Long-Term Expression of Two Interacting Motor Pattern-Generating Networks in the Stomatogastric System of Freely Behaving Lobster

STEFAN CLEMENS, DENIS COMBES, PIERRE MEYRAND, AND JOHN SIMMERS
Laboratoire de Neurobiologie des Réseaux, Centre National de la Recherche Scientifique et Université de Bordeaux I, Unité Mixte de Recherche 5816, F-33120 Arcachon, France

Clemens, Stefan, Denis Combes, Pierre Meyrand, and John Simmers. Long-term expression of two interacting motor pattern-generating networks in the stomatogastric system of freely behaving lobster. *J. Neurophysiol.* 79: 1396–1408, 1998. Rhythmic movements of the gastric mill and pyloric regions of the crustacean foregut are controlled by two stomatogastric neuronal networks that have been intensively studied in vitro. By using electromyographic recordings from the European lobster, *Homarus gammarus*, we have monitored simultaneously the motor activity of pyloric and gastric mill muscles for ≈3 mo in intact and freely behaving animals. Both pyloric and gastric mill networks are almost continuously active in vivo regardless of the presence of food. In unfed resting animals kept under ‘‘natural-like’’ conditions, the pyloric network expresses the typical triphasic pattern seen in vitro but at considerably slower cycle periods (2.5–3.5 s instead of 1–1.5 s). Gastric mill activity occurs at mean cycle periods of 20–50 s compared with 5–10 s in vitro but may suddenly stop for up to tens of minutes, then restart without any apparent behavioral reason. When conjointly active, the two networks express a strict coupling that involves certain but not all motor neurons of the pyloric network. The posterior pyloric constrictor muscles, innervated by a total of 8 pyloric (PY) motor neurons, are influenced by the onset of each gastric mill medial gastric/lateral gastric (MG/LG) neuron powerstroke burst, and for one cycle, PY neuron bursts may attain >300% of their mean duration. However, the duration of activity in the lateral pyloric constrictor muscle, innervated by the unique lateral pyloric (LP) motor neuron, remains unaffected by this perturbation. During this period after gastric perturbation, LP neuron and PY neurons thus express opposite burst-to-period relationships in that LP neuron burst duration is independent of the ongoing cycle period, whereas PY neuron burst duration changes with period length. In vitro the same type of gastro-pyloric interaction is observed, indicating that it is not dependent on sensory inputs. Moreover, this interaction is intrinsic to the stomatogastric ganglion itself because the relationship between the two networks persists after suppression of descending inputs to the ganglion. Intracellular recordings reveal that this gastro-pyloric interaction originates from the gastric MG and LG neurons of the gastric network, which inhibit the pyloric pacemaker ensemble. As a consequence, the pyloric PY neurons, which are inhibited by the pyloric dilator (PD) neurons of the pyloric pacemaker group, extend their activity during the time that PD neuron is held silent. Moreover, there is evidence for a pyloro-gastric interaction, apparently rectifying, from the pyloric pacemakers back to the gastric MG/LG neuron group.

**INTRODUCTION**

Since Delcomyn (1980) established the term ‘‘central pattern generator’’ (CPG) to describe rhythmic networks that can operate without patterned peripheral or central inputs, considerable attention has been devoted to uncovering the neural basis of rhythmic motor activity. Using a wide range of approaches on invertebrate (e.g., Byrne 1983; Calabrese and De Schutter 1992; Katz et al. 1994; Ramirez and Pearson 1989) and vertebrate (e.g., Cazalets et al. 1995; Grillner and Wallén 1985; Richter et al. 1992) preparations, significant information about CPG operating principles has been obtained, and some of these systems have been described on the level of individual identified neurons, synaptic relationships, and intrinsic membrane properties (Calabrese et al. 1995; Frost and Kandel 1995; Getting 1988; Marder and Calabrese 1996; Servierston and Moulins 1985).

One of the best understood of these model systems is the stomatogastric nervous system (STNS), which controls rhythmic food processing in the crustacean foregut. In this system, a limited number of identified neurons are assembled into four motor pattern-generating networks, each responsible for different regional behaviors of the foregut (for review, see Harris-Warrick et al. 1992; Servierston and Moulins 1987). Until now, most studies on the stomatogastric system have been restricted to in vitro approaches focusing on one of these networks at a time. Much less work has been carried out in vivo, and these studies again focused on individual motor networks, either the gastric mill, responsible for breaking up food (Heinzel 1988; Heinzel et al. 1993), or the pyloric valve, which filters food to the midgut (Rezer and Moulins 1983, 1992). Moreover, these experiments were performed on animals held immobilized during the recording sessions and thus undoubtedly did not reflect ‘‘normal’’ behavioral conditions.

Although the various stomatogastric CPG networks were once considered individual functional entities responsible for separate behavioral tasks, it has become increasingly evident from in vitro experiments that these networks are far less independent than previously thought. For example, Hooper and Moulins (1989) found that individual neurons can participate in the activity patterns of more than one network, and Dickinson et al. (1990) showed that neuropeptide application can induce two generally distinct networks to combine their activity patterns. Moreover, Meyrand et al. (1991, 1994) reported that stimulation of extrinsic modulatory neurons can completely reconfigure multiple networks into a new functional entity. A complete understanding of stomatogastric motor activity therefore will require simultaneous monitoring of the underlying networks.

