A Rhythmic Modulatory Gating System in the Stomatogastric Nervous System of Homarus gammarus. II. Modulatory Control of the Pyloric CPG

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SUMMARY AND CONCLUSIONS

1. In the European rock lobster, Homarus gammarus, two bi-laterally symmetrical pairs of commissural neurons, P and commissural pyloric (CP), evoke excitatory postsynaptic potentials in the neurons of the pyloric motor network. The present paper shows that the two commissural neurons also exert a modulatory control over the pyloric network.

2. The P and CP neurons were active during ongoing pyloric rhythms. Ongoing pyloric activity was terminated when the neurons were hyperpolarized to inhibit their firing.

3. When the pyloric network was quiescent, depolarizing either the P or CP neuron induced a robust pyloric rhythm.

4. We studied the actions of the P and CP neurons on individual pyloric neurons isolated in situ from network interactions by a photostimulation technique. The P neuron induced oscillatory properties in the pacemaker pyloric dilator (PD) neurons and in the motor neuron, ventricular dilator (VD), whereas the CP neuron induced rhythmic properties in all the network neurons but VD. Together, the P-CP neurons modulated the entire pyloric network. The modulatory effects of the P-CP neurons did not outlast the duration of their discharge.

5. The P and CP neurons also controlled the firing frequency of all the pyloric neurons. They may, in addition, control phasing of the constrictor neurons discharges, but this effect was state-dependent and occurred only when the pyloric central pattern generator was functioning weakly. Their role in providing flexibility to the network operation appeared relatively limited.

6. We conclude that the P and CP neurons are good candidates for insuring long-term maintenance of pyloric network activity patterns.

INTRODUCTION

Modulatory inputs influence the operation of most rhythmically active pattern-generating networks (Getting 1989; Grillner and Matsushima 1991; Harris-Warrick and Marder 1991; Selverston and Moulins 1985), including the pyloric network in the crustacean stomatogastric nervous system (Harris-Warrick et al. 1992). All the pyloric neurons are cellular oscillators (Bal et al. 1988; Miller 1987; Miller and Selverston 1982). The importance of their endogenous ability to produce oscillations and rhythmic bursts for the elaboration of pyloric activity patterns has long been recognized (Miller 1987) and confirmed at the theoretical level (Epstein and Marder 1990; Marder and Selverston 1992). Moreover, these active properties are conditional, i.e., depend on modulatory inputs. When the stomatogastric ganglion (STG) is disconnected from anterior ganglia, pyloric neurons become passive neurons that are either silent or tonically firing, and the rhythm stops (Bal et al. 1988; Moulins and Cournil 1982; Nagy and Miller 1987; Russell 1979). Under these conditions, long-lasting pyloric rhythmicity can be restored by brief electrical stimulation of the STG input nerve (Bal et al. 1988; Russell 1979) or by bath application of a number of modulatory substances (Bal et al. 1994; Flamm and Harris-Warrick 1986; Harris-Warrick et al. 1992; Marder 1987; Nagy et al. 1985).

The pyloric network can produce rhythmical output for hours in the intact system, in vitro as well as in vivo, after feeding (Rezer and Moulins 1983). It is therefore possible that some modulatory inputs projecting to the STG fire continuously for long periods of time to maintain the active properties of the pyloric network neurons and insure long-term operation of the pyloric CPG. The present paper shows that the commissural pyloric (CP) and P neurons are good candidates to exert this fundamental control. As shown in the companion paper (Nagy et al. 1994), the P and CP neurons project onto the pyloric network in the STG, and each of them evokes excitatory postsynaptic potentials (EPSPs) on a subset of pyloric neurons. We show here that the P and CP neurons also exert modulatory control over the pyloric network with their specific effects being analyzed on individual pyloric neurons isolated in situ from network interactions.

METHODS

Experiments (n = 71) were performed on male and female European rock lobsters, Homarus gammarus, purchased from commercial suppliers (Aiguillon-Marée, Arcachon). Animals were maintained for several days in large tanks of running, filtered, and aerated seawater. Preparation and recording procedures have been described in the companion paper (Nagy et al. 1994).

