1 Overview of the Stomatogastric Nervous System

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The stomatogastric nervous system (STNS) is the set of neural elements that generates and coordinates the motor patterns producing the different, but interrelated, movements of the crustacean foregut. The crustacean foregut has attracted the attention of biologists since at least the time of Aristotle. He described what is thought to be the gastric mill of a crayfish in his *Historia Animalium* (Felgenhauer and Abele, 1989). Since then, many distinguished biologists, including Cuvier, Milne-Edwards, Huxley, and Mocquard, have been interested in the crustacean foregut (reviewed by Felgenhauer and Abele, 1989).

The neural components of the STNS were intensively studied around the turn of this century in the classic methylene blue studies of Allen and Orlov (reviewed by Bullock and Horridge, 1965). These studies indicated even then that the STNS could be a model neurobiological system. For example, a methylene blue study of the crayfish stomatogastric ganglion (STG) by Sigmund Freud provided important results that we now take for granted and that supported the development of the neuron doctrine: he found that nerve fibers are processes extending from ganglionic cell bodies (Shepherd, 1991). This work was done, of course, before Freud chose another model system more appropriate to his level of analysis.

Modern interest in the STNS as a model neurobiological system dates from Maynard (1966). He proposed that analysis of crustacean stomach behaviors and the neural mechanisms controlling them could provide general insights about how rhythmic motor patterns for locomotion are produced. It may at first seem odd that the study of movements of an internal intestinal tract can shed light on external locomotory actions. However, like external crustacean appendages, the foregut is derived from ectoderm, chitinized and operated by striated muscle. The crustacean foregut thus has developmental, anatomical, and functional similarities to external locomotory appendages (Selverston, 1974). Indeed, the basic mechanisms underlying the foregut motor patterns may be applicable for understanding not only crustacean locomotion, but also other rhythmic movements such as vertebrate locomotory (Kiehn, 1991) and respiratory (Feldman et al., 1990) motor patterns.

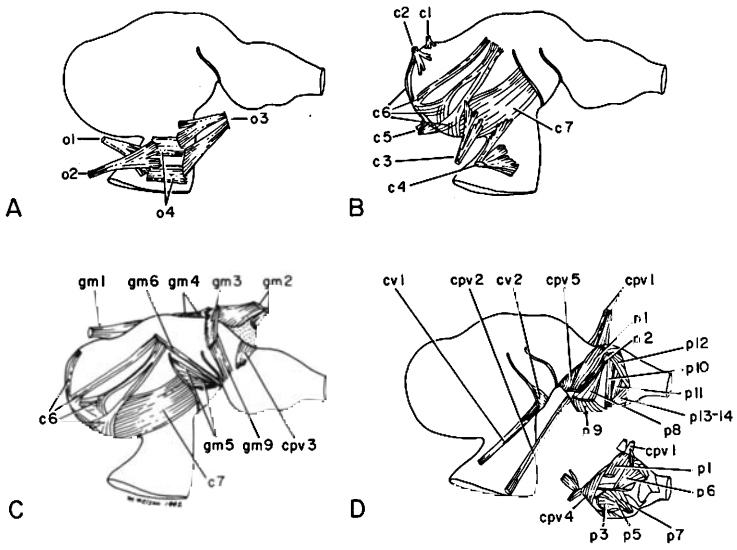

Figure 1.1 Diagrammatic view of the lobster stomach and the major muscles important for movements of the esophagus (A), cardiac sac (B), gastric mill (C), and pylorus (D). Inset in (D) shows the inner layer of muscles moving the pyloric filter. Adapted from Moulins and Vedel (1977), Maynard and Dando (1974), and Dickinson and Marder (1989).

In this chapter, we describe the general anatomy, function, and motor patterns of the crustacean foregut, the neurons and the neural circuits underlying the production of the STNS motor patterns, and the cellular and synaptic properties of STNS neurons and muscles that are important for rhythmic motor pattern generation. We will focus on the anatomy and physiology of reptantian crustacea (lobsters and crabs) because the STNS is best understood in these animals. Our goal is to present a general framework of knowledge to prepare the reader for more detailed discussions of the STNS in the other chapters of this book.

FOREGUT ANATOMY AND FUNCTION

The foregut, midgut, and hindgut compose the crustacean intestinal tract. The foregut has two main divisions: the first, a short muscular tube, the esophagus, leads to the second, the stomach chamber. The stomach chamber has three distinct regions: cardiac sac, gastric mill, and pylorus (figure 1.1). The foregut is suspended from the thoracic wall by bilaterally paired extrinsic muscles (those that attach to the thoracic wall and the stomach). These muscles usually operate antagonistically to intrinsic muscles, which have both their origins and insertions on the stomach wall. Hard, chitinized ossicles, interconnected by ligaments, line the last two areas of the foregut, the gastric mill and pylorus (Maynard and Dando, 1974). These ossicles have the following functions: (1) flexible support for the stomach wall, (2) muscle attach-

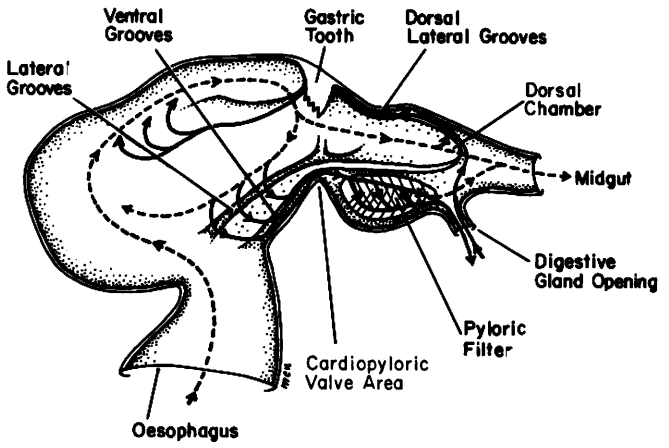

Figure 1.2 Diagrammatic scheme of food and fluid movements in the crustacean foregut. Dotted lines, movement of food; solid lines, movement of fluid. Modified from Dall and Moriarty (1983).

ment points, (3) a complex system of levers, joints, and fulcrums against which the muscles operate, (4) teeth-like structures (gastric mill) for cutting and grinding food, and (5) attachment points for chitinous combs in the pylorus used to filter food particles (Claiborne and Ayers, 1987).