In a more general context, functional interactions between neural networks is a widespread phenomenon in nervous
INTERACTING NETWORKS IN INTACT ANIMALS

systems, particularly in the coordination of complex behaviors. Examples include interactions between functionally different networks such as those responsible for locomotion and respiration (Berger et al. 1969; Carrier 1996; Perségol et al. 1991), and the coordinated activities of the neuronal oscillators that control different limbs or drive different segments of the same limb (Grillner 1981, 1985). However, in most cases, because of the complexity and inaccessibility of the networks involved, the cellular and synaptic mechanisms responsible for these interactions are poorly understood.

In this study, our aim was to record chronically and simultaneously the motor patterns of the gastric mill and pyloric muscle assemblages in lobster. This approach enabled us to examine not only the operation of each network in vivo but also to assess the functional relationships between these neighboring networks during long periods in the freely behaving and undisturbed animal. Because the neural networks responsible for these rhythmic programs are composed predominantly of motor neurons, activity patterns recorded from their target muscles provide a clear insight into the network operation from which these motor patterns arise. By using implanted extracellular electromyographic (EMG)-electrodes that did not hinder the animals from expressing their normal behavioral range, we found that the gastric mill and the pyloric filter of the STNS operate almost constantly and independent of the presence of food. We also show that for a few neurons of each network, a strict coupling between the two networks exists in vivo, and in vitro analysis allowed us to establish the synaptic pathway by which this gastro-pyloric interaction occurs. Some of the data presented here have been published in abstract form (Clemens et al. 1996).

METHODS

Experiments ($n = 58$) were performed on young adult (400–600 g) intermolt European rock lobsters, Homarus gammarus, of either sex purchased from commercial suppliers (Aiguillon-Marée, Arcachon). Before experiments, animals were maintained for several days in large tanks of running, filtered, and aerated sea water.

Electromyographic recording

For operations, animals were held immobilized with the dorsal carapace maintained above the waterline in a small aerated aquarium. Teflon-insulated silver wire electrodes with a core diameter of 125 µm (AM Systems) were implanted into the appropriate pyloric and gastric mill muscles via small holes (0.5 mm diam) drilled through the cephalothorax with a fine syringe needle (Rezer and Moulins 1983). To record the activity of the muscles innervated by the lateral pyloric (LP), pyloric (PY), and pyloric dilator (PD) motor neurons, electrodes were implanted near the midline between the cervical and subcervical grooves (Fig. 1A). As pyloric activity always is expressed spontaneously, electrodes were positioned using an audio monitor as a steering device. In some cases, the electrode could be placed sufficiently near the neuromuscular junction that presynaptic action potentials in the motor neuron terminal could be clearly distinguished in the recording. In these cases, the term “EMG” refers to recordings of corresponding pre- and postjunctional potentials.

![Fig. 1](https://example.com/fig1)

**Fig. 1.** Stomatogastric system of the European lobster, Homarus gammarus. A: for in vivo recordings, wire electrodes were inserted through the dorsal carapace anterior to the cervical groove and fixed with dental cement above the heart. Their fixation and the outgoing leads to amplifiers did not hinder the animal from performing normal behavior (hiding, digging, eating, molting). B: location of the foregut and muscles of the gastric mill and pylorus in the intact animal. The different stomach regions are indicated in clear italics: CS, cardiac sac, GM, gastric mill, Oes, esophagus, PYL, pylorus. Stomatogastric nervous system (STNS) is represented by: CoG, commissural ganglion; lvn, lateral ventricular nerve; OG, esophageal ganglion; STG, stomatogastric ganglion; stn, stomatogastric nerve; SOG, subesophageal ganglion. Stomach muscles are identified by the motor neurons that innervate them (see text for details and abbreviations). C: dissected STNS in vitro. STG receives descending input from the brain and other STNS ganglia via the single stomatogastric nerve (stn). In some in vitro experiments, a Vaseline well containing an isotonic sucrose solution placed on the stn was used to block axonal conduction and thus to isolate the STG from higher centers. D: synaptic connectivity pattern of the pyloric and gastric CPG networks. Pyloric network consists of 11 motor neurons and 1 interneuron (anterior burster, AB) that is coupled electrically to the 2 PD motor neurons. Gastric network is composed of 15 motor neurons and 1 interneuron (Int 1). Stick and ball connections denote chemical inhibitory synapses, resistor symbols represent electrical connections, diode indicates rectifying electrical coupling.
Electrodes for recording from the gastric mill muscles innervated by the medial gastric (MG) and lateral posterior gastric (LPG) neurons generally were implanted near the midline and anterior to the cervical groove. To record the activity of the muscles innervated by gastric mill (GM) neurons, electrodes were implantedpostero-medial to the supra-ocular spine (s.-o. spine, Fig. 1A). Recordings from the gastric muscles innervated by dorsal gastric (DG) or lateral gastric (LG) neurons were not obtained because these muscles are either too thin to implant with an electrode (in the case of DG) or are hidden in a hollow of the stomach behind mandibular muscles (LG). Because GM activity spontaneously appeared only rarely in immobilized animals, electrodes were placed in gastric muscles according to their known insertions beneath the carapace. Electrodes were glued to the carapace with tissue glue (Histocryl; Braun Melsungen, Germany) and connected to highly flexible wires (1 mm diam) to maintain mechanical stability without restricting the animal’s movements. The free ends of the electrodes were waterproof sealed with a commercial silicon glue and fixed to the carapace with dental cement (Durelon, ESPE, Germany). They then were connected to amplifiers (Grass P5 AC-preamplifier), and recorded data were displayed on a Tektronix 5113 oscilloscope, simultaneously stored on a Schlumberger tape recorder (Enertec S.A., France), and printed using a Gould ES 1000 electrostatic chart recorder. After electrode implantation, animals were kept singly in isolated, darkened (light intensity above the tanks 50 ± 100 lx at noon) 50-l tanks, placed on vibration isolated tables, of DG) or are hidden in a hollow of the stomach behind mandibular muscles (LG). Because GM activity spontaneously appeared only rarely in immobilized animals, electrodes were placed in gastric muscles according to their known insertions beneath the carapace. Electrodes were glued to the carapace with tissue glue (Histocryl; Braun Melsungen, Germany) and connected to highly flexible wires (1 mm diam) to maintain mechanical stability without restricting the animal’s movements. The free ends of the electrodes were waterproof sealed with a commercial silicon glue and fixed to the carapace with dental cement (Durelon, ESPE, Germany). They then were connected to amplifiers (Grass P5 AC-preamplifier), and recorded data were displayed on a Tektronix 5113 oscilloscope, simultaneously stored on a Schlumberger tape recorder (Enertec S.A., France), and printed using a Gould ES 1000 electrostatic chart recorder. After electrode implantation, animals were kept singly in isolated, darkened (light intensity above the tanks 50–100 lx at noon) 50-l tanks, placed on vibration isolated tables, with a “natural-like” aquatic environment in which animals could move freely and hide. Water supply was achieved by an external 50-l reservoir, maintained at 16 ± 1°C (mean ± SD) under closed circuit (~80% of experiments) or running sea water conditions (~20%).