In situ isolation of a pyloric neuron from network interactions was achieved by photoinactivation (Miller and Selverston 1979) of neurons to which it is electrically coupled [with the exception of the eight electrically coupled pyloric (PY) neurons that were not isolated from each other] and of some presynaptic neurons. Isolation was completed by blockade of synaptic inputs from remaining glutamatergic neurons in the network with 10 μM picrotoxin (PTX) in the perfusion saline (Bidaut 1980). The detailed procedure for isolation of each pyloric neuron is that of Bal et al. (1988).
Reversible interruption of impulse traffic in the stomatogastric nerve (STG, Fig. 1A), the single input nerve to STG, was performed by means of a sucrose block, whereby the STG was reversibly disconnected from anterior ganglia. A desheathed portion of the stn was surrounded by a petroleum-jelly chamber and superfused with an isotonic (750 mM) sucrose solution (pH adjusted to 7.45 with NH₄OH). Additional petroleum-jelly chambers eventually were built around the STG for bath application of drugs diluted in the saline and around the anterior ganglia [commissural ganglion (COG) and esophageal ganglion (OG), Fig. 1A] for superfusion with a low-Ca²⁺ saline to which 10 mM Mo⁴⁺ was added to block chemical synapses. Lucifer yellow CH dilithium, PTX, and d-tubocurarine chloride were purchased from Sigma (St Louis, MO).

Data were recorded on a electrostatic recorder (ES 1000, Gould) and simultaneously stored on video cassette recorder coupled to a digital converter (Neurocorder DR 886, Neurodata). Histograms and statistical values were processed with a computer (AT 286, IBM) using homemade statistical software written by C. Chassaret. Error bars in figures and ± numbers in text are standard deviations.

**RESULTS**

Commissural P and CP neurons are involved in maintenance of the rhythmic pyloric motor pattern

The commissural P and CP neurons appear to fire whenever the pyloric central pattern generator (CPG) is operating. In 47 preparations we found at least one of the two pairs (in left or right COG) of CP and P neurons was firing whenever the pyloric network was rhythmically active in vitro. The possibility that these neurons are involved in maintenance of pyloric rhythmicity was tested, therefore, first by silencing the two commissural neurons during spontaneous activity of the pyloric CPG, and second by firing the commissural neurons when the pyloric network was quiescent.

The experiment in Fig. 1 demonstrates the drastic effects that inhibition of P and CP firing evoked on rhythmic activity of the pyloric CPG. The contralateral esophageal nerves, inferior esophageal (ion) and superior esophageal (son), were transected (Fig. 1A) to disconnect the contralateral COG from the STG, whereby the influence of the contralateral CP and P neurons was prevented. Rhythmic activity of the pyloric network was monitored simultaneously in a motor nerve [anterior ventral nerve, (avn), Fig. 1B] and by intracellular recording of a pacemaker neuron [pyloric dilator (PD), Fig. 1B], while intracellular activity of both ipsilateral P and CP neurons (Fig. 1B) also was recorded. The CP neuron then was hyperpolarized by current injection, and as soon as its discharge stopped, the pyloric pacemaker neuron stopped oscillating and rhythmic activity of the pyloric network ceased. Similar results were obtained with all six preparations in which CP was similarly hyperpolarized.

In Fig. 1, B, hyperpolarizing the CP neuron caused a substantial decrease of the P neuron firing via the electrical synapse connecting the two neurons (Nagy et al. 1994). This low-frequency firing of the P neuron was not sufficient to maintain the activity of the pyloric network. A certain rate of continuous firing (which varied with the preparation) of the commissural P and CP neurons appeared crucial for maintaining the expression of the rhythmic pyloric motor pattern.

In the experiment depicted in Fig. 2, A, the pyloric network did not display any spontaneous activity. Under these conditions, firing the P neuron quickly induced (within the first second after the beginning of its discharge) rhythmic activity of the entire pyloric network. The effects of that discharge, however, did not peak instantaneously. Oscillatory activity of the pacemaker neurons built up progressively (oscillation amplitude, burst duration) and attained a steady state ~10 s after the beginning of P neuron firing. The achieved steady state corresponded to their normal behavior in a spontaneously active pyloric network. This suggests that the excitatory influence of the P neuron is mediated by modulatory mechanisms and not by a simple depolarization of the pyloric neurons. Another striking characteristic is that the activation of the pyloric network lasted only as long as the P neuron kept firing. This indicates that the putative modulatory processes involved in the effects of the P neuron are short lasting.

