8 Extrinsic Inputs

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8.1 Introduction

The pyloric and gastric networks are amazing little machines which can produce rhythmic motor patterns for hours in the isolated stomatogastric nervous system. When the stomatogastric ganglion (STG) itself is completely isolated, there is, however, complete cessation of activity in both CPGs. Just as the most sophisticated engine needs gas to function, the pyloric and gastric CPGs require extrinsic inputs in order to operate and produce a rhythmic output. Moreover, in the intact animal (as shown by electromyographic recordings) as well as in isolated preparation of the stomatogastric nervous system, pyloric and gastric networks do not produce only a stereotyped pattern, but a relatively large "repertoire" of outputs. It is also the activity of extrinsic inputs projecting onto the stomatogastric CPGs that determines such a flexibility of expression.

It appears more and more evident that a large number of inputs project on stomatogastric networks (see Chap. 9). Until now, only a few of them were identified and characterized physiologically, but their study introduced two fundamental changes in our idea concerning mechanisms that underlie the functioning of neuronal networks (and central pattern generators). First, it is well established that the intrinsic properties of the neurons in a circuit play a major role in the elaboration of the patterned output of this circuit. More or less intuitively, these properties were thought to be invariant. However, consideration of the effects of extrinsic inputs to the stomatogastric networks has shown that, in fact, these properties manifest a wide range of expressions which are controlled by modulatory inputs. These modulatory inputs thus play a major role in the control of the pattern produced by a circuit. Second, it was included in the concept of CPG that the latter does not receive independent rhythmic inputs from other regions of the CNS. Investigation of the effects of extrinsic inputs in the stomatogastric nervous system shows that the pyloric and gastric CPGs do receive such rhythmic inputs from premotor oscillators, which take advantage of the nonlinear properties of the CPG neurons in order to modify the output of the two circuits. These new findings provide us with an understanding of how a neuronal network, previously represented by a fixed wiring diagram, can be responsible for a flexible output.

Finally, several proprioceptive inputs have been identified, which influence the gastric and pyloric activities. Proprioceptors are generally considered to be the main source of flexibility in the expression of a motor pattern. The stomatogastric nervous system reveals some mechanisms underlying the proprioceptor-derived flexibility.
The identified proprioceptive inputs do not have access to the stomatogastric CPGs themselves, and their ability to shape the motor outputs is essentially determined by the complex properties of the premotor elements (including the premotor oscillators) on which they project.

The aim of this chapter is to characterize, in the context of the preceding findings, the identified extrinsic inputs, and analyze how they can control the output of the stomatogastric CPGs. Section 8.2 considers those endogenous properties of the stomatogastric CPGs that are susceptible to modulation by extrinsic inputs, and how some characteristics of these CPGs determine their reaction to extrinsic perturbations. In other words, what are the potentialities for flexibility built into the motor networks? Section 8.3 addresses modulatory inputs which gate and modulate the ability of stomatogastric CPGs to generate rhythmicity. In Section 8.4 are considered rhythmic inputs which provide timing cues to stomatogastric CPGs. Section 8.5 deals with an extensively studied premotor pathway which influences the pyloric and gastric CPGs via several mechanisms and probably subserves different functions, while identified sensory inputs are considered finally in Section 8.6.

8.2 Potentials for Flexibility Built into the Stomatogastric CPGs

The neurons of the pyloric and gastric CPGs possess endogenous properties which allow a complex control of their output patterns by extrinsic inputs and determine their response to these inputs. First, most of these neurons are conditional oscillators and their ability to oscillate, as well as the parameters of their rhythmic activity, are under control of modulatory inputs. Second, as oscillators, they react nonlinearly to external phasic perturbations.

8.2.1 Conditional Oscillations in Pyloric and Gastric Neurons

As stated in the preceding chapters, the pyloric and gastric CPGs require inputs in order to operate. If the axonal conduction in the single input nerve, the stomatogastric nerve (sax), is experimentally interrupted with a nerve block, the gastric rhythm stops in Panulirus (Russell 1979) and Homarus (Robertson and Moulins 1981a, 1984) as well as the pyloric rhythm in Homarus (Moulins and Courell 1982), Janus and Patiria (Dickinson and Nagy 1983) and Panulirus (Nagy and Miller, App. A to Chap. 5). With a delay varying with the preparation, all the neurons become silent or tonically active. Under these conditions, a tonic electrical stimulation of the sna reintroduces more or less reliably the pyloric and gastric rhythms. Both rhythms also resume within minutes after unblocking the sn. It is now well established that preventing axonal conduction in the sn suppresses inputs that are necessary for the expression of the regenerative bursting properties of most of the neurons comprising the two CPGs.

Five of the eleven neurons in the gastric network and all the neurons of the pyloric network can display "plateau potentials" (Russell and Hardline 1976, 1982, 1984). A plateau potential consists of a sudden regenerative depolarization which develops when the membrane potential reaches a given threshold, followed by a plateau underlying a burst of spikes. Spontaneous repolarization occurs after a delay depending on the neuron. As described in Chap. 5 of this volume for the pyloric network, several neurons also possess conductances which slowly depolarize the resting membrane to threshold for plateau potential, thereby rhythmically producing plateaus and bursts. In other words, most of the pyloric neurons and some gastric neurons can display "bursting pacemaker potentials" (BPPs) (Miller, this volume), and this ability is the essential mechanism underlying their rhythmic discharges. In addition, plateau potentials can be triggered or shortened by respectively depolarizing or hyperpolarizing synaptic inputs (Russell and Hartline 1978). So the neurons whose rhythmic regenerative depolarizations have the shortest period (the dilators in the pyloric network) behave as pacemakers entraining and shaping plateaus of the other neurons via chemical synapses.

The ability to produce BPPs for those gastric neurons that can display them and for all the pyloric neurons is, however, dependent on inputs from higher centers. Blocking conduction in the sn suppresses intrinsic burstiness of the gastric burst-generating neurons (Russell and Hardline 1978, 1984), of the pyloric contristator neurons (Russell and Hardline 1978, Dickinson and Nagy 1983), of the pyloric dilator neurons (J.P. Miller and Selverston 1982a, Moulins and Courell 1982), and of the interneuron AII (Moulins and Courell 1982, Appendix A to Chap. 5). So all the burst-generating neurons of the stomatogastric CPGs are in fact conditional bursters. The pyloric network is, in particular, an interesting model of a rhythmic pattern generator, whose operation in mainly dependent on the intrinsic burstiness of all its component neurons, although for each neuron this bursting property is a potential capability only and requires conditioning inputs for its expression.

The conditioning inputs can also exert a graded control over the expression of BPP, once unmasked, of the stomatogastric neurons. Moreover, a number of putative neuromodulators (Chap. 9), although not necessarily able to activate a CPG, do exert this graded control, and can smoothly modify all the parameters of the ongoing rhythmic activity of the CPG. Section 8.3 describes how a modulatory input can, via these controls, first turn on a CPG, then provide a wide range of flexibility to the final motor pattern generated.

Once the ability to generate BPPs is unmasked by modulatory substances, the gastric and pyloric CPGs behave like classical oscillators and react nonlinearly to extrinsic phasic perturbations.

8.2.2 Nonlinear Input-Output Relations of Stomatogastric Oscillators

Ayers and Selverston (1979, 1984) demonstrated that the pyloric and gastric networks display nonlinear responses to both inhibitory and excitatory inputs. These reactions are typical of biological oscillators (von Holst 1973, Pavlidis 1973, Finkler 1977a,b) and are particularly obvious when considering the pyloric pacemaker neurons (Ayers and Selverston 1979).

First a PD neuron presents a periodically varying sensitivity to external inputs. Depending on when an input occurs in its cycle, a PD neuron will more or less advance
or delay the occurrence of its next oscillation (and burst). Thus, there can be either a positive or a negative phase shift of the pacemaker discharge following a single input. This response is a characteristic of the oscillator and can be provoked either by an excitatory or an inhibitory input (Fig. 8.1). If the input occurs repeatedly, the phase shifts of PD discharges also occur repetitively. In other words, the pacemaker neuron follows the rhythm of the cyclic input, and it does so at a constant phase (relative to the stimulus) which depends on the stimulus cycle frequency. When these two conditions are met, an oscillator is said to be entrained. A rhythmic input can shorten the period and so accelerate the rhythm of a target oscillator, or, on the other hand, slow down the rhythm of the target oscillator. When this happens in a stable 1:1 relation, there is an absolute coordination between the two rhythmic events. However, absolute coordination is only possible if the entraining period stays within a certain range around the free-run period of the target oscillator. When input period is too different, the target oscillator momentarily escapes from the influence of the entraining input, and only a relative coordination is realized. Therefore, the closer the period of the entraining rhythm is to the period of the entrained rhythm, the better the entrainment, with long periods of absolute coordination. Finally, there can be several modes of coordination when the period of one of the linked oscillators is roughly a multiple of the period of the other. The coordination mode (1:1, 1:3, ..., ) depends then on the value of this multiple.

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8.3 Modulatory Inputs

Although considerable efforts have been made to identify some of the extrinsic inputs able to modulate the burstiness of stomatogastric neurons, until now only two of them have been characterized physiologically: the modulatory interneuron APM (Nagy et al. 1981), and the ivn Through Fibers (Russell and Hartline 1981).

8.3.1 The Anterior Pyloric Modulator (APM)

The anterior pyloric modulator (APM) was first identified in the cestode ganglion (COG) of Haima (Nagy et al. 1981b) and then found in Panulirus (Nagy and Dickinson 1983) and Panulirus (Nagy and Miller, unpublished). This neuron sends two axons which, in the STG, via the ivn’s commissural ganglia (COGs), the ivn’s and the stn’s. Its discharge influences all the neurons of the pyloric network. In the PF neurons an EPSP can be correlated with each spike of APM and, in fact, several indirect lines of evidence indicate that APM monosynaptically activates every pyloric neuron (Nagy and Dickinson 1983). Recent data show that APM also influences the gastric network (F. Nagy and P.S. Dickinson, unpublished).

8.3.1.1 Induction of Burstiness in Pyloric Neurons

Fortunately, in Jamn it is possible, without blocking axonal conduction in the stn, to suppress all the permissive inputs from the COGs which normally sustain the ability of the pyloric neurons to produce BPPs. This is achieved by superfusing the COGs with a CaCl 2 + CoCl 2 -containing saline, which blocks synaptic activity in these premotor centers. The interneuron APM can therefore still influence the pyloric network (its axonal conduction being preserved) under conditions where pyloric neurons do not produce any rhythmic pattern and just behave passively. Under this condition a relatively brief and low frequency discharge of APM restores the ability of all the pyloric neurons to produce plateau potentials (Dickinson and Nagy 1983). A slightly longer discharge of APM brings the membrane of the pyloric neurons to a state where plateau potentials are spontaneously and rhythmically produced. In other words, the discharge of APM can by itself induce the ability for the pyloric neurons to produce BPPs and can restore rhythmicity in a previously silent pyloric network. Thus the discharge of APM alone is able to turn on the pyloric CFP (Fig. 8.2A).
brane potential of deafferented pyloric neurons under the threshold for plateauing (Nagy et al. 1985).

8.3.1.2 Modulation of Burstiness in Pyloric Neurons

Although a discharge of APM is sufficient to induce the ability of pyloric neurons to generate BFPs, its firing is not a prerequisite for pyloric cycling. In most isolated preparations of the stomatogastric nervous system APM is silent, but the pyloric CPG continues to display its normal rhythmicity, providing the STG remains connected to the CGGs. In these instances, however, a discharge of APM, experimentally provoked, always strongly augments the BFP activity of pyloric neurons, and modifies the characteristics of their burstiness (Dickinson and Nagy 1983).

