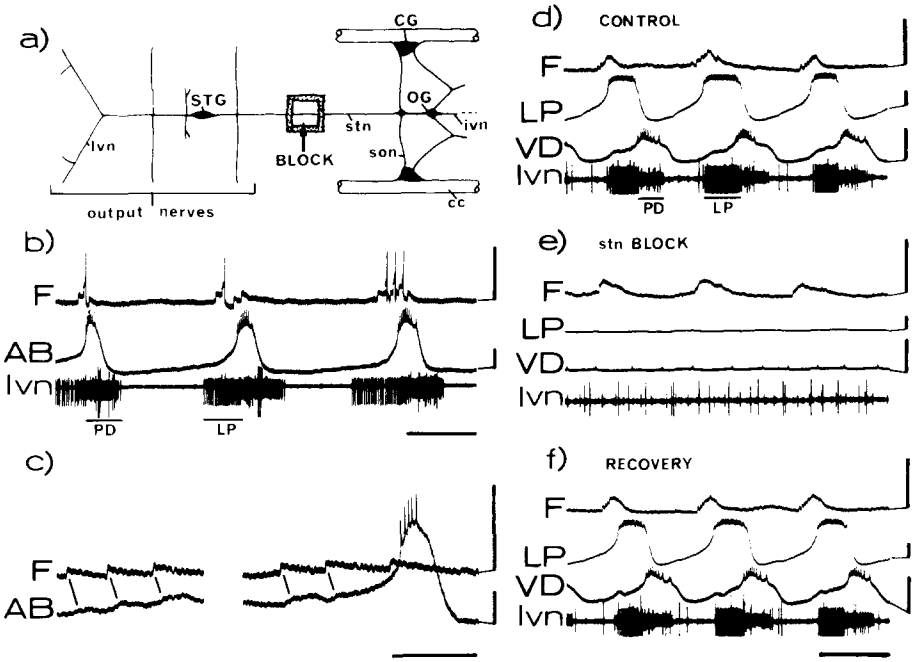

Fig. 1. The commissural pyloric oscillator. a: the preparation and procedure. The stomatogastric nerve (stn) is the only link between the stomatogastric ganglion (STG) and higher centres (i.e. the commissural ganglia (CG) which are situated on the circumoesophageal connectives (cc) linking the brain with the suboesophageal ganglion). ivn, inferior ventricular nerve; lvn, lateral ventricular nerve; OG, oesophageal ganglion; son, superior oesophageal nerve. b: the pyloric rhythm generated by stomatogastric ganglion neurones and recorded in an output nerve (lvn) consists of a burst of the dilator PD neurones followed, after a silent period, by a burst of the constrictor neurone (LP). PD and AB are bursting pacemaker neurones electrotonically coupled which periodically inhibit the constrictor neurones. The activity of the commissural pyloric oscillator, as monitored by bursts of EPSPs in a follower neurone (F) in the commissural ganglion, is locked in phase with the activity of the AB pacemaker neurone. c: in each burst the EPSPs causing F activity are time-locked with EPSPs exhibited by AB. d–f: the commissural pyloric rhythm is not generated by a feedback mechanism coming from the stomatogastric ganglion. d: the pyloric rhythm emanating from the stomatogastric ganglion is monitored extracellularly (lvn) and intracellularly by the activities of a dilator neurone (VD) and a constrictor neurone (LP). e: conduction along the stn is blocked by isolating a small desheathed portion of the nerve in a vaseline chamber and perfusing it with isotonic (750 mM) sucrose: the F activity remains unaltered while the pyloric rhythm stops. f: recovery from the block (by washing with fresh saline) is complete. Calibrations: horizontal bar, 1 sec except, in c, 500 msec; vertical bar, 10 mV.

reasons: it bursts in phase with gastric neurones as GM neurones [5] in the stomatogastric ganglion (Fig. 2a); its axon travels only in the ipsilateral superior oesophageal nerve and the stn to the stomatogastric ganglion (Fig. 2b); each action potential recorded in its cell body can be matched 1:1 (after a fixed latency of about 30 msec) with an EPSP in identified gastric neurones in the stomatogastric ganglion, indicating a probable monosynaptic relationship (Fig. 2b); and manipulation of its firing rate by current injection causes similar effects on the membrane potentials and firing rates of gastric neurones in the stomatogastric ganglion (Fig. 2c).