**FIG. 1.** Decrease or cessation of firing of the commissural pyloric (CP) and P neurons, provokes the immediate termination of the ongoing pyloric activity. A: diagram of the in vitro preparation of the stomatogastric nervous system, with P and CP axonal pathways; only one commissural ganglion (COG) was left connected to the stomatogastric ganglion (STG). B: after inhibition of CP firing by hyperpolarizing current injection, the pyloric dilator (PD) neuron ceased oscillating, and spontaneous pyloric rhythm stopped [anterior ventral nerve (avn)]. Note that CP hyperpolarization decreased P firing rate through their electrical synapse. AB, anterior buenter neuron; IC, inferior cardiac neuron; ion, inferior esophageal nerve; LP, lateral pyloric neuron; LPn, LP nerve; mvm, medial ventricular nerve; OG, esophageal ganglion; PDn, PD nerve; PY, pyloric neuron; PHn, PY nerve; son, superior esophageal nerve; stn, stomatogastric nerve; VO, ventricular dilator neuron.
P and CP neurons control rhythmogenic properties of the pyloric network neurons

Because all the pyloric neurons are conditional oscillators, it is probable that their activation by the commissural P and CP neurons is performed via the control of their active rhythmogenic properties. In Fig. 2, B, it is shown to be the case for the pacemaker neurons of the network. A single COG was left connected to the STG. The pyloric network was quiescent and the pacemaker neurons were silent or fired tonically. Current was injected in the soma of one of the two PD neurons (Fig. 2, B, left) to depolarize the three electrically coupled pacemaker neurons. When the P neuron fired (Fig. 2B, 2), injection of a brief pulse of depolarizing current in a pacemaker neuron generated a passive depolarization followed by a full active oscillation, although before firing of the P neuron (Fig. 2B, 1), the sustained response of the pacemaker neuron was not present.

It seems, therefore, that P neuron firing induces slow rhythmogenic properties of the pyloric pacemaker neurons. In the preceding experiment, however, the ipsilateral CP neuron, not recorded, could have been coactivated during depolarization of the P neuron (both neurons are electrically coupled) and could have contributed to the observed effects. Moreover, the pacemaker neurons were not isolated from each other, nor from other pyloric neurons, so that a network effect cannot be excluded. Therefore we analyzed the respective effects of the two commissural neurons on every pyloric neurons isolated in situ from their partners in the network.

P NEURON INDUCES ACTIVE PROPERTIES OF THE PD AND VD NEURONS. In the experiment shown in Fig. 3, P neuron activity was recorded intracellularly in the left COG. The two contralateral esophageal nerves as well as the ipsilateral sON were transected (Fig. 3A, 1) so that the recorded P neuron was the only element of the two P-CP couples that projected to the pyloric network. Moreover, the PD and ventricular dilator (VD) neurons were isolated in situ from the other pyloric neurons by photoactivating the anterior burster (AB) neuron (Fig. 3A, 2; see METHODS), and superfusing the STG with 10 μM PTX (Fig. 3A, 1) to block glutamatergic synapses from the pyloric constrictor neurons. P-tubocurarine (1 mM) also was added to block the discrete EPSPs that the P neuron evokes on the pyloric neurons. Under these conditions, electrical coupling between the PD and VD neurons remained, and we had to strongly hyperpolarize one of them to dissociate the effects exerted by the P neuron. Figure 3B shows that tonic discharge in the P neuron evoked a sustained oscillatory activity in a previously passive PD neuron, which fired tonically at low frequency. This effect was not indirectly because of activation of the VD neuron, which was strongly hyperpolarized by current injection and did not produce either oscillation or discharge. Similarly, when the PD neuron was hyperpolarized and VD was left free to react, discharge of the commissural P neuron evoked large-amplitude oscillations and rhythmic firing of the VD neuron (Fig. 3C). In conclusion, the commissural P neuron can induce and maintain the active rhythmogenic properties of the pyloric PD and VD neurons although the discrete P-evoked EPSPs on the same neurons were blocked by P-tubocurarine. P neuron can therefore exert on the same target neurons two types of synaptic effects, i.e. classical excitation (EPSP) and permissive modulatory control (induction of active properties).