Promoting the ability for BFP in the pyloric neurons enhances all those parameters of the pyloric rhythm that are dependent on the BFP properties (Nagy and Dickinson 1983). It increases the frequency of the oscillations (hence of the bursts), the amplitude of the oscillations and spike frequency within bursts (Fig 8.2B). As for the induction of burstiness in pyloric neurons, the promotion of burstiness is a long-lasting effect persisting several tens of seconds after a discharge of APM lasting a few seconds only.

APM is more than just a general activator of the pyloric rhythm. Its firing also qualitatively modifies the pyloric output and provides a great deal of flexibility in the expression of the pyloric pattern. The first type of flexibility derives from a differential sensitivity of the pyloric neurons to APM inputs, the most sensitive neurons being, as in Janus, the PD constrictor neurons (Dickinson and Nagy 1983). Depending on the frequency and the duration of the APM discharge, burstiness can be promoted in a variable number of neurons in the network, including or not the pacemaker group. As a consequence, a discharge of APM can either provoke an increase in intensity of discharge of the constrictor neurons without altering the overall rhythm, or provoke both an increase in the intensity of the discharge of all the pyloric neurons and an increase in the frequency of the rhythm, or even switch off momentarily the pyloric rhythm and induce strong tonic discharge in the constrictor neurons (Nagy and Dickinson 1983).

A second factor in the flexibility of the pyloric output is a modification, after an APM discharge, of the sensitivity of the pyloric neurons to synaptic inputs. Within the network, the pyloric neurons are interconnected by electrotonic synapses and inhibitory chemical synapses (see Chap. 3). The latter do not only passively hyperpolarize the post-synaptic neurons but trigger the active repolarization of their plates, thereby terminating their discharge. It is mainly the way the pyloric pacemakers entrain rhythmic discharges of the constrictor neurons. A discharge of APM significantly slows down this active repolarization that provokes the transition between firing and non-firing state of pyloric neurons (Fig 8.3C) (Dickinson and Nagy 1983). So in order to trigger this repolarization, an input must be longer or stronger than before APM firing. This renders the pyloric neurons less sensitive to inhibitory synaptic inputs. For instance, after APM firing, the constrictor neurons are more or less incompletely repolarized by the discharge of the pacemakers (Fig. 8.3A), so that the constrictors discharge earlier in the

The induction effects of APM probably involve cholinergic muscarinic receptors on the pyloric neurons, for they are specifically blocked by atropine, a muscarinic antagonist (Nagy and Dickinson 1983). Moreover it is possible to mimic APM’s effects by superfusing a deafferented STG with several muscarinic agonists like oxotremorine or pilocarpine (Nagy et al. 1985). The mechanisms mediating the effects of APM are not completely elucidated, but appear to result from the suppression, via muscarinic receptor activation, of a steady hyperpolarizing conductance that clamps the mem-

Fig. 8.2A,B. APM can switch on the pyloric CPG (A) and lastingly activate as coping pyloric rhythm (B). When the pyloric network is silent (A1), a tonic low-frequency discharge of APM induces and sustains a pyloric rhythm (A2). When the pyloric rhythm is spontaneously slow, a 5 s discharge of APM accelerates the rhythm and activates silent neurons (B). Calibrations = horizontal bar 2 s, vertical bar 20 mV. (A modified from Moulins and Nagy 1983; B modified from Nagy and Dickinson 1983)
pacemaker cycle (Fig. 8.3D,E) (Nagy and Dickinson 1983). Due to the same cause, when neurons are interconnected by mixed synapses (both electrotonic couplings and inhibitory chemical synapses), like neurons AB and VD (Eisen and Marder 1982), the electrotonic coupling is favored after APM firing and significant phase shifts between the discharges of these neurons also occur. It must be pointed out, however, that when an inhibitory input is strong enough or long enough to depolarize a postsynaptic neuron, due to the APM-mediated increase of its burstiness the postsynaptic neuron is depolarized much faster and to a more hyperpolarized level. Therefore an APM discharge leads to an increase in the contrast to synaptic effects on the pyloric neurons by augmenting the response to strong inputs and decreasing the response to weak inputs. The interneuron APM provides the pyloric neurons with a means of filtering their synaptic inputs.

In summary, the interneuron APM modifies the relative effectiveness of the synaptic connections in the pyloric CPG, which in turn results in a functional rewiring of the pyloric network. Because all but one of the neurons in the pyloric network are motoneurons, this functional rewiring is a powerful way of modulating the motor behavior of the pyloric filter. A point to be emphasized is that for this functional rewiring of the pyloric network it is not necessary to postulate any modification of the efficacy of the synaptic transmission (i.e., the size of the postsynaptic potential sensu stricto (Kandel 1976, Kuffler et al. 1984). The modification of the effectiveness of synaptic connections within the pyloric network can simply be accounted for by changes, associated with APM firing, in the burstiness of the postsynaptic neurons.

8.3.1.3 Gastric Activation

Recent data (F. Nagy and P. S. Dickinson, unpublished) indicate that, in *Junus and Palmaris*, APM could exert effects on the gastric network that are similar to the long-lasting effects exerted on the pyloric CPG. Firstly, tonic firing of APM induces a sustained rhythm from a previously silent gastric network. Secondly, when the gastric rhythm is spontaneously operating, a discharge of APM increases both the frequency and the intensity of gastric bursts. Thirdly, it is possible to correlate each spike of APM with a discrete EPSP in the gastric neurons GM and LPG. Fourthly, superfusion of a deafferented STG with octopamine or pilocarpine also induces a sustained gastric rhythm. Further studies are necessary in order to elucidate the mechanisms underlying APM control on the gastric output.

8.3.2 The innervating Fibers

First described by Dando and Selverston (1972) in *Panulirus*, these two neurons, whose cell bodies are located in the brain (Claborn and Selverston 1984b), send an axon toward the STG via the innervating fibers (from which they are named), the OG and the stn. In the STG, the innervating fibers (innTF) monosynaptically synapse with several pyloric and gastric neurons (Fig. 8.13). The complex effects exerted on these neurons by the innTF will be presented later on (see Sect. 8.5). We consider hereafter only one of these effects, the promotion of burstiness in the pyloric pacemaker neurons (PD-AB).
fiber "gastric command fiber." They also demonstrated that a gastric rhythm could operate as long as the STG remains connected to one COG via a single ion, and that subsequent section of this ion causes cessation of gastric cycling. These observations suggest that the ion "command fiber" could control the burstiness of gastric neurons, i.e., could act as a modulatory input to the gastric CPG.

Immunohistological and pharmacological data obtained in the last few years (see Chap. 9) suggest that both pyloric and gastric CPGs receive a large number of modulatory inputs. It is predictable that several of these inputs will be identified over the next few years, revealing how these inputs, when acting in combination, are able to shape the outputs of the two CPGs.

8.4 Rhythmic Inputs

The pyloric and gastric network fulfill the main criteria for CPGs (D.M. Wilson and Wyman 1965, Delcomyn 1980, Silverston and Moulins 1985). As reported in the preceding paragraph, they can be activated by tonic inputs, and then produce good replicas of the motor patterns recorded in the intact animal. In other words, they do not require timing cues in order to operate. However, they do receive additional timing cues from higher-order oscillators located in premotor centers, challenging the commonly held idea that a pattern generator does not receive independent phasic inputs acting as timers (Grillner 1977). As expected, these phasic inputs exert a frequency control of the pyloric and gastric motor patterns. But they also provide a greater flexibility in the expression of these patterns by exerting a differential influence on the individual neurons of both CPGs. This, in turn, determines the number of neurons involved in a motor sequence, and control the intensity and the phase of their discharge.

8.4.1 Rhythmic Control of the Pyloric Network

8.4.1.1 The Commissural Pyloric Oscillator (CPO)

Each COG contains an oscillator which bursts in phase with the pyloric motor pattern and continues to burst after isolation of the COG. This commissural pyloric oscillator (CPO) (Robertson and Moulins 1981a) is a master oscillator entraining the rhythm of the pyloric neurons. The CPO was first characterized in H. maruana by monitoring its activity via records of follower neurons (commissural F cells) (Robertson and Moulins 1981a,b) which exhibit bursts of EPSPs time-locked with the pyloric rhythm. Subsequently, one of its constituting elements, the commissural pyloric neuron (CP), has been identified and used to directly demonstrate that the CPO entrains the neurons of the pyloric CPG (Nag 1981, Moulins and Nagy 1983).

Neuron CP, whose cell body is located in the COG, sends an axon that projects directly to the STG via the son and the snt (Fig. 5A,B). It displays a complex discharge characterized by high-frequency bursts of spikes interspersed with lower-fre-
pears to be a master oscillator entraining the rhythm of the pyloric CPG. One may wonder about the relative insensitivity of CP bursting in regard to the strong inhibition it receives from AB. This inhibition could operate as a feedback loop which regulates CP bursting, although not modifying its overall burst frequency. Analogies may be drawn, in the pyloric network itself, with the inhibition of the pyloric pacemaker neurons by the follower LP.

Besides CP, other neurons related to the CPO were identified in the COG (Robertson and Moulins 1981a) (Fig. 8.6A). They are, however, follower neurons (F cells) that display rhythmic bursts of EPSPs with the CPO rhythm, but whose hyperpolarization or depolarization is unable to reset this rhythm. Some send an axon in the inn probably carrying to the brain efferent copies of the CPO activity. These could be different types of F cells, for some receive, from the CPO, EPSPs time-locked with EPSPs recorded in AB, although other F cells have their bursts of EPSPs in phase with bursts of the pyloric constrictor LP (Robertson and Moulins 1981b).

The CPO is a higher order oscillator entraining the pyloric CPG. One can ask what is the physiological significance of such a hierarchy of oscillators. It seems that the CPOs firstly serve as timers for the pyloric CPG and secondarily, by a differential entrainment of the individual pyloric neurons, can provide a greater flexibility to the pyloric motor pattern. A striking feature of the CPO is the great stability of its rhythm. From preparation to preparation its frequency remains between 0.8 and 1 Hz (Robertson and Moulins 1981b, Moulins and Nagy 1983). In contrast, there is no such inherent stability in the free-run frequency of the pyloric CPG, and the CPO could be considered as a time base by which the potentially variable frequency of the pyloric CPG can be entrained. This entrainment, however, can occur with different modes of coordination. In Homarus, a stable pyloric frequency usually corresponds, in isolated preparations, to a 2 or 1 coordination between CPO and the pyloric pacemaker(s) (the bursting frequency of the latter being about 0.4 Hz). In these conditions, modification, by current injection, of the membrane potential of the pyloric pacemakers induces nonlinear variations of their bursting frequency (unlike for independent endogenous oscillators) (P. Cardi, F. Nagy and M. Moulins, in preparation; see Fig. 8.6B). The discontinuities in the relationship between burst frequency and mean membrane potential are accounted for by jumps through different coordinating modes (1 : 1, 1 : 2, 1 : 3) between pyloric pacemakers and the governing CPO. In other words, the period of the pyloric pattern is roughly a multiple of the CPO bursting period, and the value of this multiple depends on the sensitivity of the pyloric pacemakers to the CPO inputs.

The CPOs, however, do not influence the pyloric CPG as a whole, but have access to each pyloric neuron individually. Interestingly enough, the sensitivity to CPO inputs is an individual characteristic of the follower neuron, so that in some instances the CPOs can entrain different pyloric neurons with different coupling ratios, such as 1 : 1 for a constrictor neuron, at the same time as 1 : 2 for a dilator (Robertson and Moulins 1981b; see Fig. 8.6A). This feature provides more flexibility to the pyloric pattern, allowing for instance the constrictor neurons to burst with the CPO rhythm, although the pyloric pacemakers are silent (in Homarus, the constrictor IC is usually the most sensitive to CPO inputs). The participation of a variable number of neurons in the pyloric sequence is not a curiosity occurring only in isolated preparations, but,
as shown by electromyographic recordings, also happens in the intact animal (Rezef and Moulins 1983). In summary, the frequency of the pyloric rhythm and the structure of the pyloric sequence both depend on the sensitivity of the pyloric neurons to the CPG inputs. As reported here, this sensitivity can be a function of their mean membrane potential (see Fig. 8.6B). However, the sensitivity of pyloric neurons to excitatory inputs can be more accurately controlled by additional inputs (like APM) that modulate their burstiness.