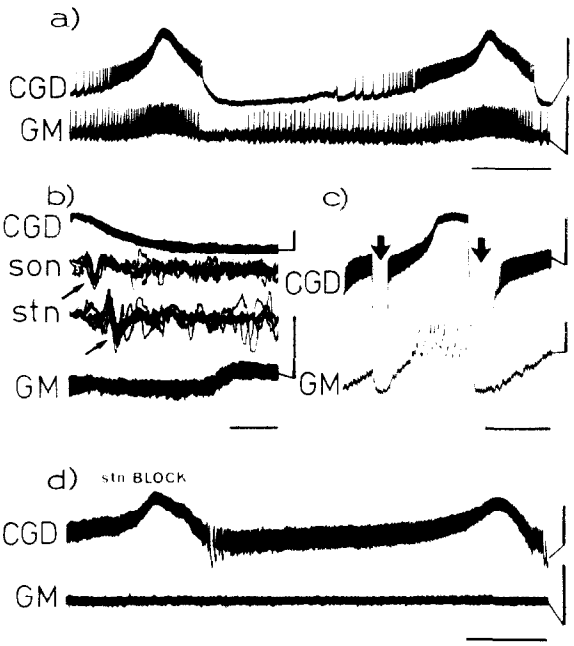

Fig. 2. The commissural gastric oscillator: activity of a higher level gastric oscillator as monitored by the bursting of a CGD neurone. a: recordings from CGD in the commissural ganglion and from a gastric neurone (GM) in the stomatogastric ganglion show simultaneous bursts. b: triggering of the oscilloscope sweep by the intracellular spike of CGD reveals monosynaptic excitatory input to the gastric neurone via the son and the stn (5 superimposed sweeps, arrows indicate the extracellular spike of CGD in the son and stn). c: temporary experimental hyperpolarization (arrows) of CGD to prevent it from firing induces a similar arrest of the depolarization of GM. d: blockage of conduction in the stn stops GM activity but CGD bursting activity remains, indicating that the latter is not generated by a feedback mechanism coming from the stomatogastric ganglion (compare with a). Calibrations: horizontal bar, 5 sec except, in b, 10 msec; vertical bar, 5 mV except, in b, 2 mV.

Blockage of, or cutting the stn stops the gastric rhythm but has no readily apparent effect on CGD burst parameters (Fig. 2d), indicating that CGD cannot be the previously described E neurones [7] and that CGD does not rely on stomatogastric neurones for the generation of its bursts.

The justification for the characterization of the commissural oscillators as providing a phasic input to, rather than being part of, the pyloric and gastric pattern generators, is that both of these rhythms can be initiated and maintained in the totally isolated stomatogastric ganglion. For the pyloric rhythm it is established that an experimentally delivered rhythmic input can drive the pattern generator [1]. Before making definite conclusions about the significance of the commissural oscillators described above it must be established that their primary function is to drive the pattern generators located in the stomatogastric ganglion. That is to say it must be shown that: (1) they do not drive motoneurones involved in the pyloric or gastric rhythms and which arise in the commissural ganglion, and (2) they are not elements of the pattern generators of other motor rhythms which can modulate or entrain the pyloric or gastric rhythms. Concerning the first point, the innervation of

the pyloric and gastric muscles is well known [5, 9], and all the identified neurones are contained in the stomatogastric ganglion. Furthermore, recordings from the output nerves of the commissural ganglion never reveal any bursting activity which can be correlated with the gastric or the pyloric rhythms. Concerning the second point, the commissural and oesophageal ganglia contain two other pattern generators which rhythmically drive the oesophagus and the cardiac sac [6, 9]. The oesophageal rhythm can be recorded concurrent with bursts of EPSPs in F and with bursting of CGD, but it is not in phase with them and can occur with a totally different burst period. The cardiac sac rhythm is generally not spontaneously active in our experimental conditions. However the characteristic indicators of such activity (e.g. bursts in the inferior ventricular nerve (ivn)) [6] have occasionally been observed though never concurrent with CGD bursts or bursts of EPSPs in F. Finally there are no other described foregut rhythms which have a frequency similar to the pyloric or gastric and while it is conceivable that one may be discovered this seems unlikely.

In conclusion, the above results are taken as evidence for the existence of independent interneuronal oscillators which are capable of driving from higher centres (the commissural ganglia) the pyloric and gastric pattern generators in the stomatogastric ganglion. This phenomenon may be unique to this and similar systems in which the pattern generators are composed almost entirely of motoneurones. It is possible that sensory and command information is best integrated prior to the motoneuronal level and, for rhythmical systems, this may be most effective if it acts on oscillatory neurones. Nevertheless, in the respiratory system of mammals the existence of a spinal cord pattern generator in addition to the medullary pattern generator has been proposed [11, 12]. However, linkage between the two has not been established. The results presented here demonstrate that a rhythm can be controlled by independent linked oscillators of which the higher-level oscillator is not part of the output pattern generator. Such a control mechanism may be found to have fundamental importance in the generation of rhythmical motor activity.

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