Because of postoperative stress, spontaneous pyloric activity oc-

**TABLE 1.** Postoperative evolution of pyloric activity in unfed animals

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ LP, s</td>
<td>0.6 ± 0.17 (15)</td>
<td>0.7 ± 0.14* (18)</td>
<td>0.89 ± 0.09 (10)</td>
<td>0.87 ± 0.16 (5)</td>
<td>0.86 ± 0.07 (4)</td>
</tr>
<tr>
<td>Δ PY, s</td>
<td>0.68 ± 0.18* (19)</td>
<td>0.89 ± 0.33 (20)</td>
<td>0.95 ± 0.11 (12)</td>
<td>0.96 ± 0.23 (7)</td>
<td>0.93 ± 0.29 (5)</td>
</tr>
<tr>
<td>τ pyl, s</td>
<td>1.92 ± 0.1* (20)</td>
<td>2.28 ± 0.12 (21)</td>
<td>2.31 ± 0.13 (13)</td>
<td>2.5 ± 0.27 (8)</td>
<td>2.5 ± 0.17 (6)</td>
</tr>
</tbody>
</table>

Parantheses enclose n values. *Significantly different from following value, P < 0.05, Mann-Whitney. Δ, burst duration; τ pyl, pyloric period; LP, lateral pyloric; PY, pyloric.
then was sealed with sterile styptic cotton (Coalgan, Brother S.A., The salines used included artificial sea water [which contained (in mM) 450 NaCl, 100 glucose, 26 citric acid, 3.91 Na₂SO₄, and 5 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid, adjusted to pH 7.45], and anti-coagulation Ringer [which contained (in mM) 479.12 NaCl, 12.74 KCl, 13.67 CaCl₂, 4.79 Na₂SO₄, 2.5 NaHCO₃, and 0.58 NaBr·2H₂O, adjusted to pH 7.8], Panulirus saline, and 0.58 NaBr·2H₂O, adjusted to pH 7.8].

**TABLE 2.** Postoperative evolution of gastric mill activity in unfed animals

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ GM, s</td>
<td>—</td>
<td>16 ± 3.9 (7)</td>
<td>13 ± 0.8 (7)</td>
<td>17 ± 1.4 (5)</td>
<td>15 ± 2.2 (4)</td>
</tr>
<tr>
<td>Δ MG, s</td>
<td>—</td>
<td>13 ± 1.4 (3)</td>
<td>13 ± 0.8 (4)</td>
<td>14 ± 2.9 (3)</td>
<td>14 ± 1.5 (2)</td>
</tr>
<tr>
<td>τ gastr, s</td>
<td>—</td>
<td>30 ± 5.4 (9)</td>
<td>28 ± 7.7 (8)</td>
<td>31 ± 6.5 (7)</td>
<td>29 ± 5.4 (5)</td>
</tr>
</tbody>
</table>

Values are means ± SE. Parentheses enclose n values. Δ, burst duration; τ gastr, gastric period; GM, gastric mill; MG, medial gastric.
RESULTS

Basic features of the stomatogastric system and in vivo recordings

In reptantian decapods, rhythmic movements of the four stomach regions (esophagus, cardiac sac, gastric mill, and pyloric filter; Fig. 1B) are governed by both intrinsic and extrinsic striated muscles. Intrinsic muscles originate and terminate wholly on ossicles of the stomach, whereas extrinsic muscles originate on the internal wall of the carapace and insert onto stomach ossicles (Maynard and Dando 1974). The gastric mill and pyloric motor patterns are generated by separate neural networks located in the STG, which is situated on top of the stomach (see Fig. 1B). As in other decapods, the pyloric and gastric mill networks of the European rock lobster are composed mainly of the motor neurons themselves (Cazalets et al. 1990; Combes 1993) (Fig. 1D).