Is the pyloric control by a pair of CPOs a feature common to other decapod crustaceans? Most of the available experimental data concerning the CPOs come from Homarus gammarus, but indirect evidence speaks for the existence of a CPO in both Jasus lalandii and Palinurus vulgaris. Firstly, in the two species, two types of rhythm can be recorded in the isolated preparation (Nagy 1981, Moulins and Nagy 1982) as well as in the intact animal (Rezef and Moulins 1983): a fast and regular rhythm that involves all the pyloric neurons, and a slow and irregular rhythm in which a variable number of motoneurons participate. The period of the first rhythm is always around 1 s (the CPO period in Homarus) and those characterizing the second rhythm are roughly multiples of 1 s. Secondly, when the rhythm is slow, activation of the pyloric CPG (for instance via the discharge of a modulatory input such as APM) shortens the pyloric period to a minimum of about 1 s (Nagy and Dickinson 1983). Thirdly, the rhythm of the pyloric CPG can be entrained by rhythmic sensory inputs (see Sect. 8.6). The stimulus period causing the best entrainment of the pyloric pacemakers is also around 1 s, even if the free-run pyloric period is twice as slow (Nagy 1981). By contrast, no such lines of evidence are available in Panulirus interruptus. Moreover, in this species, when the membrane potential of the pacemaker neuron AB is modified by current injection, the frequency of the pyloric rhythm varies linearly with respect to the potential of the pacemaker neuron (i.e., AB behaves like an independent oscillator) (J.P. Müller and F. Nagy, unpublished). However, some caution should be taken before rejecting the possible existence of a CPO in Panulirus. In isolated preparations of Panulirus the mean pyloric period is much shorter than in Jasus, Palinurus or Homarus (0.5 to 0.6 s). In isolated preparation of Homarus, if the pyloric CPG produces such a rapid rhythm (obtained when using the Panulirus perfusion saline), neuron AB displays a linear variation of its burst frequency when injected with current, just like in Panulirus. However, if enough hyperpolarizing current is injected simultaneously in two of the three electrically coupled pacemakers in order to bring the period of the pyloric rhythm above 1 s, the relation between period and potential of the pacemaker neurons becomes nonlinear, as it usually does in Homarus saline (P. Cardi, F. Nagy and M. Moulins, in preparation). It is therefore possible that the pyloric CPG is frequency controlled by the CPOs as long as its endogenous rhythm is slower than the CPO rhythm, and that it operates independently when, activated by other inputs, its frequency becomes higher than the CPO frequency.

8.4.1.2 The P Cells

In Panulirus, the COGs contain cells firing with the pyloric pattern (P cells) but which appear to be different from the CP neuron. A P neuron has its cell body located
in a COG and projects to the STG (Selverston et al. 1976, Russell 1977). It receives trans of IPSPs in phase with the dilator bursts of the pyloric sequence, so that a P cell fires in bursts with the pyloric rhythm. When the pyloric pacemaker neurons are silenced by intracellular current injection, the P neuron fires tonically. (Modified from Selverston et al. 1976)

Fig. 8.7. The commissural neuron P is rhythmically inhibited by the pyloric interneuron AB. When the pyloric pacemaker neurons are silenced by intracellular current injection, the P neuron fires tonically. (Modified from Selverston et al. 1976)

Extrinsic Inputs

It is in fact a higher order oscillator which drives the rhythm of the gastric CPG (Robertson and Moulins 1984).

Neuron CG sends an axon toward the STG via the son and the stn. Intracellular recordings in its cell body show rhythmic oscillations of its membrane potential, which are in phase with the gastric rhythm. During most of these oscillations spikes are produced at relatively high frequency. In most of the isolated preparations there is a 1:1 coordination between bursting of CG and of gastric neurons. Blocking axonal conduction in the stn proves, however, that the rhythmicity of CG is not due to influences from the gastric CPG (Fig. 8.8). In these conditions CG keeps on bursting with an almost unmodified frequency, although the gastric CPG stops cycling. Even if isolated from gastric influences, CG can express regenerative properties. Injection in its cell body of brief pulses of depolarizing or hyperpolarizing current can respectively trigger or shut off a plateau potential. Prolonged injections of current can speed up or slow down the frequency of spontaneous CG oscillations. As, in addition, CG does not seem to receive, in isolated preparations, any rhythmic excitatory input which could be responsible for its bursting discharge, it is most probably an endogenous oscillator (Robertson and Moulins 1984).

When the COG is normally connected to the STG, CG activates the gastric network. Its most obvious targets are the GM motoneurons that control the powerstroke (protraction) of the medial teeth of the gastric mill. An EPSP in all GM neurons is correlated 1:1 with each CG spike (Fig. 8.8B). These EPSPs, following the rhythm of CG oscillations, rhythmically depolarize the GM neurons above threshold, so that the GM motoneurons burst in phase with CG. As CG, whether oscillating or not, usually fires at high frequency, it keeps the GM neurons continuously depolarized even when not cycling (and reduction of CG firing frequency by current injection always provokes a clear hyperpolarization of GM neurons). Projections of CG on the other gastric neurons are less documented, but it seems that CG influences several of them. Depolarizing a CG neuron activates simultaneously LPG and LG (Robertson and Moulins 1984). CG puts a monosynaptic EPSP on LPG and causes a depolarization of DG; moreover, the gastric Intergemuron 1 feeds back on CG where it puts monosynaptic IPSPs (A.J. Simmers and F. Nagy, unpublished). There are no known gastric motoneurons in the COG. So CG cannot be considered as a local center of a distributed gastric CPG. It is merely a higher order oscillator controlling a motor CPG, the gastric network (Robertson and Moulins 1984).

What do we know about CG action on the gastric CPG? First, when neither the CGOs nor the gastric neurons are cycling, experimentally inducing the CGs to oscillate provokes a sustained gastric rhythm (Robertson and Moulins 1984). Second, in situations where the CGs are firing tonically although the gastric CPG is cycling, subsequent experimental triggering of CG's oscillations provokes entrainment of the gastric rhythm on the rhythm of the CGs (and increases the discharge of all the gastric neurons) (A.J. Simmers and F. Nagy, unpublished). So the gastric CPG can be frequency-controlled by the CG neurons (and hence by the CGOs), which is reminiscent of the control of pyloric frequency by the CPs. Is it the CG influence exerted individually on the gastric motoneurons that is able to organize the gastric pattern? Because a CG seems to provide a direct excitation to most of the gastric neurons, the organization of the gastric pattern results from both the synaptic interconnections.
motor output produced, depends on the CG firing frequency. In this respect it is worth noting that the neurons which are the most activated by the CGs are the GM neurons which are, on the other hand, the most inhibited elements within the gastric network (Mulloney and Silverston 1974b). Because the GM neurons are also completely deprived of any regenerative property (Russell and Hartline 1984) which could underlie their firing when released from inhibition, the necessity for them to receive a stronger excitatory input from the CGs is understandable. The CG neurons are inhibited by the gastric interneuron 1. This inhibition, however, does not provoke strong modifications of the CGs cycling frequency. Once again this loop allowing the gastric CPG to feed back on the CGOs is reminiscent of the action exerted by the interneuron AB on the CPFs. To summarize, the CGOs are higher order oscillators which can exert a control over the frequency and the intensity of the rhythmic motor output of the gastric CPG. In fact, this control can also provide flexibility to the expression of the gastric motor pattern because of another bioelectrical particularity of the CGs, the existence of a double threshold for spike inactivation (Robertson and Moulins 1981c).

CG has a membrane potential threshold for spiking (-60 mV) above which it begins to fire. Further depolarization of CG is correlated with a regular acceleration of spike frequency, until the membrane potential reaches another threshold (30 mV), where CG firing stops abruptly. In other words, CG can only spike within a limited range of membrane potential. When membrane potential is above -30 mV, CG spikes suddenly disappear not only in the cell body but also in the axons (in the son and the stn), and the GM neurons deprived of EPSPs abruptly hyperpolarize (Robertson and Moulins 1981c; see Fig. 8A,B). This has important implications for the control of the gastric CPG. Depending on their activation by different inputs, the mean membrane potential of the CG neurons is such that their oscillations can rhythmically cross either the lower or the upper threshold for spike inactivation. As a consequence, they may produce bursts of spikes either in the troughs or at the peaks of their oscillation (Robertson and Moulins 1984; see Fig. 8C,D). This implies that the CG neurons can burst either in phase or in anti-phase with the GM motoneurons (activated only when CG spikes are produced) (Fig. 8E,F), and that their relative phasing with every neuron of the gastric CPG can switch by 180°, when the mean membrane potential around which they oscillate is modified by some inputs.

The bioelectric properties of the CG neurons underlie the complexity of the control exerted by the CGOs over the gastric CPG. As we have seen, these properties are voltage-dependent and so are sensitive to inputs which control the potential of CG neurons. In this respect it appears particularly interesting that the CG neurons are the principal elements of integration for the inputs to the gastric CPG. First, they are the only intermediate between the gastric CPG and two identified gastric proprioreceptors, the Posterior Stomach Receptors (PSRs, Nagy 1981) and the Anterior Gastric Receptor (AGR) (Appendix A to this Chap.). Second, the CG neurons are slightly influenced by the pyloric network. Small fluctuations in phase with the pyloric rhythm are superimposed on the oscillations of their membrane potential. These fluctuations produce rhythmic decreases in CG spike frequency, which are reflected by rhythmic decreases in the activation of the gastric neurons (Robertson and Moulins 1984). Such divisions of the gastric motoneuronal discharges in subbursts following the py-
Iotic rhythm must have a physiological significance, for they are also recorded in the intact animal (Powers 1973, E. Rezzer, unpublished). The pyloric influence exerted on the gastric CPG via the CG neurons could be imposed partly from the pyloric CPG itself via the interneuron AB, but mostly from the CPD (Robertson and Moulins 1984). This is an interesting example of central organization where coordination between two CPGs occurs mainly at the level of two premotor oscillators.

8.4.2.2 The E Cells

In Panulirus, each COG contains the cell body of a neuron which fires rhythmically with the gastric CPG, and projects to the STG, the E neuron (Russell 1976a). It puts simultaneous EPSPs onto the gastric neurons GM, LG and LPG. The E neuron is rhythmically inhibited by the gastric Interneuron 1, and so fires in phase with the GM neurons (Selverston et al. 1976; see Fig. 8.10). When Interneuron 1 is experimentally silenced, the E neuron fires tonically (Russell 1976a).

It is tempting to draw some analogies between the E and the CG neurons (Fig. 8.10c). They both have their cell body in the COG, project to the STG via the som and the stn, burst with the gastric CPG, put monosynaptic EPSPs on the gastric neurons GM and LPG, and finally are both inhibited by Interneuron 1. Moreover, in Homarus, experimentally silencing the CG neurons suppresses all EPSPs in the GM neurons, indicating the absence of any active E neuron. There is, however, the same striking difference between neurons CG and E, as between neurons CP and P: CG is an independent endogenous burster, whereas E appears to be a passive follower of the gastric CPG. A possibility arises that the E neuron could display two states, one oscillating and one nonoscillating, and that the first one was not observed in the experiments where E neuron was actually recorded. But because of the scarcity of experimental data concerning the E neuron, it seems premature to consider E and CG neurons as being the same element.