Movements of the foregut are responsible for specific components of crustacean feeding behavior that include swallowing, chewing, and internal sorting of food particles for further chewing, assimilation, or processing as waste material. These movements result not only in the progressive digestion of food and its movement through the digestive tract, but also in circulation of digestive fluid forward from the digestive gland duct openings to the cardiac sac and then back to the digestive gland duct (figure 1.2; Dall and Moriarty, 1983).

Descriptions of the foregut motor patterns have focused on the rhythmic movements of the different foregut areas: the esophagus, cardiac sac, gastric mill, and pylorus. Rhythmic activity of the external mouthparts is also involved in feeding behavior and may be integrated with activity of the other foregut rhythms. However, since rhythmic mouthpart activity has received only limited attention and is probably generated by circuits extrinsic to the STNS (Macmillan et al., 1976; Wales et al., 1976a,b; Robertson and Laverack, 1979a), it will not be considered further here.

Esophagus

A particle of food gathered by one of the clawed appendages of a crustacean is passed to the mandibles and maxillipeds (external mouth parts) to be ripped into strips and chunks. These are then passed to the maxillae, which push them into the ventrally oriented mouth and

on to the esophagus. A peristaltic wave of muscle contractions traveling down the esophagus moves food dorsally into the cardiac sac of the stomach. Numerous gland openings into the esophagus are thought to add a lubricant that facilitates ingestion (Barker and Gibson, 1977). Near the entrance to the cardiac sac, dorsal and lateral invaginations of the posterior esophageal walls form a one-way valve, the esophageal-cardiac sac (OCS) valve, which prevents food from reversing its direction once it has entered the cardiac sac (Conklin, 1980).

The peristaltic wave moving food through the esophagus is brought about by alternating contractions of dorsal and ventral extrinsic muscles (figure 1.1A, o1–o3) that dilate the esophagus, and dorsoventral intrinsic muscle fibers (figure 1.1A, o4) that constrict the esophagus. Recordings from nerves containing the axons innervating the esophageal muscles show that the esophageal motor pattern has a relatively slow period of 5 to 10 sec (Moulins and Vedel, 1977; Robertson and Laverack, 1979b; Moulins and Nagy, 1981a; figure 1.3A).

Cardiac Sac

The cardiac sac region of the stomach functions as a storage area for food waiting to be macerated by the gastric mill, and as a mixing area for food and gastric juice from the digestive gland. Gastric juice is pumped out of the digestive gland openings and travels forward to the cardiac sac in dorsolateral grooves that run along the dorsal chamber of the pylorus (figure 1.2). The cardiac sac is expanded to accommodate food by the contraction of six pairs of cardiac sac dilator muscles (figure 1.1B, c1–c5) and the cardiopyloric valve muscle cv4 (Maynard and Dando 1974; Moulins and Vedel, 1977). Food is then sent posteriorally to the gastric mill for mastication by constrictions of the cardiac sac. This constriction can be passive, or induced by contraction of intrinsic muscle fibers (c6 and c7, figure 1.1B; Maynard and Dando, 1974; Moulins and Vedel, 1977; Dickinson and Marder, 1989). The cardiac sac motor pattern is more irregular than the esophageal motor pattern, having periods ranging from tens of seconds to minutes (figure 1.3B; Moulins and Vedel, 1977; Vedel and Moulins, 1977; Dickinson and Marder, 1989).

Gastric Mill

Reptantian crustaceans do not chew their food extensively with the external mouthparts, but instead leave this processing to the gastric mill. The gastric mill (named by Huxley, 1880) lies in the dorsal posterior region of the stomach (figure 1.1) and is composed of three specialized ossicles, called teeth. A lateral pair generally functions to grasp food strips, and a medial tooth rasps the secured strips (figure 1.4). Early cinematic analysis of gastric mill operation in semiintact lobsters indicated that the two lateral teeth opened and closed rhythmically with a period of about 3 sec, while the medial tooth rhythmically ground back

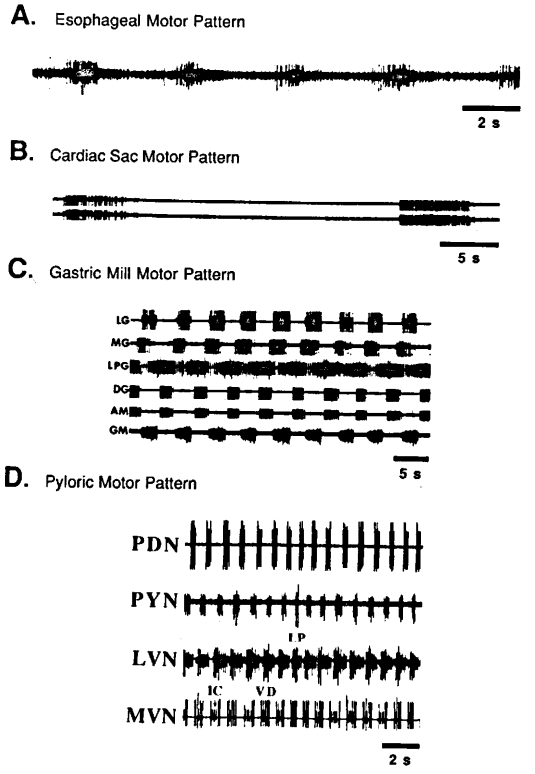

Figure 1.3 Examples of motor patterns produced by the stomatogastric nervous system. (A) Esophageal dilator motor neuron activity recorded from the *son* (from Moulins and Vedel, 1977). (B) Cardiac sac dilator motor neuron activity recorded simulataneously from the *dpon* (above) and *stn* (below) (from Moulins and Vedel, 1977). (C) Simultaneous activity of the gastric mill motor neurons recorded from the appropriate motor nerves (from Selverston, 1987b). (D) Simultaneous activity of the pyloric motor neurons (Johnson, Peck and Harris-Warrick, unpublished data). PD neuron spikes on the *pdn* trace, PY neuron spikes on the *pyn* trace, LP neuron the largest spike on the *lvn* trace, and IC (small spike) and VD (large spike) neurons on the *mvn* trace. Note the different time scales for the different motor patterns.