As seen in the preceding experiment, the P neuron can exert its modulatory control by firing tonically. Figure 4 shows the strength of this control depends on P tonic-firing frequency. In this experiment, a pacemaker PD neuron fired tonically at low frequency. When 1 nA of
depolarizing current was injected in the P neuron cell body, resulting in a mean P neuron firing frequency of 8 Hz, small-membrane potential oscillations, were triggered in the PD neuron. The oscillation amplitude increased progressively (Fig. 4A). When the P neuron was induced to fire at 20 Hz (Fig. 4B, i = −2 nA), the oscillations evoked in the PD neuron showed a higher frequency and larger amplitude, thereby driving discharges of higher intensity. The oscillatory activity of the PD neuron was increased further when the P neuron fired at higher frequency (not shown). It is noteworthy that, in Fig. 4B, the ionic component of PD discharge disappeared between the oscillations (compare with Fig. 4A). This further indicates that the active properties of the neuron actually were developed.

By contrast, the P neuron does not influence the active properties of the pyloric constrictor neurons. This is shown in Fig. 5 for the lateral pyloric (LP) neuron. The experimental conditions were identical to those in Fig. 3, but superfusion saline on the STG did not contain α-tubocurarine. In addition, chemical synapses in the rostral centers (COGs, OG; Fig. 5A) were blocked by superfusion with a low-Ca²⁺ saline to which 10mM Mn²⁺ was added, reducing the possibility that the P neuron activated an additional intervening neuron in these centers that would project on the pyloric neurons. The P neuron then was depolarized by current injection, and its discharge rapidly induced the oscillatory behavior of a pyloric pacemaker neuron (PD, Fig. 5B). The effects of the P discharge on the constrictor LP neuron clearly were different. The latter received a burst of EPSPs correlated one-for-one with P spikes (Nagy et al. 1994), which induced a tonic low-frequency firing of the pyloric neuron. This tonic discharge was interrupted rhythmically by the PD-generated inhibition, but did not show any underlying active component. Under these conditions, no slow regenerative depolarizations such as plateau potentials or oscillations can be triggered in the LP neuron by current injection (not shown). A similar conventional excitation was shown in isolated PY neurons (n = 5), and no effect was detected in the inferior cardiac (IC) neuron (n = 2). Therefore the P neuron does not appear to modify the active properties of the pyloric constrictor neurons.

CP NEURON INDUCES ACTIVE PROPERTIES OF ALL PYLORIC NEURONS EXCEPT VD. The commissural CP neuron also exerts a modulatory control over the pyloric network. In Figure 6B, the STG was connected only to the ipsilateral COG containing the recorded CP neuron (Fig. 6A), and synaptic activity in this COG was blocked by superfusion with a low-Ca²⁺ saline to which 10mM Mn²⁺ was added. The ipsilateral ion was transected to prevent the effects of the ipsilateral P neuron. The AB and PD neurons were photoactivated and the STG was superfused with 10 μM PTX. The PD neurons therefore were isolated in situ, and the con-
strictor neurons received only cholinergic inhibition from the PDs. Under these conditions, no pyloric neurons showed slow oscillatory properties. The CP neuron then was stimulated to fire (note that its discharge was tonic in the absence of the AB feedback) resulting in oscillations and rhythmical bursting in the remaining pyloric neurons. Note also that the first oscillations of the PD neurons appeared several seconds after the beginning of the CP discharge and that the duration and amplitude of these oscillations progressively increased. Moreover, the constrictor LP neuron developed a long plateau potential underlying an intense discharge, which peaked after several seconds. Every inhibition from the PDs triggered an active repolarization of the LP neuron, that transiently terminated its plateau. This is in contrast to what occurs during discharge of the P neuron alone (compare with LP, Fig. 5B), when the PD bursts produce only inhibition of the LP neuron. Under conditions of Fig. 6B, a brief pulse of depolarizing current triggered a plateau from LP held hyperpolarized by constant current injection (not shown).