8.4.2.3 Other Inputs

Besides the P cells that excite the AM, DG, and LPG neurons, other inputs, whose spikes are recorded in the stn, were reported to inhibit the motoneurons LG and MG. Because these motoneurons control the powerstroke of the lateral teeth of the gastric

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Fig. 8.9A–F. Neuron CG has two thresholds for spike inactivation, 60 mV and 30 mV. A,B When CG membrane potential is above -30 mV, it stops firing; the large remaining events are EPSPs from the sensory neurons AG; see App. A to this Chap., and gastric neurosecretory GM are deinnervated; C,D Depending on the mean membrane potential around which it oscillates, neuron CG can produce spikes either at the peaks (C) or in the troughs (D) of its oscillations; E,F As a consequence, CG oscillations are either in phase (E) or in phase-opposition (F) with bursts of the gastric motoneuron GM. Calibrations = horizontal bars 0.5 s in A,B, 10 s in C,D, 5 s in E,F. (Modified from Robertson and Moulins 1984)
mill, the inhibitory inputs were named Lateral Inhibitor (LI) neurons (Selverston et al. 1976). Nothing is known concerning either their mode of operation or their function.

8.5 inn Through Fibers

The output of the gastric and pyloric CPGs, although flexible, are well defined rhythmic motor patterns. Within each sequence of these rhythms, the phase and the intensity of the discharges of their constituent neurons can be controlled by modulatory inputs, and the frequency of these sequences can be entrained by higher order oscillators. Nevertheless, it is still possible to recognize a gastric or a pyloric pattern. A different situation arises when gastric and pyloric networks are influenced by the discharge of a pair of input fibers, the inn Through Fibers (innTF) (Dando and Selverston 1972) and strongly modify their firing. The innTF exert a complex action on different stomatogastric neurons, like promoting burstiness (Russell and Hartline 1981) or putting compound PSPs (Sigvardt and Mullenoy 1982a, Claiborne and Selverston 1984a). The innTF could subserve at least two types of function. First, because they project on several neurons of both CPGs and in some circumstances they can trigger a particular motor sequence, they may have a command function (Dando and Selverston 1972, Sigvardt and Mullenoy 1982a,b). Second, they can be involved in the generation of the cardiac sac rhythm, and could mediate the influence exerted by the cardiac sac CPG on the gastric and pyloric networks. They may therefore exert a coordinating function (Moulins and Vedel 1977). We will first describe briefly the innTF and their projections on the stomatogastric CPGs. Then we will consider both their command and coordinating functions.

As already mentioned (see Sect. 8.3), in Panulirus the cell body of each innTF is located in the brain, close to the base of the inn (Claiborne and Selverston 1984b). They project in the STG via the inn and the inn, but send also axonal branches in both soms and ions toward the COGs (Dando and Selverston 1972, Selverston et al. 1976; see Fig. 8.11A). The inn neurons can fire in long bursts (several tens of second long) which surprisingly enough are generated in the STG (where innTF probably have integrative regions) and travel toward the brain, the COGs and the STG (Selverston et al. 1976, Moulins and Vedel 1977). The innTF contact monosynaptically some pyloric (PD, VD) and gastric (GM, LPG, Int) neurons (Sigvardt and Mullenoy 1982a, Claiborne and Selverston 1984a), providing either excitation or inhibition, depending on the target. The most interesting, however, are the complex synaptic relationships between the innTF and the pacemaker neurons PD. As already reported, innTF firing promotes bursting abilities in the PD neurons (see Sect. 8.3). Second, it is possible to correlate each spike in an innTF with a compound PSP in PD neurons consisting of a fast EPSP followed by a slow IPSP (Sigvardt and Mullenoy 1982b; see Chap. 4), a situation which was also described in different molluscan preparations (Wachtel and Kandel 1967, Berry and Cottrell 1975). The excitatory component of the compound PSP (Russell and Hartline 1981) as well as the inhibitory component (Sigvardt and Mullenoy 1982b) are mediated by a conventional synapse.
The complex postsynaptic effects of the ivnTF could be mediated by several transmitters, a situation which is found to characterize an increasing number of neurons (M.E. Adams and O'Shea 1983). It is, at least, well established that the inhibitory responses are due to release of histamine by the ivnTF (Claiborne and Selverston 1984c; see Chap. 9). The authors propose that the ivnTF use another transmitter to mediate the EPSP and the promotion of burstiness in the target neurons. This transmitter could activate respectively nicotinic-like and muscarinic-like receptors of the PD neurons (Russell and Hartline 1981). This situation therefore appears different from what is known of multicomponent synapses in mollusca, where a single transmitter activates different types of receptors on the postsynaptic neuron (Kehoe 1972, Gardner and Kandel 1977), and is more reminiscent of what happens at skeletal neuromuscular junctions of some invertebrates, where different functions are devoted to several co-transmitters (O'Shea 1985).

Whatever is their causal element, the ivnTF-mediated EPSPs and IPSPs have different kinetics. So during a repetitive firing of the ivnTF, neurons that receive both types of PSPs are first activated, then inhibited when the IPSPs sum and short-circuit the excitatory component. In fact, depending on the firing frequency of the ivnTF, their overall effect is either an excitation (low-frequency firing of ivnTF) or an inhibition (high-frequency firing) (Sigvardt and Molloney 1982a, Ciabirone and Selverston 1984b; see Fig. 8.11B), but whatever the effect is, the target neurons are not oscillating any more (i.e., become tonically firing or silent). In addition, the ivnTF put IPSPs on some gastric and pyloric neurons and EPSPs on some others. A burst of discharge of the ivnTF thus results in a profound modification of both pyloric and gastric motor patterns. Translated in terms of behavior, the effect of an ivnTF burst can be, on the gastric mill, interrupting the gastric rhythm and inducing a stereotyped rest pos-

tion for the gastric teeth (Sigvardt and Molloney 1982a). For the pyloric filter the ventral gutter, which brings digestive enzymes forward from the hepatopancreas, remains open and, depending on ivnTF spike frequency, either the dilator muscles or the constrictor muscles are hyperactivated, bringing food in or pushing it out of the pylorus (Sigvardt and Molloney 1982a).

The modifications of activities of the stomatogastric CPGs can occur in two ways, either due to an isolated ivnTF burst which can be triggered by a propriospinous input (Sigvardt and Molloney 1982a) or due to rhythmic, centrally driven ivnTF bursts, which occur with the cardiac sac rhythm (Molloney and Vedel 1977). In the first case the ivnTF behave like command fibers, whereas in the latter case their action is characteristic of coordinating fibers.

An ivnTF burst which induces the gastric and pyloric CPGs to produce a well-characterized stereotyped motor sequence can be triggered by stimulation of pyloric proprioceptors (Sigvardt and Molloney 1982a; see Fig. 8.12A,B). There is no direct sensory-motor interactions, in the STG, between these proprioceptors and the gastric and pyloric CPGs. The sensory fibers project in the OG and activate there an inhibitory zone of the ivn neurons, which in turn influence the motor CPGs. According to the authors, in these conditions the ivnTF meet the main criteria for command neurons outlined by Kupfermann and Weiss (1978): (1) they are activated by a natural stimulus, stretch of the pylorus; (2) they are the necessary link between the propriospinous input and the resulting motor sequence; (3) their direct electrical stimulation provokes on the gastric and pyloric motor patterns effects similar to those of a naturally occurring ivnTF burst.

IvnTF bursts can also occur rhythmically in de-afferented preparations with a period of 20 to 70 s. Moreover, they are concomitant with long bursts of spikes in two unidentified motoneurons of the cardiac sac, CD1 and CD2 (Molloney and Vedel 1977; see Fig. 8.12C). The cell body of CD1 is located in the OG from where originate the ivnTF bursts. The soma of CD2 lies in the STG, but this neuron fires in long bursts with each cardiac sac rhythm, from an aconal spike initiating site also located in the OG (Vedel and Molloney 1977, 1978). In short, the oesophageal ganglion contains the cardiac sac CPG, whose activity involves the ivnTF. The occurrence of a burst of the cardiac sac CPG is correlated with profound modifications in the activity of the gastric and pyloric CPGs. These modifications are quite similar to those produced by direct stimulation of the ivnTF. For instance, the pyloric pacemaker neurons are activated when spike frequency of cardiac bursts is low, and inhibited when spike frequency of cardiac burst is high (Molloney and Vedel 1977; see Fig. 8.12D,E), and during cardiac sac activity the dilator neuron (VD) fires a long burst just like after an ivnTF stimulation (that neuron VD can display either a pyloric or a cardiac firing is interesting because it innervates a bifunctional muscle also innervated by the cardiac sac motoneuron CD2) (Molloney and Vedel 1977). The authors propose that the ivnTF are elements of the cardiac sac CPG which mediate the cardiac sac-induced control of the gastric and pyloric CPGs. In addition, the ivnTF contribute a complex input to the gastric and pyloric CPGs (Fig. 8.13). They can exert a command function, putting the target CPGs in operation to perform a particular behavioral sequence. They can also, during operation of the cardiac sac CPG, exert a coordinating action, associating activities of different CPGs.
8.6 Sensory Inputs

Considerable attention has been paid in many motor systems to the contribution of sensory (mainly proprioceptive) inputs to the generation of the final motor output. Most of the time, however, the central organization is not known enough to provide understanding of the mechanisms by which sensory inputs influence the CPG. The stomatogastric nervous system is one of the few preparations where some of these mechanisms can be analyzed. The foregut of Crustacea possesses a large variety of putative sense organs (see Dando and D.M. Maynard 1974) that most probably influence the gastric and the pyloric CPGs. Very few of them, however, were experimentally studied, and the action of only two gastric proprioceptors is relatively well understood: the Posterior Stomach Receptor (PSR) (Dando and Laverack 1969) and the Anterior Gastric Receptor (AGR) (Appendix A to this Chap.). Although less well characterized anatomically, another pyloric proprioceptor was reported to influence the stomatogastric CPGs (Sigvardt and Mulloney 1982a). There are two common features characterizing these three proprioceptive inputs. Firstly, they do not project directly to the motor CPGs themselves, but on premotor elements which are the CPO, the CGO and the invTF. Secondly, they produce various effects on the motor CPGs, which are all determined by the complex properties of the premotor elements (regenerative properties of the CPO and the CGO, synaptic properties of the invTF). We will first consider the effects of the PSR on the gastric and pyloric CPGs.
8.6.1 The Posterior Stomach Receptors (PSRs)

The PSRs influence both the gastric and pyloric CPGs. They provoke several effects. First, they can strongly and lastingly activate both gastric and pyloric CPGs. Second, they can trigger the gastric rhythm when the gastric CPG is silent. Finally, when they are rhythmically stimulated, they can entrain the rhythms of both the gastric and pyloric CPGs.

8.6.1.1 Rhythmic Discharge of the PSRs

On each side of the stomach, a group of about 180 mechanoreceptive neurons, comprising a PSR, send dendrites to the stomach wall, which ramify around the posterior

arch supporting the gastric teeth. The cell bodies of the sensory neurons are grouped in the distal part of the posterior stomach nerve (pan) (Dando and Laverack 1969), which at this level is a purely sensory nerve. Centrally the pan merges with the outer mandibular nerve which, in *Homo*rus, enters the circumoesophageal connective (Wals et al. 1976a) and in *Jaurus* and *Pulmarus* directly enters the suboesophageal ganglion (Nagy 1977). Recordings of the pan in semi-intact preparations show that the PSRs monitor the gastric rhythm, firing in anti-phase with the GM motoneurons (Nagy and Mullins 1981; see Fig. 8.14A). The PSRs can also monitor the cardiac sac rhythm (Nagy and Mullins 1981). In fact, they are probably stimulated by any mechanical disturbance imposed upon the soft stomach wall (as, for instance, by the pyloric motor activity). In summary, the PSRs discharge cyclically and provide a rhythmic input to the stomatogastric nervous system. The frequency of their bursts depends on the neuromuscular system which activates them (Nagy and Mullins 1981), the most common being, however, a gastric frequency.