The gastric mill network consists of two subsystems that, respectively, control movement of the two lateral teeth and the medial tooth. We have obtained EMG recordings from a pyloric dilator muscle (innervated by the 2 PD motor neurons), two pyloric constrictor muscles (innervated by the unique LP motor neuron and 8 PY motor neurons), and gastric mill muscles innervated by the 8–10 GM motor neurons and the unique MG motor neuron (which, respectively, innervate the power stroke muscles of the medial and lateral teeth), and the 2 LPG motor neurons (return stroke muscle of the lateral teeth).

Pyloric activity in vivo

In all animals examined, pyloric rhythmicity was expressed continuously throughout recording sessions, which lasted for \( \leq 3 \) mo. Differences between the sexes were not observed. Although we obtained recordings from all three main pyloric muscle types (Fig. 2A), only recordings from the LP and PY neuron innervated constrictor muscles could be followed for prolonged periods. Recordings with good signal-to-noise ratio from the fine PD neuron-innervated dilator muscles generally lasted no longer than 24–48 h after electrode implantation \((n = 3)\), presumably due to the electrode slipping away from the muscle bundle.

In some experiments \((n = 6)\) on freely behaving animals, we recorded simultaneously the activity of the lvn (Fig. 1, B and C) and of the muscles innervated by the pyloric motor neurons the axons of which this nerve carries (Fig. 2B). Such mixed neuro-myo-grams indicate first that in vivo the pyloric network generates, in a very stable manner, a rhythmic triphasic motor pattern qualitatively similar to that previously described in vitro for \(H.\) gammarus (Cazalets et al. 1990; Nagy et al. 1994). Second, as in vitro, the sequence of LP, PY, and PD neurons is followed by a brief, constant duration pause before the onset of the LP neuron burst of the next cycle. Within individual bursts there exists a strict one-to-one correlation between spikes occurring in the nerve recording (Fig. 2B, top) and muscle activity in one or other of the pyloric EMG recordings (Fig. 2B, bottom). Because pyloric motor neurons themselves form the central pattern generator responsible for pyloric rhythmicity, these data confirm that activity patterns of the various stomatogastric muscles represent a mirror image of ongoing network activity within the STG. Moreover, because of the highly conservative and strictly sequential nature of pyloric rhythmicity, activity in the LP and PY neuron-innervated c1 and c2 muscles provides sufficient information for analysis of pyloric network activity from long-term recordings in vivo.

FIG. 4. Spontaneous gastric rhythmicity in vivo. A: gastric mill network is silent on the day of operation but thereafter starts oscillating regularly. B: episodic nature of gastric activity. In a typical continuous recording, the gastric period (measured between the onsets of 2 consecutive bursts in a given muscle) may vary from \( \sim 20 \) s to \( > 40 \) min. These long periods occur randomly and are unrelated to the time of day (night). C: graded distribution of gastric cycle periods. A period of 100 s, corresponding to mean value \( \pm 2 \) SE, was chosen arbitrarily to distinguish on and off states of the gastric network.
Occasionally, during longer-lasting operations to implant electrodes, pyloric activity could slow to cycle periods of 6–8 s, and the regular pattern could disappear temporarily (data not shown). This generally was accompanied by prolonged PY neuron bursts of up to several seconds in each cycle. The subsequent recovery of stable pyloric activity is summarized in the single experiment shown in Fig. 2C and the pooled data shown in Fig. 2D. Figure 2C also compares the evolution of the pyloric period in an animal that was kept immobilized in the operation tank for 5 days with a typical experimental animal that was free to move under “natural-like” conditions after the operation. Under these different postoperative conditions, pyloric period displayed an opposite evolution. In the immobilized animal, the shortening effect of the operation led to a dramatic prolongation of pyloric period, whereas during the same time, an acceleration of pyloric activity was observed in the “free” animal. After ~12 h, the activity patterns became inverted in that the restrained animal expressed faster pyloric rhythmicity than its freely behaving counterpart. These results indicate that the pyloric network is not only affected by the operation itself but also by the conditions under which the experimental animal subsequently is maintained.

To obtain comparable steady state baselines of stomatogastric activity in freely behaving animals, we always started our analyses ~3 h after the animals had been placed into isolated tanks. After this recovery period, typical pyloric activity had reappeared, albeit with significantly shorter cycle periods than were expressed during the ensuing days (1.9 vs. 2.3 s, Fig. 2D), indicating a persistent influence of the initial operation. One to two days were typically required for pyloric activity to stabilize with mean period lengths of ~2.5 s and mean LP and PY neuron burst durations of 0.9 and 0.96 s, respectively (see Table 1).

**Gastric mill activity in vivo**

Despite remaining unfed for >2 days before experimentation, animals also express (after recovery from electrode implantation) spontaneous gastric mill rhythmicity. Figure 3 illustrates the typical pattern of gastric activity, here represented by powerstroke muscles innervated by the MG and GM neurons, from the two gastric mill subsystems during a continuous recording. Note that the electrode in the MG neuron-innervated muscle (Fig. 3, top) also recorded activity in a nearby pyloric LP neuron innervated constrictor muscle (see Fig. 1B). When the gastric mill network is active in the unfed animal, cycle periods are 25–35 s, with GM and MG neuron burst durations of ~15 s (see Table 2). However, gastric periods occasionally increased to as much as 60–100 s; these long gastric mill periods were associated with prolonged LPG neuron firing.