The IC neuron responded similarly as the LP neuron to CP discharge (ann, Fig. 6B). Figure 6D shows that a discharge of the CP neuron induced rhythmic activity in the IC neuron consisting of a long-lasting plateau potential that was repolarized rhythmically by pacemaker neuron inhibition.

Unlike the P neuron, the CP neuron does not influence the regenerative properties of the pyloric VD neuron (not shown). We were unable to test the effects of the CP neuron on the AB neuron isolated in situ from the other pyloric neurons. When coupled to the PD neurons in the intact pyloric network, however, the AB neuron oscillatory properties appeared to depend on the discharge of the CP neuron.

**MODULATORY EFFECTS OF THE P AND CP NEURONS ARE COMPLEMENTARY.** From the preceding results, one may draw the conclusion that the pair of commissural neurons control rhythogenic properties of all the pyloric network neurons (Fig. 7, ▲). The CP and P neurons actually control all network neurons, both conventionally by means of trains of EPSPs (Fig. 7, △), and via neuromodulatory processes. Moreover, for some neurons like the PDs, the effects of P and CP neurons are additive.

In the experiment shown in Fig. 8, the pyloric network was intact but was left connected to a single COG (as in Fig. 1A). The activities of P and CP neurons were recorded intracellularly in that COG. The pyloric pattern was monitored intracellularly in a pacemaker PD neuron and a constrictor LP neuron, while the constrictor LP, PY, and IC neurons were recorded extracellularly on the corresponding motor nerves [LP and PY nerves (LP-PYn), ann; Fig. 8]. The P and CP neurons were not spontaneously active. When the P neuron was depolarized by current injection (Fig. 8A, P, arrows), it induced the oscillatory properties of the PD. The LP neuron, however, was simply depolarized by P-derived EPSPs and produced only a few action potentials. A similarly weak discharge was produced by the PY neurons (LP-PYn, Fig. 8A), whereas the IC neuron remained quiescent (ann, Fig. 8A).

However, when both CP and P neurons were induced to fire simultaneously (Fig. 8B, arrows), the effects evoked on the entire pyloric network were qualitatively and quantitatively different. The oscillation amplitude and discharge intensity of the PDs were larger. The LP neuron produced a plateau potential (repetitively interrupted by the pacemaker neurons) that drove intense firing, and the IC neuron was activated (ann; Fig. 8B) producing a strong rhythmic discharge. It appears, therefore, that simultaneous dis-
FIG. 5. Activity of the commissural P neuron evokes only passive depolarization of the pyloric constrictor neurons. A: diagram of the preparation: same conditions as in Fig. 3, but without (DTC) on the STG and with additional blockade of chemical synapses in the central centers by modified saline (square). B: discharge of the P neuron induced oscillations of a pacemaker PD, but only train of EPSPs in the constrictor lateral pyloric neuron (LP). EPSPs summate and evoked LP tonic discharge, rhythmically interrupted by PD inhibition.

charges of both CP and P neurons are required to elicit a complete pyloric pattern, such as that spontaneously expressed in vitro in the intact preparation.

MODULATORY EFFECTS OF THE P AND CP NEURONS DO NOT OUTLAST THEIR DISCHARGE. Figure 9 shows that network activation lasts only for the duration of CP and P activity. Simultaneous intracellular recordings of a pacemaker PD neuron and a CP neuron were performed under conditions where the CP neuron was the only neuron of the two P and CP pairs that projected to the pyloric network. CP neuron depolarization induced the oscillatory properties of the PD neuron, resulting in rhythmical activity lasting only during CP discharge. Subsequently, depolarization of the PD neuron again evoked a single initial oscillation followed by a tonic discharge, resembling that in the control, and indicating that the PD neuron again was lacking rhythmic properties. The same phenomenon was seen with the P neuron (not shown).