8.6.1.2 Long-lasting Activation of the Gastric and Pyloric CPGs

The PSRs feed back on the gastric CPG. They influence several gastric neurons and particularly the motoneurons GM, on which they exert a double effect. Electrical or mechanical stimulation of a PSR provokes a short-term inhibition of the GM neurons, followed by a long-term excitation. When the gastric rhythm is very slow, stimulating briefly a pan reactivate (after the transient inhibition of gastric motoneurons) the gastric CPG for several minutes (Nagy 1981). All these effects are explained by a specific projection of the PSRs onto the two premotor neurons CG (see Sect. 8.4).

In *Homo*rus, each PSR projects bilaterally and puts complex postnaptic events on each CG neuron. These events appear clearly when the CG neurons are disconnected from the gastric CPG, during axonal conduction block in the sin. A single electrical shock on PSR dendrites induces a brief excitation, then an inhibition several tens of milliseconds long, and finally a strong post-inhibitory rebound (Fig. 8.15B). When PSR stimulation is delivered in a short train, the CG neuron is inhibited during the train, then rebounds and gives a several seconds-long regenerative depolarization (which is the equivalent of an oscillation during CG rhythmic activity). When CGOs are normally connected to the NTC, the CG neurons drive the gastric CPG, and hence allow the PSRs to influence the gastric activity. During a train of PSR stimulation, gastric motoneurons are first deactivated (due to CG inhibition), then they are induced to burst during the CG rebound oscillation. If both CG neurons are simultaneously hyperpolarized by current injection in order to prevent their firing, the same stimulation delivered to a PSR only induces a slight depolarization which lasts for the stimulus duration only (Fig. 8.14B). So the activation of the gastric CPG by the PSRs is essentially mediated by the two CGs.

The pyloric network is also influenced by the PSRs (Dando et al. 1974, Herman and Dando 1977). In isolated preparations of *Jaurus*, electrical stimulation of the PSR dendrites strongly activates the pyloric CPG (Nagy 1977). First, it increases the oscillation frequency of the pacemaker neurons (Fig. 8.15D) and so accelerates the pyloric rhythm. Second it excites firing of previously silent neurons of the network. Third, it strongly increases the intensity of the discharges of neurons already active. As for

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**Fig. 8.14.** A On a semi-intact preparation, PSR discharges are correlated with rhythmic activity of the gastric mill (monitored by bursts of the gastric motoneurons GM) and of the cardiac sac (monitored by bursts of the cardiac motoneurone CD1); B Simultaneous hyperpolarization of both CG neurons almost completely suppresses excitation of the gastric motoneurons GM following electrical stimulation of a PSR. Calibration = horizontal bar 2 s, vertical bar 10 mV for the CG neurons, 5 mV for GM (A modified from Nagy and Mullins 1981; B from Nagy 1981).
the gastric activation, these effects are long-lasting, a 0.25 s PSR stimulation activating the pyloric CPG for several tens of seconds (Nagy 1977; see Fig. 15E).

8.6.1.3 Triggering of Rhythmic Activity of the Gastric CPG

A tonic low-frequency discharge of the PSRs can induce rhythmicity in a previously silent gastric CPG. This is due to the induction by the PSR discharge of oscillatory behavior in the CG neurons (Nagy 1981). Once again this effect appears more clearly when these neurons are disconnected from the gastric CPG. If in these conditions the CG neurons are not oscillating and fire tonically, stimulation at low frequency of a PSR slowly depolarizes the CG neurons. After a while both CGs begin to oscillate, although oscillations are not necessarily synchronized (Fig. 8.15C). When the CG neurons are connected to the STG, their oscillations drive a rhythmic discharge of the gastric CPG.

Such a triggering of rhythmicity by a PSR discharge is difficult to demonstrate for the pyloric network. In Janus, the only way to stop the pyloric rhythm without blocking axonal conduction in the sin, is to block synaptic activity in both COGs by perfusing with a Ca++-CO3-containing saline (Dickinson and Nagy 1983). Unfortunately, this treatment suppresses all influences of the PSR inputs on the pyloric network. Under these conditions, it is thus impossible to check if PSR inputs can trigger rhythmicity of a totally silent pyloric CPG. However, that these inputs are able to do so is suggested by the fact that when the pyloric network is weakly active a brief PSR stimulation can trigger strong and durable plateauing activity in previously silent constricter neurons. On the other hand, prevention of PSR effects by blocking synaptic activity in COGs indicates that PSR inputs project on commissural premotor elements and that there is no direct sensory-motor interaction at the CPG level.

8.6.1.4 Entrainment of Gastric and Pyloric Rhythms

As already mentioned, the PSRs are stimulated by different rhythmic movements of the stomach and so provide a rhythmic input to the stomatogastric nervous system. It would be worth looking at what rhythmic stimulation of the receptors could do to the rhythms of the stomatogastric CPGs. Indeed, such a repetitive stimulation of a PSR might entrain, over a certain range of frequency, both the gastric and pyloric patterns.

The premotor neuron CG is transiently inhibited during a short train of stimuli delivered to a PSR, then, on rebound, it produces an oscillation. Delivering stimuli in repetitive trains engenders repetitive transient inhibitions, followed by oscillations of the CG neurons. By controlling the train frequency of PSR stimulations it is possible to impose a rhythm of oscillation on both CG neurons. In addition, their discharges become synchronized (Nagy 1981). Because each CG oscillation delivers a burst to the gastric motorneurons, the gastric rhythm is finally entrained by the rhythm of the PSR firing (Fig. 8.16A,B).
Rhythmic stimulation of a PSR with single electric pulses can also entrain the pyloric rhythm over a certain range of frequencies. An interesting feature of this sensory-motor interaction is that the best possible entrainment (i.e., a 1:1 coordination between stimuli and pyloric pacemaker discharges) occurs when the stimulus period is close to 1 s whatever the control pyloric period (Nagy 1981; see Fig. 8.16C,D). This period corresponds to the stable period of the CPG rhythm (see Sect. 8.4). Because an oscillator undergoes the best entrainment when the frequency of the entraining input is close to its free-run frequency (see Sect. 8.2), it seems probable that the oscillator entrained by the rhythmic PSR stimulation is the CPG and not the pyloric pacemakers themselves. This is also suggested by the fact that, in Homarus, repetitive stimula-

8.6.1.5 Functional Significance

On the intact animal the PSRs are mainly activated by rhythmic movements of the gastric mill (Dando and Leverack 1969, Nagy 1981). So functionally the PSRs participate in a proprioceptive loop by which the gastric rhythm is self-regulated (because the gastric CPG and the CG neurons, like every other oscillator, tend to keep a constant phase relative to a rhythmic input able to entrain them). It can be remarked that during delivery of repetitive trains of PSR stimulation, the activation of the CG neurons gradually builds up and the gastric motoneurons undergo a parallel increase in activation (Fig. 8.16A). After a while, however, the CG neurons can, at the peak of their oscillations, cross their upper threshold for spike inactivation (see Sect. 8.4) and transiently stop activating the gastric neurons (Fig. 8.16B). So along the sequence of PSR stimulation the motoneuronal bursts gradually change. In other words, the proprioceptive input can not only regularize the gastric rhythm but can also, via the properties of the premotor neurons, shape the motor output of the gastric CPG. Another interesting feature is that the direct PSR effect is an inhibition of the CG neurons, and that the overall activation of these premotor neurons appears to result from an endogenous rebound of activity when they are released from inhibition. So again it is the properties of the premotor element which determine the response of the motor system to the proprioceptive input.

The functional significance of PSR control over the pyloric rhythm is less clear. One can speculate that it contributes to coordination between pyloric and gastric rhythms. Another possibility is that some of the 180 sensory neurons of a PSR can be more specifically devoted to pyloric control, and are involved in a loop for self-regulation of the pyloric rhythm.

The PSRs exert a rhythmic influence on the gastric and pyloric pyloric neurons via projections on the gastric and pyloric premotor oscillators CGO and CPO. However, these proprioceptors also provoke a long-lasting activation of both rhythms, an effect which is reminiscent of a modulatory control. So far, no experimental data demonstrate a PSR projection on an identified modulatory neuron. Because a tonic discharge of a PSR can induce rhythmicity in the CG neurons, it is possible that the proprioceptive input itself can participate in the induction of the regenerative properties of the premotor neurons. This problem has, however, still to be worked out.

8.6.2 The Anterior Gastric Receptor (AGR)

In Homarus, a single mechanoreceptive neuron was recently identified by Simmers, the Anterior Gastric Receptor (AGR). It is described in Appendix A to this Chap. and we will just briefly summarize its principal characteristics. This proprioceptor is stimulated by movements of the anterior gastric muscles (gm1) and provides a monosynaptic EPSP on both CG neurons. Its tonic discharge can induce rhythmic oscillations of
both CG neurons and so can induce a gastric rhythm from a previously silent gastric CPG. A rhythmic discharge of AGR can also synchronize the discharges of both CG neurons, and entrain the rhythm of their oscillations. Finally, depending on how much it activates the CG neurons, and AGR discharge can control the intensity and phase of the gastric motoneuronal discharges.

We may remark that the PSRs and the AGR, which are both gastric propriocceptors, have the same overall effect on the gastric CPG, namely activation and frequency control of the gastric rhythm, and intensity and phase control of the motoneuronal discharges in the gastric sequence. However, they do so in different ways: AGR via direct excitation of CG neurons, and the PSRs via indirect excitation (inhibition then rebound) of these neurons. It would be interesting to verify on the intact animal if the two receptors fire in phase opposition while at the same time exerting a synergistic control of the gastric CPG.

8.6.3 Other Inputs

We already mentioned that other pyloric propriocceptors were able to strongly influence the pyloric pattern via the sriTF (Sigvardt and Mulloney 1982; see Sect. 8.5). One does not know too much, however, about either their anatomy and the exact nature of the stimulus which elicits their discharge.

Finally, a third sensory input is known to influence the pyloric CPG. On each side of the pyloric region there are two multipolar sensory neurons, the dendrites of which ramify around the hepatopancreatic duct (Dando and D.M. Maynard 1974). Stimulation of these neurons, known as HD cells, was reported to strongly enhance plateau potential amplitude and burst intensity in some pyloric constrictor neurons (Hartline and Russell 1978; see Appendix A, Chap. 2).

8.7 Conclusion

CPGs designed to generate long-lasting rhythmic activities in vivo generally produce stereotyped motor patterns when isolated. It is a function of extrinsic inputs to modify transiently the parameters of these stable motor patterns, in order to adapt them to behavioral contingencies. In the stomatogastric nervous system, extrinsic inputs control the characteristics of the motor patterns, essentially by influencing the production of BPPs by stomatogastric neurons. Some of these inputs induce or modulate the ability of stomatogastric neurons to produce BPPs. They are involved in control of both intracycle pattern generation and rhythm generation by stomatogastric CPGs. Other inputs, the discharges of which are rhythmic, can entrain the rhythms of stomatogastric oscillatory neurons (i.e., influencing the frequency of BPPs), and are more particularly involved in the control of rhythm generation by stomatogastric CPGs.