and forth (Hartline and Maynard, 1975); the medial tooth chewed the food clasped between the lateral teeth. Recent views of gastric mill operation in intact lobsters using endoscopic cameras extend these earlier observations by documenting two fundamentally different modes of spontaneous gastric movements (Selverston, 1989, see chapter 6). The first is the "squeeze" mode. In this mode, the lateral teeth are initially spread apart and the medial tooth is held in a dorsocaudal position. The three teeth simultaneously converge to squeeze food held between them and then move apart again. In the second mode of operation, the "cut and grind" mode, the lateral teeth open slightly from the rest position before they close simultaneously. The medial tooth moves forward with a slight delay, and as it moves, the serrated edges of the lateral teeth grind posteriorally along the file of the medial

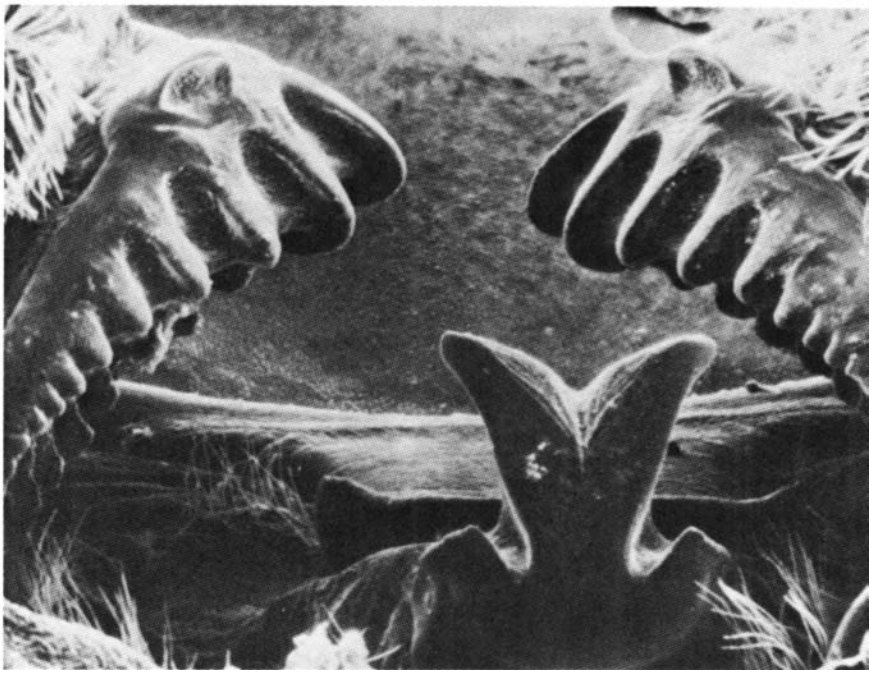

Figure 1.4 SEM micrograph of the lateral and medial teeth of the crayfish gastric mill. From Schramm (1986).

tooth. The initial closing of the lateral teeth is thought to effect cutting, while the movement of the medial tooth effects grinding (Selverston, 1989). Other movements are also seen (chapter 6). For example, the lateral and medial teeth can move independently of one other. The lateral teeth can make cutting movements without medial tooth motion and the medial tooth can make full forward and backward movements while the lateral teeth remain at rest (Selverston, 1989).

The muscles that move the teeth are shown in figure 1.1C and the motor neurons innervating these muscles are listed in table 1.1. Contraction of part of the extrinsic muscle group gm3 (innervated by the LPG neuron) pulls the lateral teeth apart, and contractions of the intrinsic gm5, gm6 (innervated by the LG neuron), and cardiopyloric valve cpv3 (innervated by the MG neuron) muscles close the lateral teeth. The medial tooth is moved forward by contraction of the extrinsic muscles gm1, and gm2 and part of the gm3 group (all of these are innervated by the GM neuron), and is pulled backward or retracted by contraction of the intrinsic muscles gm4 (innervated by the DG neuron) and c6 and c7 (both innervated by the AM neuron). The activity of the gastric motor neurons during the "cut and grind" motor pattern is shown in figure 1.3C; this pattern has a period of 5–10 sec (Hartline and Maynard, 1975). Continual maceration and mixing of food with gastric fluid in the gastric mill produces a suspension of fine food

particles that travels in the ventral grooves of the cardiac sac toward the pylorus (figure 1.2).

Pylorus

The pylorus has two distinct areas, a dorsal chamber that leads to the midgut and a ventral region, the pyloric filter (ampulla), that leads to ducts entering the digestive gland (figure 1.2). It is the most complicated region of the foregut and its functions are not well understood. It appears to act as a press and filter apparatus for sorting food particles (van Weel, 1970; Schaefer, 1970) and as a pump to help circulate digestive fluid (Dall and Moriarty, 1983). Food enters the pylorus through two main routes, the dorsal cardiopyloric valve and the ventrolateral grooves (figure 1.2). Food particles too coarse to enter the ventrolateral grooves pass through the cardiopyloric valve and into the midgut. Fine particles and liquid, produced by the mixing of the gastric fluid with food macerated by the gastric mill, enter the ventrolateral grooves in the cardiac sac and proceed to the end of the grooves, which open into the pyloric filter. Here, overlapping rows of hairs mat together to form a filter press whose pumping action strains and divides food particles on the basis of size. This allows only the smallest particles (less than 1 μm) to enter the digestive gland for final digestion and absorption. Larger particles may be directed back to the gastric mill for further chewing. Intermediate size particles go through the pyloric valve into the midgut, where, along with debris from the digestive gland, they are compacted into fecal strands in the hindgut. The pyloric apparatus differs little from species to species (Schram, 1986; Felgenhauer and Abele, 1989), perhaps because it has a filtering function independent of the original food ingested (Schaefer, 1970).