The gastric mill network in vivo is not continuously active. As seen in Fig. 3, rhythmicity can stop suddenly and restart, equally abruptly, some time later. During such pauses, which could occur without any apparent behavioral reason, pyloric activity continued to be expressed (see LP neuron activity in Fig. 3, top trace). Further analysis (Poincaré map, Dekhuijzen and Bagust 1996, data not shown) confirmed that a cessation or resumption of gastric activity could not be predicted by changes in the immediately preceding active or inactive state; a slow fading out of gastric activity with periods becoming progressively longer before activity ceases altogether or a progressive acceleration of gastric activity after a pause, indicating waning and waxing of excitability within the gastric network, was not observed. Rather these data strongly suggest that the activity of the gastric network is subject to an active switching command, presumably from higher centers. The overall expression of gastric motor activity with its sudden and unpredictable interruptions was similar in all animals observed (n = 22). Moreover, the sudden switching off and on of gastric activity did not appear to be due to operation-induced stress. Once gastric network activity recommenced after operation, which never occurred during the first 24 h (Fig. 4A), the off/on transitions occurred with equal regularity during the ensuing weeks.

To better understand the switching of the gastric network between active and silent states, we measured all cycle periods in continuous recordings for ~24 h (n = 5) and analyzed their distribution patterns. As in the typical experiment illustrated in Fig. 4B, gastric periods (measured between the onsets of consecutive GM neuron bursts) can extend over a continuous range from 20 s up to ~40 min. Because ~90% of these periods were <100 s (Fig. 4C), which corresponds to the mean value ±2 SE, and no clear discontinuity in the distribution pattern could be observed, we designated intervals of 100 s as an arbitrary cut-off between an active (rhythmic) and nonactive (silent) state of the gastric network. By this definition, periods >100 s are considered to be a pause in activity rather than a long-lasting cycle of a still active network. On this basis, the gastric mill expresses

![FIG. 5. Gastric mill network influences pyloric activity. Combined recordings of 2 pyloric constrictor muscles innervated by the LP and PY neurons and 3 gastric mill muscles, the lateral and medial teeth powerstroke muscles (MG and GM neuron innervated) and the lateral teeth returnstroke muscle (LPG neuron innervated). At the onset of MG neuron activity (see bottom) the PY neuron burst is prolonged significantly. However, LP neuron burst duration and the interval between the end of the PY neuron burst and onset of the next LP neuron burst are unaffected.](image-url)
rhythmic motor activity 18–20 h/day. Comparable results were obtained from long-term recordings \( (n = 4) \) lasting 1 wk with samples of 5 min/h; in \( \sim 70–80\% \) of these samples, the gastric mill was active (data not shown). The distribution of active/silent states seems to follow no obvious temporal pattern in that activity phases are distributed randomly and exhibit no correlation with either animal behavior or time of day (Fig. 4B).

**Gastro-pyloric interactions in vivo**

When both the gastric mill and pyloric networks are active, a strong interaction occurs between the two networks at onset of MG and LG motor neuron activity (Fig. 5). Two pyloric pattern parameters clearly change during this interaction: pyloric cycle period and PY neuron burst duration. As seen in Fig. 5 (bottom), although the duration of each LP neuron burst and its overlap with the following PY neuron burst remain relatively unchanged during each MG/LG neuron timed perturbation, the transient increase in pyloric cycle period is due to an increase in PY neuron burst duration, which attains \( >300\% \) of its mean value.

Because of this network interaction, it is possible to distinguish two types of pyloric activity that depend strictly on the functional state of the gastric mill (Fig. 6). When the gastric mill is silent (Fig. 6A1), the pyloric network expresses an extremely regular (mean cycle period of \( 2.2 \pm 0.02 \) s), unimodal activity pattern (Fig. 6A, 2 and 3). In contrast, when the gastric mill network is coactive in the same preparation (Fig. 6B1), the pyloric network expresses prolonged cycle periods that are coordinated to the onset of each gastric cycle (Fig. 6B2; here monitored from the GM neuron-innervated muscle). As seen in Fig. 6B, 1 and 2, only a single pyloric period is maximally affected in each gastric cycle. The resulting distribution pattern of pyloric activity (Fig. 6B3) now shows a bimodal distribution (normality-test Kolmogorov-Smirnov failed at \( P = 0.0001 \) ) with two discrete populations centered around modes of 2.4 and 4 s. The first population corresponds to pyloric activity between gastric-timed perturbations, the second population to the first pyloric period after the onset of gastric neuron bursting. In keeping with the tight correlation of altered PY neuron activity and MG neuron bursting, the period of the second population always equaled the cycle period of the ongoing gastric rhythm (data not shown).

An analysis of activity in PY and LP neuron innervated muscles confirmed that the gastric network acts differently on these two classes of constrictor motor neurons. As seen in Fig. 6A1, the burst-to-period relationships of the LP motor neuron without gastric network activity (square) and those in unperturbed cycles (see left hand mode in Fig. 6B3) during an ongoing gastric network (circle) are virtually identical. This

![FIG. 6. Pyloric network expression with and without gastric network activity.](image-url)
result is similar for the PY motor neurons (Fig. 7B1). However, in pyloric periods associated with gastric perturbation (after the onset of MG/LG neuron activity), LP neuron burst duration is independent of cycle period (Fig. 7A1, ▲). In contrast, (Fig. 7B1, ▲), in the same gastric-perturbed cycles, the duration of PY neuron bursts remains closely correlated with the corresponding cycle period.