P and CP neurons control the expression of the pyloric motor pattern

Because the expression of the pyloric neurons' oscillatory properties depend on CP and P firing frequency, these neurons also can modify several parameters of spontaneous ongoing pyloric patterns. This is analyzed for the CP neuron in Figs. 10 and 11.

In the experiment shown in Fig. 10 A, the spontaneous pyloric rhythm was monitored on a ventral lateral ventricu-
FIG. 6. Activity of the commissural CP neuron induces oscillatory properties of all pyloric neurons except VD. A: diagram of the preparation in B; chemical synapses in the COG (circle) were blocked by modified saline: the pyloric AB and VD neurons were photoinactivated and glutamatergic synapses were blocked in the STG by 10 μM PTX (square); all synaptic interactions within the pyloric network were suppressed except inhibition of the constrictor neurons by the pacemakers PD. B: depolarizing the commissural CP neuron induced oscillatory activity of the 2 PDs and LP neurons (intracellular records) and rhythmic firing of the IC neuron (extracellular record in avm); the LP neuron was repolarized rhythmically by the PD neurons. C: diagram of the preparation in D; the pyloric network was intact. D: CP neuron discharge induced rhythmic oscillations in the IC neuron. Note that in the 2 experiments, modulatory effects did not outlast the duration of CP discharge.

FIG. 7. Summary of excitatory and modulatory synaptic projections of the commissural CP and P neurons on the pyloric network neurons. Excitatory connections, (●); modulatory influences leading to induction of rhythmogenic properties, (●); inhibitory connections, (●); electrotonic synapses, (resistance symbols); connections not tested on AB isolated in situ (?).
plied, modulate pyloric network activity (Harris-Warrick 1988; Harris-Warrick et al. 1992; Marder 1987) and, for some of them, turn on a quiescent pyloric network (Bal et al. 1994; Hooper and Marder 1987). However, knowing about the implication of a modulatory substance in long-term processes, requires direct activation of the corresponding neuron, because pharmacological approaches eliminate the temporal characteristics of discharge of the natural modulators. Besides the P and CP neurons, five types of modulatory neurons have thus far been identified, of which three exert a permissive control over the pyloric network operation. These include the cholinergic neuron, anterior pyloric modulator (APM); the proctolinergic neuron, modulatory proctolin-containing (MPN); and the serotoninergic receptor neuron, gastro-pyloric receptor (GPR) (Dickinson and Nagy 1983; Katz et al. 1989; Nagy and Dickinson 1983; Nusbaum and Marder 1989a,b). These neurons strongly influence the expression of ongoing pyloric patterns, but

FIG. 8. P and CP modulatory effects on pyloric network are complementary: A. only one COG was left connected to the STG; the P and CP neurons were silent, and the pyloric network was quiescent (intracellular record in PD and LP; extracellular record in LP-PY neurons (LP-PYN) and avn); when depolarized by current injection (arrows), the PD neuron fired tonically as a passive neuron; discharge of the commissural P neuron only, induced oscillatory activity of the PD neuron, passive depolarization of the LP neuron, and rhythmic firing of the PY neuron (LP-PYN). B: simultaneous discharge of the P and CP neurons reinduced stronger oscillatory activity of PD, active oscillations of LP (note progressive increase in amplitude of LP depolarizations), and rhythmic activity of IC (avn).

FIG. 9. CP modulatory effects do not outlast the duration of its discharge. Same experimental conditions as in Fig. 6.1, but with the AB neuron not photoactivated; a pacemaker PD produced a tonic discharge when depolarized by current injection (arrows); discharge of the CP neuron induced PD oscillatory activity, that ceased when the CP neuron stopped firing; subsequent depolarization of the pacemaker PD evoked tonic discharge; right after the end of CP firing, the PD neuron again was lacking its oscillatory properties.
their discharge characteristics or type of effect, do not indicate that they are involved in long-term maintenance of pyloric activity. APM does not usually fire spontaneously in the in vitro preparation (Nagy and Dickinson 1983) and modulatory effects vanish when APM is induced experimentally to fire tonically more than a few minutes (Bal 1991). The MPN neurons also rarely fire spontaneously in vitro and do not control regenerative properties of all the pyloric network neurons (Nusbaum and Marder 1989a,b). Finally, serotonergic GPR neurons, stimulated by rhythmic movements of the gastric mill (Katz et al. 1989), may fire for long periods of time, but do not project on all pyloric neurons, do not control active properties of all their target neurons, and cessation of their firing does not terminate pyloric activity (Katz and Harris-Warrick 1990b).