The muscles important for pyloric movements are shown in figure 1.1D and the motor neurons innervating them are listed in table 1.1. The cv1 (innervated by the VD neuron) and cv2 (innervated by the IC neuron) muscles govern the position of ossicles whose movement appears to open and close the ventrolateral grooves of the cardiac sac (Maynard and Dando, 1974). Thus, these muscles may control the movement of liquified food into the pylorus. Contractions of the cardiopyloric valve muscles (cpv1 and cpv2, innervated by the PD neurons) open the cardiopyloric valve to allow food to enter the dorsal chamber of the pylorus. The valve action, however, may be more complicated than this, with gastric mill and cardiac sac muscles participating in opening the valve, clearing the gastric mill, and moving food into the anterior pylorus (Claiborne and Ayers, 1987). The food in the pyloric press and filter apparatus is processed by contractions of the remaining pyloric constrictor muscles (the LP muscle pair cpv4 and cpv5 and the PY muscle group p2–14). It is not understood how the contractions of these muscles result in the sorting of food particles.

Recordings from the pyloric muscles in intact lobsters show a three-

Table 1.1 Identified Neurons of the Stomatogastric Nervous System[a,b]

Neuron Soma Location		Axon Location	Projection	Function
Commissural ganglion				
Large cell	L	Circumesophageal connective	**Brain, subesophageal ganglia**	?
Pyloric	P	*ion, stn*	**STG**	Pyloric oscillator
Commissural pyloric	CP	*son, stn*	**STG**	Pyloric oscillator
Excitatory	E	*son, stn*	**STG**	Gastric oscillator
Commissural gastric	CG	*son, stn*	**STG**	Gastric oscillator
Follower neurons	F (n?)	*ion, ivn*	**Brain**	?
GABA neurons	GN5, GN6	*ion, stn*	**STG**	Pyloric modulation
Esophageal ganglion				
Anterior pyloric modulator	APM	*ion, son, stn*	CG, STG	Gastric and pyloric modulation
Modulatory proctolin neuron	MPN (2)	*stn*	STG	Pyloric modulation
Esophageal dilator 1	OD1	*on, son*	o1, o2, o3	Esophageal dilation
Cardiac dilator 1	CD1	*on, son, dpon, stn, aln*	c1, c2, c3, c5	Cardiac sac dilation
GABA neurons	GN1, GN2	*stn*	STG	Pyloric and gastric modulation
GABA neurons	GN3, GN4	*ion, son, stn*	STG	?, But probably modulatory
Stomatogastric ganglion				
Cardiac dilator 2	CD2	*stn, son, dpon*	c4, c5, cv1	Cardiac sac dilation
Gastric network				
Anterior median	AM	*amn*	c6, c7	Cardiac sac contraction, medial tooth retraction

Lateral gastric	LG	*lvn, lgn*	gm5, gm6	Close lateral teeth
Median gastric	MG	*lvn, gpn*	gm9, cpv3	Close lateral teeth
Laterial posterior gastric	LPG (2)	*lvn, lpgn*	gm3	Open lateral teeth
Gastric mill	GM (4)	*aln, lvn, dlvn*	gm1, gm2, gm3	Medial tooth protraction
Dorsal gastric	DG	*dgn*	gm4	Medial tooth retraction
Interneuron 1	Int1	*stn, son*	CoG	Rhythm organization, feedback to CoG neurons
Pyloric network				
Anterior burster	AB	*stn, son*	CoG	Pyloric pacemaker, feedback to CoG neurons
Pyloric dilator	PD (2)	*lvn, pdn, ddn*	cpv1, cpv2	Part of pacemaker group, control of cardiopyloric valve
Ventricular dilator	VD	*lvn, mvn*	cv1	Control of ventral stomach grooves leading to pyloric filter
Inferior cardiac	IC	*lvn, mvn*	cv2	Control of ventral stomach grooves leading to pyloric filter
Lateral pyloric	LP	*lvn, lpn*	p1, cpv4, cpv5	Pyloric filter movements
Pyloric	PY (8)	*lvn, pyn*	p2–p14	Pyloric filter movements
Brain-ivn *nerve*				
ivn-through fiber	*ivn*-TF (2)	*ivn, stn*	Pyloric neurons	Interrhythm coordination, components of cardiac sac network
Pyloric suppressor	PS (2)	*ivn, stn*	Pyloric neurons	Modulate pyloric rhythm organize "swallowing" network
Sensory cells				
Anterior gastric receptor	AGR	*stn, ion*	CoG	Modulate gastric rhythm
Gastropyloric receptor	GPR	*lvn, stn, son*	STG, CoG	Modulate gastric and pyloric rhythms

[a] Updated and modified from Moulins and Vedel (1977) and Claiborne and Ayers (1987). See text for details.
[b] All neurons have not been found in all decapod crustaceans studied and the number within a neuron type may vary from species to species.

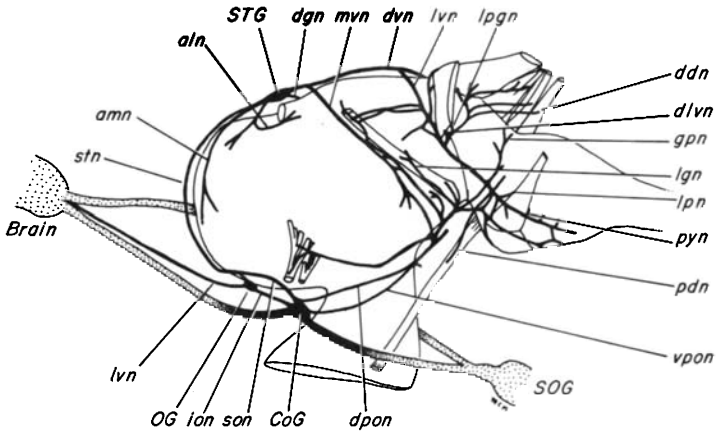

Figure 1.5 Diagrammatic view of the STNS indicating its ganglia and major nerves. Modified from Moulins and Vedel (1977) and Maynard and Dando (1974).