8.7.1 Control of Intracycle Pattern Generation

In the stomatogastric CPGs, the parameters characterizing the organization of every single sequence of a rhythmic pattern (number of motoneurons involved, phase relationships between different motoneuronal discharges, intensity of motoneuronal discharges) can be strongly influenced by the discharge of a single "modulatory" neuron. The important initial question was how a single neuron can exert such a wide range of effects, not to mention that it can influence most if not all neurons of a CPG? The data collected concerning the modulatory control of the pyloric CPG have provided some of the answers. Most of the parameters of the motor pattern depend on one endogenous property, more or less similarly expressed by all the neurons of the CPG: the ability to produce bursting pacemaker potentials (see Sect. 8.3). If a modulatory neuron can alter (induce, amplify, decrease or prevent) the ability to produce BPP on several neurons of the CPG, it can control most of the characteristics of the ensuing motor pattern. This is the case for the pyloric CPG, where burstiness of pyloric neurons can be either induced or increased by the single modulatory neuron APM, or by one of several other, yet unidentified, modulatory inputs (Marder and Hooper 1985; see Chap. 9). Burstiness of pyloric neurons can even be subject to suppressive influences from an input which, although not fully identified, appears to be most probably GALaergic (Caralea et al. 1985).

Modulation of endogenous burstiness in neurons by putative neuromodulators has been reported in several preparations (Parnas et al. 1974, J.L. Barker and Smith 1976, J.M. Cooke and Hartline, 1973, Mayeri et al. 1979, M.W. Miller et al. 1984, W.B. Adams and Benson 1985). The physiological implications of such a control, however, are not always clear. On the other hand, long-lasting modifications of rhythmic behaviors by putative neuromodulators have also been reported (Kristan and Nusbaum 1983, Lent and Dickinson 1984, Truman and Weeks 1985), but in these cases the cellular mechanisms underlying the modifications often remain to be elucidated. The stomatogastric nervous system thus appears to be an outstandingly favorable preparation where modulatory control of nonlinear endogenous properties of identified neurons by identified neurons is understood in terms of organization of motor activity. It provides, in fact, a powerful model where general principles for neuromodulation can be tested.

With the stomatogastric model a question remains, however, almost completely unanswered: do sensory inputs have access to the modulatory neurons? So far, no sensory projection on the modulatory neuron APM has been described, but the following lines of evidence indicate that propriocaptors could influence the stomatogastric CPG via some other modulatory elements. Stimulation of the HD sensory neuron (see Sect. 8.6) increases burstiness of pyloric constrictor neurons (Appendix A to Chap. 3). A brief stimulation of a PSR can trigger plateauing of a previously silent pyloric constrictor neuron for several tens of seconds (see Sect. 8.6). Finally, pyloric propriocaptors activate the sriTF which, among other effects, modulate burstiness of the pyloric pacemakers PD (see Sect. 8.5). The occurrence of such relationships between sensory and modulatory neurons is presently being investigated.
8.7.2 Control of Rhythm Generation

Pyloric and gastric networks receive rhythmic inputs from oscillators located in pre-motor centers. This organization does not fit very well with a classical model of central pattern generator (Grillner 1975) in which the CPG performs the dual functions of rhythm generation and intracycle coordination, and does not receive extrinsic timing cues from other regions of the CNS. Another model of CPG (specially related to locomotion) was recently proposed (Lennard 1985) in which the two functions can be exerted by different subpopulations of neurons. The CPG is divided into a “Central Timing Network” (CTN) producing the rhythm, and a “Central Intracycle Pattern Generator” (CIPG) organizing each motor sequence. The CPG may or may not be an oscillator entrained by the CTN. It is tempting to consider the gastric and pyloric networks as being CPGs, and the commissural oscillators as being the corresponding CTNs. Several fundamental characteristics, however, differentiate the stomatogastric organization. Firstly, the gastric and pyloric networks in isolation are actual CPGs. This is particularly clear for the pyloric network which can produce a rhythmic motor output in a de-afferented STG, in vitro, where subject to pharmacological activation that mimics the effects of modulatory inputs. This also occurs after chronic de-afferentation (several months after section of the isthmus in the animal), as shown in a subsequently isolated preparation (Appendix B to this Chap.), or by electromyography in operated animal (E. Reizer and M. Moulins, in preparation). Secondly, the pyloric network, when connected to the commissural oscillator CPO, can either be strongly entrained by the CPO rhythm, or follow it with different coordination modes, or even be faster than and independent from the CPO. In other words, the CPO does not generate the pyloric rhythm, like a CTN would, but produces a rhythm that can be either strictly followed, or transformed, or ignored by the pyloric CPG. In addition, this rhythm of the CPO is, at least under our experimental conditions, relatively stable, and this is not always the case for the pyloric rhythm. So the CPO could be considered as a “Timing Oscillator,” which the pyloric oscillator could more or less refer to.

The extent to which the CPG follows the rhythm of the Timing Oscillator depends on the burstiness of the CPG oscillatory neurons, which is itself controlled by modulatory inputs. In other words, the stomatogastric rhythms are generated by the intrinsic oscillators of the stomatogastric. CPGs more or less according to an extrinsic time base, reference to which is controlled by modulatory inputs. This is illustrated by the diagram of Fig. 8.17A, which must be considered as a dynamic model where all relationships are not necessarily functional at any one time. This model can account, for instance, for at least three types of pyloric activity recorded in isolated preparations as well as in the intact animal. When modulatory control is such that burstiness of neurons of the pyloric CPG is weak (Fig. 8.17B1), these neurons are weakly sensitive to the Timing Oscillator (CPO) inputs. The pyloric frequency is slow and variable, and there can be different coordination modes between CPO inputs and the discharges of different neurons of the CPG. This kind of variable pattern was chronically recorded for hours in intact Janus (Reizer and Moulins 1983). When the CPG neurons fully express their burstiness (under a stronger “permissive” modulatory control (Fig. 8.17B2), they are more sensitive and tightly coupled to inputs from the Timing Oscillator. The pyloric rhythm is stable and identical to the CPO rhythm. In fact, the modulatory inputs have grafted the timing inputs. Such an increase of the CPO-pyloric CPG coupling was inferred to explain the activation and
The "stomachic phenomenon," as it is sometimes referred to, occurs in the context of the gastric-epithelial pathway through the gastric mucosa. This pathway is responsible for the regulation of gastric motility, which is essential for the proper digestion and absorption of nutrients. The gastric-epithelial pathway is a complex network of cells and tissues that work together to ensure the health and functionality of the stomach. It is important to note that the exact mechanisms and pathways involved in this process are still being studied and researched.
muscle. Moreover, they are not associated with any other distal sensory structure. On the basis of the cell’s morphology and its response to specific mechanical stimulation in semi-intact and isolated preparations (e.g., Fig. 8.19a), it is concluded that this is a primary mechanoreceptor neuron whose dendrites are specialized as stretch-sensitive elements themselves. This neuron, which has been named anterior gastric receptor (AGR), is unique: no other similar cell type has been found in this region of the stomatogastric neuromuscular system.

AGR in Homarus is probably homologous to the bilateral mechanoreceptor cell recorded extracellularly in crayfish, Procambarus by Larimer and Kennedy (1966), while various anatomical studies have also shown a single bipolar neuron of unknown function in or near the STG of Astacus, Panulirus and Cancer (Glow 1927, King 1976a, Kushter 1979a, F. Nagy, personal communication).

A clue to the functional role of AGR is given in Fig. 8.19b. In this, as in most experiments to date, mechanical stimulation of the receptor terminals of AGR is replaced with direct intracellular stimulation of its cell body in standard “combined” preparations of the isolated stomatogastric nervous system. In terms of output connectivity, therefore, the first major conclusion is that discharge of AGR causes sympathetic excitation of motorneurons (GM) innervating gm1, the muscle from which the receptor originates. Although the analogy with a classical stretch reflex is immediately appealing, the precise mode of AGR stimulation in the intact animal is currently unknown. Under normal conditions, stretch-induced activation of AGR could occur in either (or both) of two ways. Firstly, AGR may be activated by contraction of gastric muscles (gm4) that are antagonistic to gm1, and cause medial tooth retraction (Selverston and Mulloney 1974). This would imply a negative feedback reflex, gm1 being excited to oppose the applied stretch. Alternatively, contraction of gm1 itself may activate AGR, the resultant positive feedback reflex serving to reinforce the ongoing movement. At present the latter possibility is favored on the basis of AGR’s close anatomical relationship with gm1, and its responsiveness to imposed medial tooth protraction in semi-intact preparations (Fig. 8.19a).

Pathway Properties of AGR. The cellular pathway by which AGR has access to GM motorneurons is summarized in Fig. 8.20a. The receptor’s axon ascends in the stn, traverses the oesophageal ganglion (OG), then bifurcates into the inferior oesophageal nerves (sons) to terminate in the left and right commissural ganglia (COGs). There it synapses with the bilateral commissural gastric (CG) interneurons, which in turn descend via the superior oesophageal nerves (sond) and stn, to synapse directly with gastric neurons in the STG. All synaptic connections on either side of CG (i.e., AGR onto CG, CG onto GM) are strongly excitatory and monosynaptic (Fig. 8.20b, C). Furthermore, there is no evidence for additional synaptic connections, either in the STG or COG, which may bypass or operate in series with CG (Fig. 8.20c, d). Thus the second major conclusion is that feedback from AGR to GM occurs via an excitatory, di-synaptic pathway involving the two CG interneurons alone.

At first sight, this tightly coupled circuit seems to provide little scope for integrative flexibility. As a passive sensorimotor relay, CG would appear to do the job well, transforming synaptic excitation from AGR into spiking activity and transmitting this information with little failure, and with the same sign, to gastric motorneurons (see Fig. 8.20b). However, CG is clearly far from being a simple “through-waypath” neuron. Not only does this cell express oscillatory properties, probably resulting from both endogenous “pacemaking” and “plateauing” mechanisms, but it also has the novel intrinsic capability of spike inactivation at both hyperpolarized and depolarized levels of membrane potential (Robertson and Moulin 1982a, 1984; reviewed in this Chap.). How do these endogenous properties relate to CG’s role in sensorimo-tor integration? The following data show that due to its strong synaptic coupling
with CG the single mechanoreceptor AGR has direct access to all three intrinsic properties of the interneuron. This basic cellular and synaptic requirements are thus available for considerable flexibility in the operation of this "simple" proprioceptive pathway.

J. Plateau Property of CG. "Passive" transfer of synaptic excitation through CG imposes linearity on the pathway, the postsynaptic GM response varying as a direct function of the duration and frequency of firing in presynaptic AGR. At other times a different type of input/output relationship is observed, whereby a short-term discharge in AGR evokes relatively long-term activation of GM motoneurons (Fig. 8.21a). The basis for this nonlinear relationship can be seen in the CG recording of Fig. 8.21b. Here a similarly brief train of spikes in AGR triggers a prolonged depolarization (and intense firing) in CG that eventually terminates several seconds after the activating AGR burst. The correlation between Fig. 8.21b and the motor response of Fig. 8.21a now becomes evident: the mechanism that explains this phenomenon is CG's ability to generate plateau potentials (see also Robertson and Moulines 1984).

The contribution of such a mechanism to sensorimotor integration is functionally important, providing amplification of both the strength and duration of the proprioceptor's input to the system. An extreme demonstration of this is shown in Fig. 8.21c. Throughout this experiment repetitive bursts evoked in AGR triggered 35 mV plateau potentials in both left and right CG interneurons. At the end of some AGR bursts, the sudden removal of synaptic excitation caused immediate plateau repolar-
zation in the two cells. At other times, the repolarizing "jump" occurs long after termination of the activating burst (e.g., 1st plateau response, left CG), or even fails to occur before the onset of the next AGR spike train (2nd/3rd cycles, right CG).

2. Oscillatory Property of CG. A further endogenous property of CG is the ability for its membrane potential to oscillate and drive gastric bursting (Robertson and Moulins 1984). As for neuronal oscillators in general, the membrane conductances underlying cyclic CG activity appear to be voltage-dependent; shifting the interneuron's membrane potential up or down with direct current injection activates oscillation, causes corresponding changes in the frequency of oscillation, or inactivates the latter altogether.