As predicted from Fig. 7A, 1 and 2, the distribution patterns of burst durations for these two constrictor neuron groups also behave differently (Fig. 7, A2 and B2). During gastric network activity (Fig. 7B2, □), PY neurons show a much larger distribution in their bursting pattern than when the gastric mill is silent (Fig. 7B2, □). For the LP neuron, however, the distribution pattern of its bursts does not change during gastric activity (Fig. 7A2).

Finally, the duty cycles of both LP neuron and PY neurons (defined as the ratio of burst duration to cycle period), which are similarly dispersed under control conditions (without gastric activity, □; and during gastric network activity, ▼) express opposing responses to gastric MG/LG perturbation (Fig. 7, A3 and B3, ▲). During the latter, an increase in cycle period is associated with a decrease in LP neuron duty cycle while PY neuron duty cycle increases.

Gastro-pyloric interactions in vitro

In the following series of experiments (n = 9), we turned to in vitro preparations to assess whether the gastric and pyloric networks in the isolated STNS express interacting activity patterns similar to those observed in the intact animal. As is illustrated in Fig. 8A, which shows extracellular motor nerve recordings from a preparation with spontaneously active pyloric and gastric mill networks, similar interactions between the two networks occur in vitro. Thus after the onset of bursts in the MG neuron (which is electrically coupled to, and fires synchronously with, the LG neuron; see Fig. 1D), the next pyloric period (►) is lengthened substantially, and the PY neuron burst is prolonged. Note that the PD neuron burst duration is not affected by the expression of the gastro-pyloric interaction.

In a subsequent approach, we used intracellular recording and stimulation of individual stomatogastric neurons to in-

![FIG. 7. Pyloric motor neurons are affected differently by an active gastric network. A1: LP neuron. A1: comparison of the burst duration vs. cycle period with and without gastric activity. LP neuron burst duration depends similarly on cycle period (dashed regression lines) when the gastric network is silent (□) and between perturbations when the latter is active (▼). However, in perturbed cycles (immediately after gastric MG neuron burst onset) LP neuron burst duration is virtually independent of the corresponding cycle period (solid regression line, ▲). A2: a distribution histogram reveals no changes in burst duration related to gastric activity. A3: LP neuron duty cycle (burst duration/cycle period) in gastric-perturbed cycles decreases as cycle period increases. B: PY neurons. B1: PY neuron bursts at MG neuron burst onset strongly depend on cycle period (— regression line, ▲). B2: resulting population distribution thus differs as a function of the expression of gastric activity, with the appearance of a population of very long-lasting PY neuron bursts during gastric-perturbed cycles. B3: in the long periods during each gastric perturbation, PY neuron duty cycle increases with cycle period.](image-url)
PY neuron but appears to be mediated by a selective inhibitory action on the pyloric pacemakers themselves.

To verify this intraganglionic pathway, we isolated the STG from other STNS centers and recorded the activity of gastric and pyloric neurons during intracellular and pharmacological stimulation. Suppressing unpatterned modulatory inputs to the STG by blocking impulse traffic in the stn stops all spontaneous rhythmicity. However, in such isolated ganglia, pyloric network activity can be induced by superfusing the STG with a muscarinic agonist, oxotremorine (10^{-5} M) (Bal et al. 1988) (Fig. 9). Under these conditions, when the silent gastric MG neuron is stimulated with depolarizing current, PD neuron bursting is again retarded, the pyloric cycle is lengthened, and the PY neurons fire longer in that cycle (Fig. 9A, →). This result demonstrates that the gastro-

![Image](image1.png)

FIG. 8. Interaction between the gastric and pyloric networks in vitro. A: simultaneous nerve recordings from an isolated STNS with a spontaneously active gastric network. Pyloric cycles after the onset of each MG neuron burst are prolonged (→) in a manner similar to that seen in the intact animal. B: simultaneous extracellular (top 3 traces) and intracellular (bottom 3 traces) recordings from a preparation with a spontaneously silent gastric network. Experimental stimulation of the MG neuron by depolarizing current injection has similar effects on pyloric output as seen during spontaneous MG bursts in vivo; i.e., a prolongation of pyloric period (→) and the PY neuron burst (●) without alteration in LP (or PD) neuron burst duration.

investigate the synaptic pathway responsible for the gastropyloric interaction. These experiments confirmed that the synaptic pathway for this interaction is not, as could be expected at first sight, due to a direct excitatory effect of the gastric MG/LG neurons onto the pyloric PY neurons. As seen in Fig. 8B, where the gastric network was spontaneously inactive, stimulating the otherwise silent MG neuron with injected depolarizing current caused transient inhibition of the PD neurons, thereby delaying their depolarization and burst firing. This delay retarded the PD neuron synaptic inhibition of the PY neurons (see Fig. 1D), which therefore fire longer than in a normal unperturbed cycle (Fig. 8B, ●). These results show that the interaction between the gastric and pyloric motor networks occurs in vitro as in vivo and therefore is not mediated by proprioceptive feedback but rather derives from neural pathways within the central nervous system. Moreover, these experiments indicate that the interaction is not due to a direct effect of MG neuron onto