phase activity pattern with a frequency much faster that of the other foregut motor patterns (Hartline and Maynard, 1975; Rezer and Moulins, 1983, figure 1.3D). Activity starts in muscles that dilate the pyloric chamber (PD muscle group cpv1 and cpv2), is followed by activity in an initial group of pyloric constrictor muscles (LP muscles p1, cpv4 and cpv5), and then finishes with activity in a second group of pyloric constrictor muscles (PY muscles p2–p14). This pattern repeats with a period ranging from 0.5 to 5 sec. In freely behaving animals, two distinct patterns of pyloric activity are seen (Rezer and Moulins, 1983). The first is a weak and irregular triphasic pattern with an average period of about 3 sec. The second is observed during feeding and is much stronger and more regular, with a period of about 1 sec. The two patterns also differ in the burst durations of the constrictors and the latency of firing of the constrictors in the cycle (Rezer and Moulins, 1983).

STNS ANATOMY

The STNS consists of the paired commissural ganglia (CoGs), the esophageal ganglion (OG), the stomatogastric ganglion (STG), and the nerves connecting these ganglia (Bullock and Horridge, 1965; figure 1.5). The paired CoGs are located on each side of the esophagus, and connect to the brain and subesophageal ganglion through the circumesophageal commissures. The OG lies on the anterior wall of the esophagus and is connected to the CoGs by the paired superior and inferior esophageal nerves (*son* and *ion*, respectively) and to the brain by the inferior ventricular nerve (*ivn*). The STG is found within the ophthalmic artery, which runs from the heart to the brain over the dorsal surface of the stomach. The STG is connected to the OG via the stomatogastric nerve (*stn*). The *stn* is the only source of central input to the STG, and this

anatomical arrangement is thus extremely advantageous for examining the role of central inputs in initiating and maintaining motor patterns produced within the STG.

When the STNS is removed from the animal, destroying all sensory feedback and influences from higher neural centers, motor patterns can still be recorded that are similar to the in vivo motor patterns. This indicates that neither the sensory input nor outside ganglia (brain) are necessary for motor pattern production by the foregut pattern generators (Selverston et al., 1976; Meyrand and Moulins, 1988a). Thus, much of the neural mechanisms required for the production of the four distinct motor patterns of the foregut resides within the neural networks of the STNS. However, sensory feedback can play instructive roles in organizing foregut motor patterns (Hooper and Moulins, 1989; Katz and Harris-Warrick, 1990c) and probably does so in vivo.

Each motor pattern generator is mostly constituted from separate sets of neurons (although there is some overlap) and these may be distributed in more than one ganglion. Although we describe the four foregut motor patterns and their neural networks as independent of one another, each individual network can be thought of as a component of a larger distributed system. By integrating neural components normally seen as semiindependent motor patterns, this system is capable of combining normally separate motor patterns (Weimann et al., 1991a), switching neurons from one network to another (Hooper and Moulins, 1989; Katz and Harris-Warrick, 1991), and creating alternative motor patterns (Dickinson et al., 1990a; Meyrand et al., 1991; see chapter 4). The STNS neurons that comprise, influence, and create these circuits are identifiable in preparations of the same and different crustacean species. We will briefly describe the individual ganglia of the STNS including the location and function of these identified neurons (table 1.1).

CoGs

The CoGs are important centers for coordination for the foregut motor patterns (Selverston et al., 1976). This is because (1) much of the sensory input from the several regions of the foregut projects to and is integrated in the CoGs, and (2) identified neurons in the CoGs can control and modulate the motor patterns generated in the STG. Only a few of the hundreds of cell bodies within each CoG are presently identified as neurons involved in or whose activity is affected by the foregut motor patterns. These neurons are listed in table 1.1 and discussed in detail in chapter 3. Some apparently do not participate in the foregut rhythms (L and F neurons) but may send efferent copies of the rhythms to the brain (Nagy and Moulins, 1987). CoG neurons that do participate in the foregut rhythms appear to be higher order to neurons generating the gastric and pyloric patterns (Selverston et al., 1976; Robertson and Moulins, 1981a,d, 1984; Cardi et al., 1990). Other identified neurons in

the CoGs show GABA-like immunoreactivity and can modulate pyloric activity (Cournil et al., 1990a).

Portions of the neural circuits generating the esophageal motor pattern and the motor neurons controlling the OCS valve are located in the CoGs but the cell bodies of these neurons have not been identified. Each CoG contains a neural circuit that can generate esophageal contractions; this is different from the other three motor patterns that are generated by single networks that project to both sides of the foregut. The two esophageal networks are coordinated for synchronized function through the esophageal commissures in crayfish (Spirito, 1975) and through the *sons* in the spiny lobster (Selverston et al., 1976).

OG

The OG contains 16–18 cell bodies (Spirito, 1975; Selverston et al., 1976) and is also an important center for foregut movements. This is because it contains the cell bodies of motor neurons innervating muscles of the esophagus and cardiac sac and of neurons that modulate the foregut motor patterns (table 1.1, see also chapter 3, table 3.2). One identified neuron (OD1) innervates esophageal dilator muscles and another (CD1) innervates dilator muscles of the cardiac sac (Moulins and Vedel, 1977). At least seven other neurons have been identified in or near the OGs. These neurons contain a variety of modulatory compounds (chapter 3, table 3.2) and can profoundly influence the gastric and pyloric motor patterns (Nagy and Dickinson, 1983; Nagy et al., 1988a; Dickinson et al., 1988; Nusbaum and Marder, 1989a,b; Cournil et al., 1990a; Cazalets et al., 1990a).

STG

The STG is the most studied ganglion of the STNS because it contains the pattern generating networks for the gastric mill and pyloric movements. It has approximately 30 cell bodies, most of which are motor neurons that participate in either the gastric or pyloric motor patterns.