The experiments of Fig. 8.22 suggest that AGR has access synaptically to these conductances in CG. A tonic train of impulses evoked in AGR can switch on oscillatory activity in CG and produce concomitant bursting in otherwise silent GM moto-neurons (Fig. 8.22a). This cyclic postspiny activity ceases abruptly when AGR is silenced (Fig. 8.22b). In addition to an on/off effect, graded changes in tonic discharge of AGR can control the cycle frequency of CG oscillation. In the experiment of Fig. 8.22c,d when AGR fires at 8 Hz, the cycle period of CG is about 30 s (Fig. 8.22c), but decreases to less than 8 s when AGR fires at 16 Hz (Fig. 8.22d). This triggering and frequency modulation of CG bursting are therefore consistent with a voltage-dependent oscillatory mechanism having a high sensitivity to tonic synaptic excitation from AGR. Moreover, given CG's response to phasic AGR input (as in Fig. 8.21c), it is probable that transient AGR discharge can reset an ongoing CG rhythm, and entrain it if the proprioreceptor is firing cyclically. Such phasic influences of proprioceptive feedback have been demonstrated for the locomotor rhythms of cat and turtle (Andersson and Grillner 1983, Lennard 1985).

3. "Upper" Threshold Spike Inactivation of CG. Under conditions where CG is not expressing regenerative properties, the relationship between AGR, CG and GM show features perhaps expected of any three spiking neurons coupled in series via strong synaptic excitation (e.g., Fig. 8.20c,d). Current-evoked increase in firing rate of the primary input element (AGR) causes strong activation of the tertiary element (GM) (Fig. 8.23a). This is due to synaptic depolarization and increased spike generation in the secondary element CG (Fig. 8.23b).

How then can such straightforward positive coupling lead to the inverted input/output relationship seen in Fig. 8.23b? When the firing rate of AGR was increased from 20 to 40 Hz, GM hyperpolarized (instead of depolarizing) and became silent. The basis of this paradoxical effect again resides with the intercalated interneuron. As described by Robertson and Moulins (1981c, 1984) (see this Chap.), CG has the intrinsic ability to suddenly "shut-off" impulse generation once the cell's membrane potential has become depolarized above a certain critical value (ca. -30mV). The result of this "upward" inactivation is to abruptly switch the neuron from an excited, high-frequency spiking state into total silence, the cell remaining in the nonspiking state despite further depolarization (Fig. 8.23d). The effect on follower GM is a sudden removal of synaptic excitation and repolarization of the cell, which otherwise was maintained relatively depolarized by the high rate of CG firing (Fig. 8.23b,d). Reactiva-
Fig. 8.23a–d. Depolarized spike inactivation of CG and its effects on sensorimotor feedback. Similar results from two different experiments without (a,b) and with (c,d) CG involvement; a: Typical positive feedback effects on GM motoneurons at moderate levels (20, 30 Hz) of AGR activity; b: At higher AGR discharge levels (40, 50 Hz), however, feedback becomes negative with GM now hyperpolarizing and becoming silent. The inversion of the motor response is due to CG; in (b) and (d) AGR has driven CG across its upper threshold for spike inactivation and causes to excite GM. Horizontal bars 1 s; vertical bars 10 mV.

Achieve functional complexity. At first sight, a hard-wired afferent pathway comprising a single mecanoreceptor and two equivalent postsynaptic interneurons seems to represent simplicity in the extreme. Yet the integrative potential of this pathway is considerable, including (1) changes in input/output gain, both in "amplitude" and "time"; (2) encoding of tonic proprioceptive information into a phasic descending drive to gastric motoneurons; (3) switching the sign of sensorimotor reflex effects from strongly positive to functionally negative feedback. That this flexibility appears largely due to special cellular properties of the intervening interneurons is consistent with a second major stomatogastric theme. Namely, the key to a precise understanding of circuit function depends not only on the relationships between component neurons but also on the contribution of mechanisms intrinsic to the elements themselves. The hope is that the pathway described here will furnish such an understanding, eventually providing a working model for the cellular basis of sensorimotor integration in general.

Appendix B: Chronic Effects of De-afferentation on the Stomatogastric Ganglion of Panulirus

S.M. ROYER

Characterization of central inputs to the stomatogastric ganglion (STG) has been the object of much of the recent research on the stomatogastric nervous system. The experiments described here were motivated by a desire to study the significance of central connections in the initiation and maintenance of the rhythmic activity of the STG (Royez 1981). The approach selected was to eliminate these central connections by in vivo transection of the stomatogastric nerve (stn), to follow the subsequent effects on spontaneous STG activity, and to examine the morphological changes in the ganglion resulting from stn transection. At selected intervals after such transection, the de-afferented ganglia were removed and examined electrophysiologically and histologically.

Since only the pyloric rhythm is usually seen in the in vivo isolated STG, electrophysiological studies of chronically de-afferented ganglia were focused on the activity of the cells of the pyloric network. Using in vitro preparations, comparisons were made between the spontaneous pyloric output of isolated operated ganglia and that of controls consisting either of isolated STG preparations or combined preparations (STG attached to more rostral ganglia). In particular, detailed data were collected regarding bursting activity of the PD-AB "pacemaker" cells in all three types of preparation.

The extent to which the operated ganglia might still be subject to influence from extrinsic inputs was assessed both physiologically and anatomically. The stn stump remaining with the STG was electrically stimulated to test for the presence of non degenerated input axons which might affect ganglion activity. Fluorescence histochemistry (Falck-Hillarp technique) was used to discover whether operated ganglia exhibited specific catecholamine fluorescence indicating remnants of input processes containing the neurotransmitter dopamine. Light and electron microscopy were used to
determine the extent of degeneration in the snn stump and STG, and to look for possible persistent extrinsic input processes.

1. Electrophysiological Results. Electrophysiological studies of control-combined and isolated stomatogastric preparations confirmed the observations of others that transection of the snn in vitro reliably results in diminished pyloric rhythmicity. The present experiments indicated that control-isolated STG preparations displayed increased pyloric cycle periods, increased interburst intervals, decreased numbers of PD spikes per burst, and decreased PD spike frequencies when compared with combined controls. Figure 8.24A illustrates a typical pyloric output pattern obtained in vitro for (1) a combined control stomatogastric preparation and (2) the same preparation 20 min after isolation of the STG by transection of the snn.

Rhythmic pyloric activity was present in 15 out of 18 chronically de-afferented stomatogastric preparations examined from 3 to 243 days after snn transection in vivo. There was a roughly linear relationship between cycle period and the duration of the de-afferation. The operated preparations differed significantly from combined controls in cycle period, interburst interval, PD burst duration, and PD spike frequency, but not in number of PD spikes per burst. The operated preparations differed significantly from isolated controls in interburst interval, number of PD spikes per burst, and PD spike frequency, but not in cycle period or PD burst duration. The increase in cycle period of both isolated controls and operated preparations when compared with isolated controls was accounted for more by an increase in interburst interval than an increase in the PD burst duration. Chronically de-afferented preparations and isolated controls were found to display more cycle-to-cycle variability than combined controls, particularly in cycle period, PD burst duration, and number of PD spikes per burst.

Figures 8.24B-E illustrate the spontaneous rhythmic activity of chronically de-afferented stomatogastric ganglia examined in vitro 30, 116, 190, and 243 days after snn transection. Overall, the spontaneous activity of operated preparations tended to be more vigorous than that of isolated controls, with higher numbers of PD spikes per burst, and PD spike frequencies and more units participating in the rhythm in addition to the PDs. LP was participating in pyloric cycling in 5 out of 15 chronically de-afferented preparations, compared with 1 out of 17 isolated controls. In general, VDN bursting was also more vigorous in chronically de-afferented preparations than in isolated controls, both in number of spikes per burst and spike frequency.

Stimulation of the snn stumps of operated preparations was effective in altering ganglion output in 13 out of 14 cases; in 11 of these 13, snn stimulation enhanced pyloric rhythmicity. The effects of stimulation were usually very similar to the effects of snn stimulation of isolated controls and were not mimicked by stimulation of the anterior lateral nerve (aln) or dorsal ventricular nerve (dvn). These results suggested that functional input processes persisted in the snn stump and STG for very long times (up to 243 days) after snn transection. Figure 8.24F illustrates the effect of snn stimulation on a stomatogastric ganglion removed from the animal 243 days after snn transection.

2. Fluorescence Histochemistry. Ten stomatogastric preparations (three control and seven chronically de-afferented for 15 to 199 days) were examined for specific cate-
the STG may have contributed to the maintenance of pyloric rhythmicity in chronically de-afferented preparations and the efficacy of STN stimulation in many of these preparations.

3. Morphological Observations. Examination of sections of the STN (stump and central segment), STG, and DUN of operated stomatogastric preparations from lobsters sacrificed 16 to 243 days after STN transection revealed degenerative alterations in the nerves and ganglia. With increasing time after operation, a great number of axons in the STN stump underwent degeneration. The glial sheaths of the axons were hypertrophied and lost their concentric arrangement, and many of the enwrapped axons were separated by enlarged extracellular spaces containing a crystalline matrix. Degenerative alterations in the neuropil of the STG appeared more slowly than in the STN stump, but by 243 days after STN transection the STG had also become greatly abnormal. In some areas of the neuropil as many as half of the neuronal processes were degenerating, and the density of synapses in the neuropil seemed to be reduced. Although many axons in the STN stump of the 243-day operated preparation had obviously degenerated, the nerve still contained several dozen intact axonal profiles. It is known that less than ten neurons of the STG have axons in the STN (see Chap. 1), and this appears to be confirmed by the fact that only a few degenerating axons were observed in the central segment of the STN. This suggests that a large number of non-degenerating axons in the STN stump belong to extrinsic fibers projecting to the STG.

Finally, special attention was devoted to three groups of axons believed to arise from somata extrinsic to the STG, including Type I and Type II dense core vesicle (DCV)-containing axons and a group of very small axons (the “fine fiber bundle”) found in the STN, STG, and DUN (Friend 1977). Type I DCV-containing axons and terminals. In control preparations, axons and synaptic terminals containing Type I DCVs are found in the STN and STG (Friend 1976). Throughout the series of preparations examined, the proportion of Type I DCV-containing processes showing degenerative features increased with time after operation. At all times up to and including 243 days after STN transection, a few intact Type I DCV-containing axons could be found in the STN stump, and synaptic terminals containing these DCVs were numerous in the STG neuropil. This observation, when compared with the relatively greater reduction of specific catecholamine fluorescence in the operated preparations, suggests that not all of the Type I DCV-containing processes in the STN and STG contain dopamine (if, indeed, any of them do). However, this indicates again that some extrinsic fibers still have not degenerated in the STG 243 days after STN transection (Fig. 8.26).

Type II DCV-containing axons. Axons containing Type II DCVs are found in the STN, STG, and DUN and in their surrounding perineurial sheaths in control stomatogastric preparations. Degenerating Type II DCV-containing axons were never observed in any operated preparation. The present study provided no firm basis for a conclusion regarding the location of the somata from which Type II DCV-containing axons originate. The generally normal appearance of such fibers when they were observed in the operated preparations could mean that they were not separated from their somata by transection of the STN, but it is also possible that these axons were long-surviving severed distal processes.
rhythmicity (PD bursting) when examined in vitro from 3 to 243 days after transection in vivo. However, the evidence from coturnix stimulation experiments, histo-fluorescence studies, and light and electron microscopic examination of operated preparations indicated the persistence of distal axon segments and neuropil processes of presumptive extrinsic interneurons in the coturnix stump and STG of these preparations. Such surviving input processes may have contributed to the observed maintenance of pyloric rhythmicity in preparations obtained from long-term operated animals.