![Image](image2.png)

FIG. 9. Gastro-pyloric interaction in the isolated stomatogastric ganglion. A: pyloric network activity in a STG bathed in 10^{-5} M oxotremorine after sucrose blockade of the stn. Depolarizing the otherwise silent MG neuron still retards PD neuron bursting and prolongs the subsequent PY neuron burst (→). Under these conditions a pyloric-timed depolarizing influence on the gastric MG neuron is apparent. B: in the same preparation, delaying PD neuron bursting by direct hyperpolarization also prolongs PY neuron firing (→), suggesting that the gastric-timed influence is mediated through the PD neuron. PD neuron hyperpolarization also retards the pyloric-timed influence on MG neuron (●). Note that the intracellularly recorded PY neuron (3rd trace) did not have its axon in the distal PY nerve at the level of the extracellular recording (top).
discussIon

Here we have explored the behavior of two related neural networks in the intact animal, identified and characterized a functional coupling between these two networks, and determined in vitro the nature and the cellular pathway responsible for this interaction. Our results show that in vivo both networks are active independently of feeding-related behavior. However, although the pyloric network oscillates without interruption, the gastric network may suddenly switch off and on. This independent activating/inactivating capability of the gastric network suggests a controlling influence arising from outside the network. In contrast, our data from in vitro experiments suggest that the gastro-pyloric interaction is strictly intrinsic to the STG and is mediated by a direct synaptic connection between discrete elements of the two networks. These findings are summarized in Fig. 10.

Pyloric network

Although a tremendous amount of work has been devoted to unraveling neural principles of stomatogastric function in vitro (Harris-Warrick et al. 1992; Selverston and Moulins 1987), much less information is available on the link between these in vitro data and stomatogastric activity in the freely behaving animal. In vivo, the pyloric network of *H. gammarus* expresses rhythmic motor output spontaneously and continuously. The strict triphasic LP, PY, PD neuron burst order (see Fig. 2, A and B) is similar to in vitro activity in this (Cazalets et al. 1990) and other decapod crustacean species (Hermann 1979; Hooper et al. 1990; Norris et al. 1996; Selverston et al. 1976; Weimann et al. 1991). However, our results show that, at least in *Homarus*, pyloric cycle period in vivo is almost two times slower than that observed in vitro (2.5 vs. 1.2 s). This suggests that, although not necessary for organizing the basic pattern, under resting conditions in the intact animal peripheral sensory feedback, hormonal or other influences contribute to setting pyloric cycle frequency (see further).

In our experiments on resting, unrestrained and unfed animals, pyloric activity is expressed continuously with little spontaneous alteration in pattern or cycle period. This is in contrast to the findings of a previous study on intact spiny lobster (Rezer and Moulins 1983), in which large spontaneous variations (1–6 s) in cycle period were reported, as well as an ability of the pyloric rhythm to lose its strict triphasic pattern. These differences with our findings may be due to interspecies variability or different experimental conditions (freely behaving vs. restrained) under which the operated animals were maintained (Fig. 2C). To minimize the effects of stress, our measurements were made after long periods (several days) of postoperative recovery. Emersion of decapods from water (which is indispensable for the placement and the fixation of the recording electrodes) affects oxygen transport in the hemolymph as well as blood glucose and lactate concentrations (Santos and Keller 1993; Taylor and Whiteley 1989), and dramatically increases crustacean hyperglycemic hormone levels (Webster 1996). Such stress-related changes may explain the need for 1–2 days of postoperative recovery before steady state values and activity patterns are reached. During this time, pyloric cycle duration

pyloric interaction described in this study takes place within the STG itself and does not involve long loop pathways via the higher centers of the STNS.

To further confirm the role of the PD neurons in the gastro-pyloric interaction, we hyperpolarized the PD neuron with injected current. As can be seen in Fig. 9B, PD neuron hyperpolarization delays the cell’s ensuing spontaneous depolarization and prolongs PY neuron activity (→). These data are therefore consistent with the conclusion that the gastro-pyloric interaction, originating from the MG neuron (and perhaps also LG neuron), is mediated by the pyloric pacemaker PD (and/or via the anterior burster, AB) neurons, and that the prolongation of the PY neuron burst is a functional consequence of this inhibition by gastric MG neuron of the pyloric pacemaker ensemble.

It is interesting to note finally that in the isolated STG, a clear pyloric influence on MG neuron’s membrane potential can be seen, consisting of cyclic depolarizations in phase with PD neuron depolarizations (Fig. 9A). Moreover, experimental perturbation of the PD neuron oscillations causes a similar modification of the pyloric-timed activity in the MG neuron (Fig. 9B, △). In contrast, even strong MG neuron hyperpolarization with injected current has no effect on PD neurons (not shown). Thus while further studies are needed to decipher this second interaction, which is not always evident in the intact STNS in vitro (see MG neuron trace, Fig. 8B), there may exist a functional pyloro-gastric connection from the pyloric pacemaker group back to the gastric MG/LG neurons.
Gastric network

After recovery from electrode implantation, the gastric mill network in unfed animals also expresses robust rhythmicity, albeit (unlike the pyloric network) with periods of spontaneous pausing (Fig. 3). During active periods in vivo, the gastric mill shows a pattern similar to that observed in vitro in Homarus (Combes 1993) and other decapod species (Elson et al. 1994). However, in a manner analogous to the pyloric system, gastric cycle period in vitro is up to three times shorter than in vivo (5–10 vs. 30 s; unpublished observations for in vitro values). In this context, it is also interesting that in vivo, the lobster gastric network switches on and off spontaneously, unlike in vitro where it is continuously active (Meyrand et al. 1994). In an earlier study on unfed, freely moving spiny lobsters, periods of gastric network activity alternating with silent intervals were also observed (Fleischer 1981), although Heinzel et al. (1993) reported that gastric activity in intact crabs is largely indistinguishable from that seen in vitro. Here again these differences may be explained by unavoidable stress-induced effects arising from the endoscopic recording techniques employed in the latter experiments.