Eleven of the neurons in the STG are components of the central pattern generator for the gastric mill (table 1.1). Interneuron 1 (Int1) plays a pivitol role in coordinating the activity of the other gastric neurons and sends an inhibitory efference copy of the rhythm to higher order cells in the CoGs via the *stn*. The other 10 cells are motor neurons that effect the teeth movements as described above and in table 1.1. Under the appropriate modulatory conditions, the AM neuron can also participate in the cardiac sac rhythm and cause active constrictions of the cardiac sac (Dickinson and Marder, 1989).

Most of the remaining neurons (14) in the STG are part of the pyloric motor network and can be divided into six major neuron types (table 1.1). The AB interneuron is a conditional burster neuron that determines the pyloric rhythm frequency (Miller and Selverston, 1982a; Bal et al., 1988). Like Int1, the AB neuron sends inhibitory feedback out the *stn*

to higher order neurons in the CoGs. The STG also contains the cell body of a cardiac sac dilatory motor neuron (CD2). This neuron projects its axon down the *stn* and out the *dpon* (figure 1.5) to its target muscle.

ivn through Fiber–PS Cells

Additional identified components of the STNS include the *ivn* through fibers in *Panulirus interruptus* and their likely homologues, the pyloric suppresser (PS) neurons in *Homarus gamarrus* (Cazelets et al., 1990a; chapter 3). The *ivn* fibers and the PS cells do, however, differ functionally in a number of ways. The two *ivn* fibers contain histamine and have their cell bodies in the brain (Claiborne and Selverston, 1984b). They project down the *ivn* and *stn* to the STGs and into the CoGs (Selverston et al., 1976; Moulins and Vedel, 1977). In the STG, they make direct synapses onto both gastric and pyloric neurons (Sigvardt and Mulloney, 1982; Claiborne and Selverston, 1984a) and can activate and restructure the pyloric and gastric rhythms. In addition to modulating the pyloric and gastric networks, the *ivn* fibers are components of the cardiac sac motor pattern (Moulins and Vedel, 1977, see Foregut Neural Networks below). The PS neurons have their cell bodies in the *ivn*, just outside the OG and project to the STG. Unlike the *ivn* neurons, the PS neurons suppress pyloric activity (Cazelets et al., 1990a). In addition, PS firing can restructure the four major motor programs of the foregut so that a novel motor pattern is produced that uses components of the other networks (Meyrand et al., 1991; chapter 4). The effects of these modulatory neurons emphasize the plasticity of the foregut motor networks.

Sensory Input

Foregut sensory input is an important functional component of the STNS. Some of this input has been demonstrated to play a modulatory role that reorganizes foregut motor patterns (Hooper and Moulins, 1989; Katz and Harris-Warrick, 1991). Sensory input into the STNS comes both from mechanoreceptors (stretch receptors and tactile hairs in the foregut wall and proprioceptors in foregut muscles) and chemoreceptors. Dando and Maynard (1974) divided the sensory innervation of the foregut into six main groups: (1) receptors that monitor movements of the lower esophagus and mouth parts, (2) chemoreceptors in the esophagus and lower cardiac sac, (3) receptors in or near the STG that monitor gastric mill movements, (4) receptors in the posterior stomach nerve that innervate the stomach wall near the gastric mill and monitor gastric mill movements, (5) proprioceptors in muscles near the cardiopyloric valve, and (6) stretch receptors on the hepatopancreatic (digestive gland) duct and initial part of the midgut. We describe below some of the receptors from these groups and the effects of their activity on the foregut motor patterns.

Esophageal Sensors Two prominent sense organs respond to and control esophageal movements. The anterior esophageal sensor (AOS) is found on either side of the midline of the anterior esophagus and the posterior esophageal sensor (POS) is found on either side of the midline of the posterior esophagus, near the entrance to the cardiac sac (Robertson and Laverack, 1979b). Both organs contain approximately 400–500 sensory cells whose dendrites project into sensilla that extend into the lumen of the esophagus. Sensilla of the AOS are bimodal receptors containing chemosensitive and mechanosensitive cells (Altner et al., 1986). Stimulation of the nerve containing AOS axons and application of mussel extract inhibits the esophageal rhythm in *H. gammarus* (Robertson and Laverack 1979b) but nerve stimulation enhances the esophageal, gastric, and pyloric rhythms in *P. interruptus* (Selverston et al., 1976). POS nerve stimulation and mussel extract enhance esophageal activity in *H. gamarrus* (Robertson and Laverack, 1979b).

Posterior Stomach Receptor The posterior stomach receptor organs (PSR) consist of a group of 180 mechanoreceptors on each side of the stomach that innervate the arch supporting the gastric teeth (Dando and Laverack, 1969). The PSRs are stimulated by rhythmic movements of the stomach wall and thus monitor the gastric and cardiac rhythms (Nagy and Moulins, 1981). A brief stimulation of the PSR nerve activates the gastric and pyloric motor patterns for a prolonged period. The effects of PSR activation are polysynaptic, requiring activation of neurons in the CoGs (Nagy and Moulins, 1981).

Anterior Gastric Receptor The anterior gastric receptor (AGR) is a proprioceptor with its cell body in the *dvn* (figure 1.5) and its processes extending into the stomach epidermis around muscle gm1. The AGR is simulated by movements of anterior gastric mill muscles and its firing activates and modulates the gastric rhythm. Like the PSR cells, the AGR acts indirectly on the STG neurons. It sends its axon to the CoGs in a polysynaptic pathway that projects back to the STG (Simmers and Moulins, 1988a,b).

Gastropyloric Receptors The gastropyloric receptors (GPR) are stretch receptors that have their cell bodies in peripheral nerves near the gastric muscles that they innervate (Katz et al., 1989). These receptors are normally activated by gastric mill movements and are of two types, giving tonic or phasic responses to applied muscle stretch. Brief stimulation of the GPRs in crabs activates the pyloric rhythm and can excite certain gastric neurons to fire in the pyloric rhythm for periods that outlast the stimulation (Katz and Harris-Warrick, 1991). These GPR neurons contain serotonin and acetylcholine as cotransmitters; it is believed that serotonin causes the prolonged effects of brief GPR stimulation. Unlike the PSR and AGR neurons, the GPRs synapse directly

onto gastric and pyloric network neurons in the STG. The GPRs appear to be endogenously rhythmic (Katz and Harris-Warrick, 1990c), suggesting that the GPRs and the gastric and pyloric networks are coupled oscillators connected via a proprioceptive feedback loop.