On the other hand, APM, MPN, and GPR may initiate rhythmic pyloric patterns. When briefly stimulated, they evoke a bout of pyloric rhythm, the duration of which outlasts the duration of their stimulation to different extents (see review in Katz and Harris-Warrick 1990a). These neurons could activate in turn neurons such as P and CP, which subsequently maintain the CPG operation. In the lobster, Palmarus vulgaris, APM evokes a strong and long-lasting activation of the P neuron, which in turn affects the pyloric network (Dickinson et al. 1988).

The commissural P and CP neurons appear to be the first known example, although most probably not the only one, of modulatory neurons that fulfill the main criteria to ensure the maintenance of pyloric rhythmic patterns for long time periods. The fact that they actually do it in vivo remains, however, to be demonstrated.

**P and CP neurons control the expression of pyloric activity patterns**

The P and CP neurons also can participate in modulation of the ongoing pyloric motor pattern, but only to a limited extent, their effect being mainly restricted to control of firing frequency during bursts. The P and CP neurons also can control phasing of constrictor neurons discharges, but mostly when the pyloric CPG is operating weakly. Clearly the effect of P-CP discharge in shaping pyloric activity patterns depends on the state and previous history of the CPG, a feature shared by other modulatory neurons in the stomatogastric system (Hooper and Marder 1987; Katz and Harris-Warrick 1990b; Nusbaum and Marder 1989b). Al-

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**FIG. 10.** Activity of the commissural CP neuron modulates expression of the ongoing pyloric pattern. A. 1: ipsilateral CP neuron was almost silent, and the pyloric pattern (monitored on the Vm and intracellularly in a PD neuron) was gated by the P-CP neurons in the contralateral COG. A. 2: CP neuron was depolarized by current injection and fired in bursts (firing frequency 35 Hz); this increased intensity of pyloric neurons’ discharges. B. 1: CP neuron was bursting weakly (mean firing frequency 10 Hz), and intensity of pyloric neurons discharges was low. B. 2: depolarizing the CP neuron (mean firing frequency 23 Hz) modified pyloric period very little, but clearly changed intensity of the pyloric discharges. A and B were from different experiments.
though P and CP are coupled electrically and can be considered as a functional unit, it remains that the P neuron alone elicits a different pyloric motor pattern than that resulting from either CP stimulation alone or coactivation of P and CP (see Fig. 8). Thus depending on the relative activity levels of these two neurons, qualitatively different pyloric motor patterns could be generated.

There are at least two ways of considering the P-CP function. They may be considered as preferentially insuring long term and stable expression of a basic pattern that can be sculpted at need by other modulatory inputs. On the other hand, one may consider a pattern-generating network to be a set of neurons temporarily selected from a wider neuronal population by selective modulatory inputs in a specific behavioral context (Dickinson and Moulines 1992; Weimann et al. 1991). For instance, to perform a swallow-
ing behavior, the modulatory neuron PS can assemble transiently neurons from different stomatogastric “circuits” in a novel pattern-generating network (Meyrand et al. 1991). In this view, the P and CP neurons could be regarded as selecting the set of neurons known as the “pyloric network”, when the pyloric filter has to operate rhythmically for prolonged times, for instance after feeding. We think that in the case of the P and CP neurons, the two theoretical conceptions are not mutually exclusive.

P and CP neurons: a gating command system?