That gastric mill activity may stop and start suddenly (Fig. 3), apparently randomly (Fig. 4B) and without gradual changes in cycle frequency before and after a pause, could be explained by an underlying neuronal command, acting as a rapid and powerful "on/off" switch. On this basis a projection neuron(s), that directly activate(s) or inactivate(s) the gastric mill network can be postulated (Fig. 10). Although a number of modulatory neurons that influence stomatogastric network activity have been identified from in vitro studies (Katz and Harris-Warrick 1990; Meyrand et al. 1991, 1994; Norris et al. 1994, 1996) to date an input neuron from the rest of the animal (Delcomyn 1980).

Interactions between the gastric and pyloric networks

When the gastric mill and pyloric networks are conjointly active in the undisturbed and unfed animal, a strong interaction between the two networks is reliably seen. Specifically, this consists of a substantial prolongation of the pyloric period uniquely at the onset of each gastric lateral teeth power-stroke burst (Figs. 5 and 6). This interaction still is observed in the isolated preparation and hence is an inherent property of the STNS (Fig. 8). Furthermore, our data reveal that this interaction takes place within the STG itself (Fig. 9). At first sight, the interaction could be explained by a direct excitatory influence of gastric MG/LG neurons on the pyloric PY neuron group. However, inspection of the pyloric network wiring diagram (see Fig. 1D) reveals that such an influence would not readily explain an increase in cycle period because the PY neurons do not themselves inhibit the pacemaker group. Indeed, our intracellular data suggest it instead is mediated by a direct MG/LG neuron inhibition of the pyloric pacemaker group (PD/AB) that prolongs the pyloric cycle and permits the already active PY neurons to continue firing until the subsequent pacemaker burst (Figs. 8 and 9). This finding indicates the importance of indirect cellular pathways in internetwork coordination, and emphasizes the caution needed in drawing conclusions about synaptic connectivity solely from the temporal features of motor output patterns.

In addition to the above gastro-pyloric interaction, our in vitro experiments with the isolated STG suggested a further intraganglionic interaction consisting of pyloric pacemaker timed depolarizations of the gastric MG neuron. However, this pyloro-gastric influence was not always evident in intact in vitro preparations or in vivo. A number of functional interactions among the pyloric and gastric systems have been reported in spiny lobster (Mulloney 1977), but we have found no evidence for them in either isolated Homarus STG or the intact animal.

In vitro studies have revealed a number of ways in which the various STNS networks can interact. Under extrinsic modulatory influence, single neurons can switch between networks (Hooper and Moulins 1989; Weimann et al. 1991), and networks can merge into a single functional entity (Dickinson et al. 1990) or be reconfigured into completely new functional networks (Meyrand et al. 1991, 1994). In contrast to these extreme examples of internetwork plasticity, our data indicate that neural networks also can interact significantly while maintaining separate functional integrities and identity. Interestingly, such a relationship between two distinct networks, again involving a gastro-pyloric interaction, has been reported recently in crab STNS (Bartos and Nusbaum 1997). In this case, an extrinsic input neuron is involved, which both modulates the respective bursting patterns of the two target networks and mediates their coordination. Whereas in crab this interaction relies strictly upon the continual firing of this extrinsic input, which modulates the burst firing of all pyloric neurons, in Homarus, the internetwork coordination involves only changes in PY and PD neuron activity and persists after suppression of all extraganglionic inputs (Fig. 9). Without excluding either possibility, it could be that in the lobster the gastro-pyloric interaction is different from that in crab, being mediated, perhaps additionally, by a direct and specific pathway within the STG (Fig. 10).

Internetwork interactions in other motor systems

The coordination of distinct motor pattern generating networks appears to be a general feature of the central nervous system. Examples range from interactions between networks responsible for different aspects of the same behavior, such as the coordination of segmental oscillators responsible for swimming in leech (Freisen and Pearce 1993), lamprey (Grillner 1985), and tadpoles (Tunstall and Sillar 1993), to networks responsible for largely different behaviors, such as...
the coordination of lobster locomotion and swimmeret beating (Cattaert and Clarac 1983), and locomotion and respiration in birds (Berger et al. 1969; Funk et al. 1989), tetrapods (Carrier 1996; Viala 1986; Young et al. 1992), and humans (Bernasconi and Kohl 1993; Perségl 1991). In most cases, however, the cellular mechanisms for coordination remain largely unknown (Dickinson 1995). In the present study, we were able to unravel the cellular pathway of such an interaction between two distinct but behaviorally related networks and demonstrated that it contributes to the steady state operation of these networks in the intact animal. Determining the actual role of this interaction in stomatogastric function now awaits simultaneous exploration of the pyloric and gastric networks under working conditions in vivo during the production of feeding-related behavior.

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Address reprint requests to J. Simmers.

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