Hepatic Duct Receptors The hepatic duct (HD) receptors are stretch receptors that innervate the ducts leading from the pyloric filter into the digestive gland (Dando and Maynard, 1974). Like the GPRs, these receptors project directly to the STG to modulate the pyloric network neurons (Hartline et al., 1987). If the foregut movements both circulate digestive fluids anteriorly from the digestive gland and liquified food posteriorly to the gland, then sensory information from the HD receptors may be important for coordinating these fluid movements.

The foregut motor patterns are subject to control from other areas of the nervous system that are not considered part of the STNS. For example, restraint of a lobster or visual stimuli can stop the gastric motor pattern, and eyestalk ablation can enhance it transiently (Fleischer, 1981). In addition, cutting the commissures between either the brain and the CoGs or the CoGs and subsesophageal ganglia can alter the esophageal motor pattern (Spirito, 1975). This indicates that other neural centers participate in structuring the foregut motor patterns.

FOREGUT NEURAL NETWORKS

Esophageal Rhythm

The neural circuit responsible for rhythmic contractions of the esophagus has not been characterized, largely because most of the components of this network are in the CoGs and it is difficult to locate them among the hundreds of other cell bodies. For example, it is known that each CoG has a network capable of generating the esophageal motor pattern (Spirito, 1975; Selverston et al., 1976). Extracellular recordings from motor nerves have identified three dilator and two constrictor motor neurons; one of the dilator motor neurons, OD1, has its cell body in the OG (Moulins and Vedel, 1977).

Cardiac Sac Rhythm

The circuit responsible for cardiac sac movements is also incompletely worked out. Like the esophageal rhythm, it is distributed across several ganglia. Three motor neurons have been identified that participate in the cardiac sac rhythm. CD1, with its cell body in the OG, and CD2, with its cell body in the STG, dilate the cardiac sac. The *ivn* fibers are also considered part of the cardiac sac network (Dickinson and Marder, 1989) and fire simultaneously with the cardiac sac dilator motor neurons (figure 1.6A). Under the appropriate modulatory conditions, the cardiac sac and gastric networks produce a conjoint rhythm. In this situation,

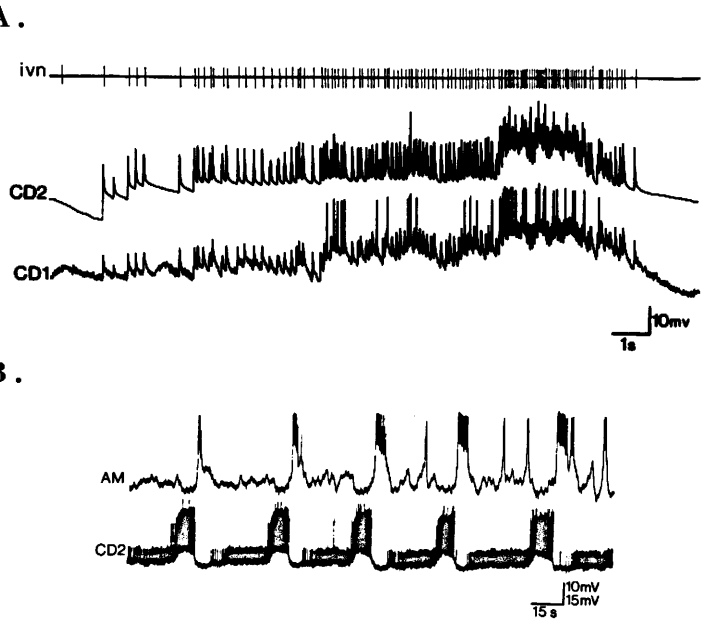

Figure 1.6 Neurons active in the cardiac sac network (from Dickinson and Marder, 1989). (A) Firing of ivn through fibers with the cardiac sac motor neurons CD1 and CD2. (B) Firing of the gastric mill motor neuron AM immediately after firing of cardiac sac neuron CD2.

the AM neuron of the gastric network also helps constrict the sac, firing in antiphase with the cardiac sac dilators (Dickinson and Marder, 1989; Dickinson et al., 1990a; compare AM activity in figure 1.3C with that in figure 1.6B). A pyloric neuron can also participate in the cardiac sac rhythm (Hooper and Moulins, 1989). Both spontaneous cardiac sac activity and stimulation of a sensory nerve, the *lpln*, induces the VD neuron to switch its activity from the pyloric network to the cardiac sac network.

STG Neural Networks

The neural components of the gastric and pyloric networks that reside in the STG have been well characterized. When the STG is disconnected from all sources of input, the appropriate modulatory conditions can cause both the pyloric and gastric networks to generate rhythmic motor patterns that resemble those seen in vivo. Thus, the capacity to generate the pattern lies within the STG networks themselves and higher order input to these networks must then play a permissive role rather than strictly setting the timing cues. Neither the gastric nor pyloric motor patterns can be explained solely on the basis of the anatomical network connections. They result from the synaptic connectivity and the synaptic and intrinsic cellular properties of the component neurons (see Cellular and Synaptic Properties of the STNS below). The pyloric and gastric