Many years ago, the concept of command neuron was introduced to characterize neurons that can elicit and/or maintain complex sequences of rhythmic movements (Davis 1977; Wiersma and Ikeda 1964). Although more appropriately used with “fixed action patterns” such as avoidance reactions (Nolen and Hoy 1984) or escape reflexes (Camhi 1980; Drewes 1984; Eaton 1982; Wine and Krasne 1982), the concept of command neuron also was used in cases of triggering more long-lasting and flexible rhythmic behaviors (Brodfuehrer and Friesen 1986; Croll et al. 1984; Delaney and Gelperin 1990; Fredman and Jahan-Parwar 1983; Lennard et al. 1980; McCrohan and Audesirk 1987; Rose and Benjamin 1981; Weeks and Kristan 1978). To be classified as a command element, a neuron must fulfill several criteria: 1) its discharge must be necessary and sufficient to trigger the corresponding behavioral sequence (Kupfermann and Weiss 1978); 2) its discharge must be appropriate, i.e., the neuron must actually fire during the behavior (Davis 1985); and 3) the neuron must have preferred access to sensory input triggering the behavior (Davis and Kovak 1981). According to that strict definition, the concept may not be applicable to the control exerted by the P and CP neurons over the pyloric CPG. It is not known if the proper sensory inputs project onto these neurons. CPG activity is strongly influenced by the stomach proprioceptor PSR (unpublished observations), but the PSR is unlikely to trigger pyloric food processing in vivo (Dando and Laverack 1969). In addition, although P-CP taken as a functional unit are sufficient to trigger and maintain a stable basic pyloric pattern in vitro, they are not necessary, because other modulatory neurons may also transiently elicit the pattern.

It remains, however, that cessation of P-CP firing provokes immediate termination of the pattern expressed spontaneously in vitro, indicating that the P and CP neurons are key elements in maintaining pyloric rhythmicity. This is reminiscent of another notion related to the command concept. Neurons capable of initiating a motor sequence may be classified in two categories, “trigger neurons” and “gating neurons”, the latter being characterized by the fact that the motor sequence they elicit is maintained only for the duration of their discharge (Stein 1978). This is particularly well exemplified by the leech swimming system, where pattern-generator neurons are activated by swim-initiating interneurons during a swim episode (Nusbaum and Kristan 1986; Weeks 1982; Weeks and Kristan 1978). The swim-initiating neurons are first excited by trigger interneurons, which initiate swimming behavior (Brodfuehrer and Friesen 1986). Drawing an analogy with the pyloric system is tempting. The present paper shows clearly that the P and CP neurons could be considered gating neurons, and they could be activated by trigger neurons such as APM.

The P and CP neurons, however, could present an interesting situation, because they exert their gating effect via modulatory mechanisms. It is known that behaviors may be turned on by neurohormones (Hewes and Truman 1991; Rezer and Moulins 1992; Truman and Weeks 1985; Turrigiano and Selverston 1990; Willard 1981), and that neuromodulation may participate in activation of CPG rhythmic activity (Alevizos et al. 1989; Charchri and Clarac 1990; Claassen and Kammer 1986; Dickinson and Marder 1989; Grillner 1985). In literature, however, the command notion is usually a “dry” concept, implying conventional synaptic potentials and command and modulatory neurons are considered as two distinct functional groups (McCrohan 1988) that may exert their control independently or in synergy (Hoyle 1985). We have shown here that P and CP are modulatory neurons that gate the pyloric pattern by inducing and maintaining endogenous active properties of the network neurons.

Gating-command neurons most often receive a feedback loop from the controlled CPG and fire rhythmically (Kupfermann and Weiss 1978; McCrohan 1988). The feedback loop may prolong firing of the gating neuron (Weeks 1982), provide stability to the pattern produced (Liberst 1992), or participate directly in pattern generation (Davis 1985; Getting and Dekin 1985; Gillette et al. 1978). The P-CP neurons also receive an inhibitory feedback loop from the pyloric CPG (Nagy et al. 1994), but their rhythmic firing may be more complex. We will show in another companion paper (Cardi and Nagy 1994) that they participate in a higher-order oscillator, constraining strongly the pyloric CPG rhythm.

In conclusion, by their impact on the pyloric CPG and by the underlying mechanisms of their control, the commissural P and CP neurons constitute an original modulatory gating system, the knowledge of which should improve our understanding of organization and central control of rhythmic behaviors.

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