Appendix C: Contingent Effects of Synaptic Input to the Pyloric Oscillator
J. AYERS and P.D. KUSHNER

A general model of central motor pattern generators, based on neuronal oscillators, has been proposed for the organization of rhythmic motor systems in both invertebrates and vertebrates (Davis 1973, Stein 1978). The stomatogastric nervous system serves as a model for neuronal oscillators (Selverston 1980). Neuronal oscillators are modulated by a multiplicity of controlling inputs during behavior including: humoral and command inputs (Pinsker and Ayers 1983, Kupfermann and Weiss 1978) and phase-modulating inputs such as coordinating system (Stein 1978). Furthermore, sensory inputs generate reflexes which may be further subdivided into phase or amplitude modulating depending on whether they control the timing or amplitude of the oscillation (Evoy and Ayers 1982).

Animals commonly utilize the same neuronal pools to produce different, but closely related behaviors. A familiar example is the control of walking, running and swimming of the mammalian hindlimb. Potential explanations for this phenomenon include: activation of different central pattern generators which impinge on the same motoneuron pools (D.M. Wilson 1962), modulation of the membrane properties of different neurons in the circuit (Russell and Hartline 1977), and modulation of synaptic connectivity within a single circuit (Ayers and Davis 1977; see Chap. 9). We have demonstrated a fourth mechanism, namely modulation by the pattern of discharge of controlling inputs. In other words, the function of the input depends on the temporal pattern in which it discharges.

Several attempts have been made to describe functions analogous to those of the proposed general model to stomatogastric elements. For example, the innervated fibers (invTF) have been classified as command neurons (Dando and Selverston 1972), can exhibit a coordinating function (Moulins and Vede 1977, Ayers and Selverston 1977, 1979, 1984), and participate in a sensory reflex (Dando et al. 1974). Clearly, the above classification scheme is inadequate to delineate the function of the invTFs.

Oscillator theory offers an alternative classification scheme which summarizes the most important forms of control of neuronal oscillators (Pinsker and Ayers 1983). According to this scheme, controlling inputs can operate: (1) as parameters to control the state and average frequency of the oscillation, (2) as perturbations to control the timing on a cycle-by-cycle basis, and (3) by superposition to control the amplitude of discharge of particular motoneuron pools. There are some similarities to the
above classification scheme. For example, command systems generally function as parameters, while coordinating systems operate by perturbations. It is proposed here that the intrinsically (TF) input to the stomatogastric system can exhibit all three forms of control contingent on modulators and the pattern of discharge, and that this capability allows them to produce metastable coordination and phase shifting of the pyloric oscillation.

**Parametric Modulation.** Parameters are those inputs which turn on and control the overall frequency of a neuronal oscillator. Parameters control the states (on or off) of the oscillator as well as its average frequency and amplitude. Examples of parameters include temperature and neuromodulators. In the pyloric oscillator, current injected into pacemaker neurons is a strong parameter of the oscillation (Ayres and Selverston 1979). Inputs from the COGs, which can be reversibly blocked by suxamethonium, such as dopamine, can also turn on and off the pyloric oscillation (see Chaps. 5 and 9).

We have recently found evidence that the intrinsically (TF) input may also function as a much stronger parameter than previously described (Russell and Hartline 1981) and that the effect of intrinsically (TF) input is profoundly enhanced in the presence of octopamine. To demonstrate this effect, we utilized combined preparations of the STG, OG and COG with a vaseline channel passing over the soma and ions so that inputs from the commissural ganglia could be reversibly suppressed. In preparations with the soma blocked with a sucrose gap, the intrinsically (TF) inputs had little effects except for generating action potentials in the OG (Fig. 8.27A). When octopamine was added to the saline, the intrinsically (TF) inputs strongly activated the rhythm, even though the octopamine unmasked the activating effects of the intrinsically (TF) cells causing them to have a strong parametric effect.

**A**

![Fig. 8.27A, B. Parametric effects of the intrinsically (TF) input are contingent on the modulatory effects of octopamine. A: A simultaneous recording from A8 and VD in a preparation where the ion and son input nerves were blocked by a sucrose gap. Note that the A8 oscillation is nearly damped. A train of intrinsically (TF) stimuli (30 Hz) is delivered at the bar; B: The bath was infused with salme containing 10^{-6} M octopamine and intrinsically (TF) stimulation has now a strong effect on A8 oscillations. Time mark = 1 sec](image)

**B**

![Extrinsic Inputs (Appendix C)](image)

**Perturbation and Entrainment.** A neuronal oscillator may be perturbed in its timing by many sources of input. Such perturbations alter the timing of the oscillation to reset it to a new phase relative to the original timing (Finsker and Ayers 1983). The perturbing effects of an input to a neuronal oscillator can be formalized by phase response curves which summarize the resetting effects in terms of the phase at which the stimulus occurs in the endogenous oscillations. If perturbing inputs occur repetitively at frequencies near that of the endogenous oscillation, they can entrain the endogenous oscillation to the oscillating stimulus to produce weak (relative) or strong (absolute) entrainment or coordination (von Holst 1939). Both the pyloric and gastric oscillations can be entrained by intrinsically (TF) stimuli which repeat at frequencies which are faster or slower than the endogenous oscillations (Ayers and Selverston 1979, 1984; see also Chap. 8).

**Superposition.** Motoneurones, functioning as the final common pathway, may integrate inputs from one or several central pattern generators. In other words, if a moto-
neuron receives inputs from two or more pattern generators, it may be amplitude modulated by both to exhibit components of both oscillations. Examples of such superposition in the pyloric oscillation are shown in Fig. 8.28. The normal spontaneous pattern of inn-TF discharge consists of 1–3 s bursts which can be monitored in intracellular or extracellular VD recordings, since the synapse between inn-TF and VD is direct. The effect of a spontaneous inn-TF burst is shown in Fig. 8.28A. The inn-TF burst causes a long high-frequency burst of VD impulses and a concomitant suppression of activity in PD.

In favorable preparations, brief trains of inn-TF impulses can be evoked by extracellular stimulation of the inn (Figs. 8.28B–D). The responses in PD and VD depend strongly on the patterning of the stimulus. When short trains are delivered which approximate the frequency and duration of the PD bursts, the stimulus can entrain the PD rhythm as well as shift the phase of VD relative to PD such that PD now discharges synchronously with PD. If the spike frequency within the train and the train duration are both increased, both PD and VD burst, but their bursts become asynchronous (Fig. 8.28C). When the inn-TFs are stimulated tonically, PD will continue to burst at a slightly elevated frequency and VD discharges tonically, following the stimulus for the most part 1:1 (Fig. 8.28D).

Of particular interest is the example shown in Fig. 8.28B, where the stimulus shifts the timing of VD discharge from out-of-phase to in-phase with PD. This effect not only requires VD to discharge when it normally would be inhibited, but also causes its activity to be suppressed when it would normally be active. Three processes appear necessary for the phase shifting of VD to occur. First, the pyloric rhythm must become synchronous with the cyclic inn-TF stimulus. This synchrony results from the previously documented ability of the inn-TF to entrain the PD-AB endogenous rhythm (Ayers and Selverston 1977, 1979). The second process involves production of a burst of VD action potentials synchronous with that in the PD-AB network. The synapse between inn-TF and VD is so strong that VD impulses generally follow the stimulus on 1:1 basis (Fig. 8.27A). If the inn-TF stimulus train entrains the PD rhythm, then the inn-TF-evoked burst of VD impulses will be synchronous with the PD burst. In other words, the inn-TF pattern is superimposed on the normal VD pattern.

The stimulus parameters which appears to be the most important in determining whether the normal burst in VD is suppressed is the inn-TF instantaneous frequency within the train. In the experiment of Fig. 8.29, the stimulation frequency within the train is gradually increased while all other stimulus parameters remain constant. At 20 Hz, the VD cell continues to burst in the inter-PD interval, but as the stimulation frequency is increased, VD becomes silenced during this interval when it would nor-

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Fig. 8.29. Phase shifting depends upon the impulse frequency within repetitive stimulus trains with interburst intervals constant. All three panels are simultaneous intracellular recordings from the pyloric dilator motoneuron (PD), the lateral pyloric motoneuron (LP) and the ventricular dilator motoneuron (VD). Repetitive stimulus trains to the inn are delivered at a frequency slightly higher than the endogenous frequency, and in the three panels the impulse frequency within the trains is increased.
mally discharge. This suppression of the normal VD bursts appears to result primarily from the parametric effects of the ivn-TF input. Notice that the absolute level of depolarization and hyperpolarization, the overall amplitude of the oscillation increases, presumably due to stimulus increased levels of endogenous currents (Pinsker and Ayers 1983). The superposition provided by ivn-TF can clearly override this hyperpolarization to generate the in-phase VD burst.

What is the Function of the ivnTFs? The effect of the ivnTF is contingent on both humoral modulation as well as on the temporal pattern in which they discharge. In fact, we have been able to demonstrate for PD-VD patterns ranging from synchronous to asynchronous bursts as well as tonic discharge. In its overall temporal organization, the pyloric motor pattern is similar to that observed in the decapod walking systems (Evoy and Ayers 1982). The program is divided into a constant duration phase (PD burst) and a variable duration phase (LP-PY burst). In animals which locomote with jointed appendages, changes in the direction of walking are produced by phase shifts of the motor discharge to different leg joints (Ayers and Davis 1977, Ayers and Clarac 1978; analogous to the phase shifts relative to PD and LP induced in VD by patterned ivnTF input.

The induction of phase shifting and metastable coordination is merely an elaboration of the cellular processes necessary for entrainment of the oscillator, a process which has typically been thought of as being in the domain of coordinating systems (Stein 1976, Davis 1976, Pinsker and Ayers 1983). If one accepts that command systems might discharge in different patterns, a switch from one pattern of motor output (here, VD out of phase with PD) to another (VD in phase with PD) can therefore be achieved by changing the pattern of discharge of one command input rather than requiring a separate command channel. A corollary of this notion is that the distinction between command neurons and coordinating neurons may depend only on their normal pattern of use.

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9 Neurotransmitters and Neuromodulators
E. MARDER

9.1 Introduction
It has been long appreciated that a large number of different substances likely to be neurotransmitters and neuromodulators are present in all nervous systems, from the simplest to the most complex. One of the fundamental questions that has puzzled neurobiologists through the decades is why there are so many different substances used as signaling molecules.

The stomatogastric nervous system provides a unique opportunity for the formulation and testing of hypotheses concerning neurotransmitter organization in nervous systems. Because of the relatively small number of individually identifiable neurons, it is possible to attempt to identify all the chemical mediators within the stomatogastric nervous system, as well as to characterize in detail their mechanisms of action. The overall aim of this chapter is dual: (1) to present what is known about the neurotransmitters and neuromodulators in the stomatogastric nervous system and (2) to highlight some ideas that have emerged from studying neurotransmitter actions in this preparation that may be difficult or impossible to achieve in other nervous systems. Future work that builds on what is currently known should provide further insights into the functional relevance of neurotransmitter diversity within neural circuits.

9.2 Identification of Neurotransmitters Used by STG Neurons
Most of the neurons of the STG are motoneurons, and make excitatory neuromuscular connections. Because neuromuscular junctions are easily studied, historically attempts to identify the neurotransmitters released by neurons of the STG took advantage of the easy experimental access the neuromuscular junctions provided. Underlying these experiments was the premise that a neuron would release the same neurotransmitter(s) at all of its terminals. Therefore, the overall strategy was to identify a neurotransmitter candidate for each of the STG motoneurons, on the basis of their excitatory neuromuscular properties. Subsequently, the strategy was to attempt to demonstrate that the same substance was mediating the central, inhibitory, synaptic connections made by the STG neurons in the neuropil of the STG by analyzing the pharmacological properties of the STG synaptic connections.