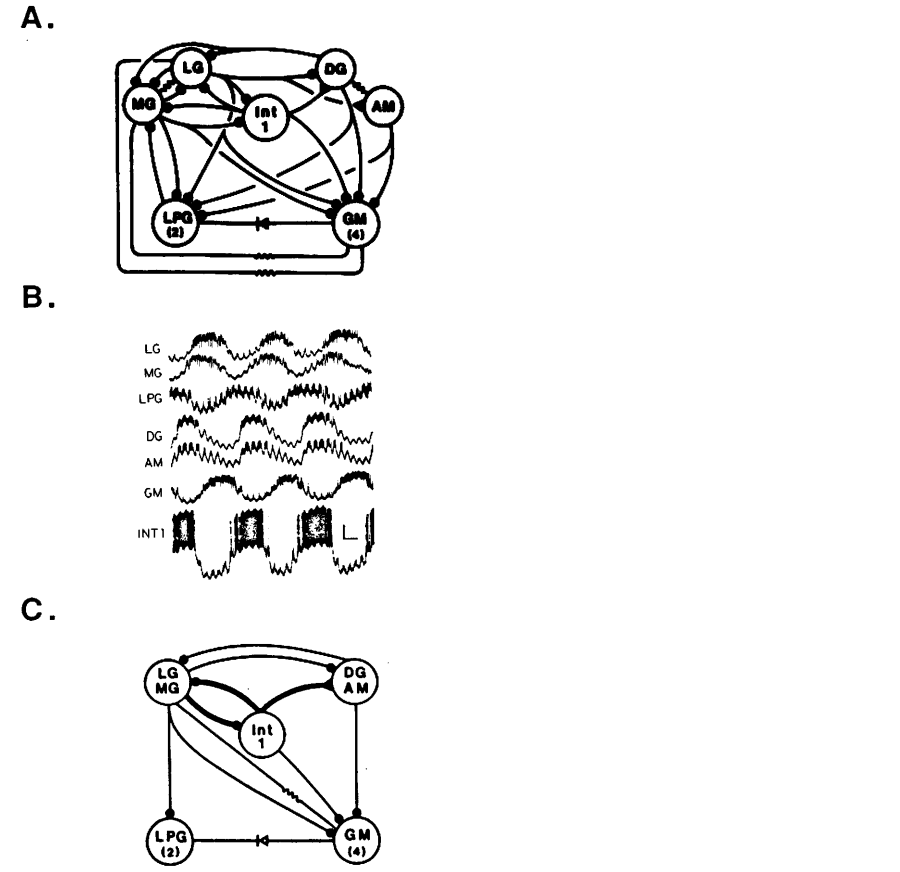

Figure 1.7 Gastric mill network. (A) Complete gastric network. (B) Simultaneous activity recorded intracellularly in all the different types of gastric mill neurons. Calibration marks: 1s, 10 mv. (C) Condensed gastric mill network. (A) and (C) From Mulloney (1987); filled circles indicate inhibitory chemical synapses, filled triangles indicate excitatory chemical synapses, resistor symbols indicate nonrectifying electrical synapses, and diode symbols indicate rectifying electical synapses. (B) From Heinzel and Selverston (1988).

networks are also capable of producing multiple outputs from the same anatomically defined circuits. These two circuits must therefore have the ability to operate in several functionally different modes (see How Are the Rhythmic Patterns Produced? below).

Gastric Network All of the anatomical connections of the seven types of gastric neurons in the STG of *P. interruptus* have been determined (Mulloney and Selverston, 1974a,b; Selverston and Mulloney, 1974) and are shown in figure 1.7A. There are extensive chemical and electrical connections between the gastric neurons. The chemical synapses are mostly inhibitory and the electrical synapses are mostly nonrectifying. The simultaneous intracellular recordings from all gastric cell types in figure 1.7B show that the LG and MG neurons fire at approximately

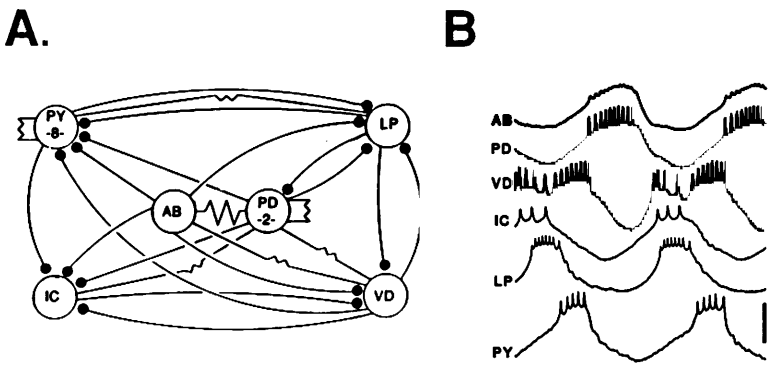

Figure 1.8 Pyloric network. (A) Synaptic connectivity between the pyloric neurons. Symbols have the same meaning as in figure 1.7 (from Johnson, Peck, and Harris-Warrick, unpublished). (B) Intracellular recordings from all the pyloric neuron types; top four traces recorded simultaneously; bottom two traces recorded 30 sec later together with the PD trace to allow proper alignment. Calibration bar: 20 mV for PD and LP and 10 mV for AB, VD, IC, and PY. Duration of recording is 1 sec. From Miller (1987).

the same time and alternate with the LPG neuron; the AM and DG neurons fire together and alternate with the GM neurons; finally, Int1 fires together (slightly phase advanced) with the AM and DG neurons (Selverston, 1989). This pattern can be partially explained by the condensed circuit of figure 1.7C (see How Are the Rhythmic Patterns Produced? below). Int1 plays a pivitol role in the gastric pattern because of the strong synaptic connections it makes with the other neurons. However, none of the gastric neurons is essential to maintain some form of gastric mill pattern.

A set of CoG neurons comprising the commissural gastric oscillator exercise higher order control over the gastric rhythm and could be considered part of the in vivo network (see chapter 3). These neurons send excitatory drive to all the gastric neurons except Int1. Feedback from the gastric mill rhythm is returned to the oscillator network in the CoGs through an inhibitory connection from Int1 to higher order neurons.

Pyloric Network The synaptic connections of the pyloric neurons have also been determined in *P. interruptus* (Maynard, 1972; Maynard and Selverston, 1975; Hartline and Gassie, 1979; Miller and Selverston, 1982a,b; Eisen and Marder, 1982; Hartline et al., 1987). Again there are extensive chemical and electrical interconnections between the six cell types (figure 1.8A). In this network, all the chemical synapses are inhibitory (see Synaptic Properties of STNS Neurons below). These anatomical connections partially explain the pattern shown in the simultaneous recordings from all pyloric cell types shown in figure 1.8B (as explained below in How Are the Rhythmic Patterns Produced?). Again, a set of CoG neurons (comprising the commissural pyloric os-

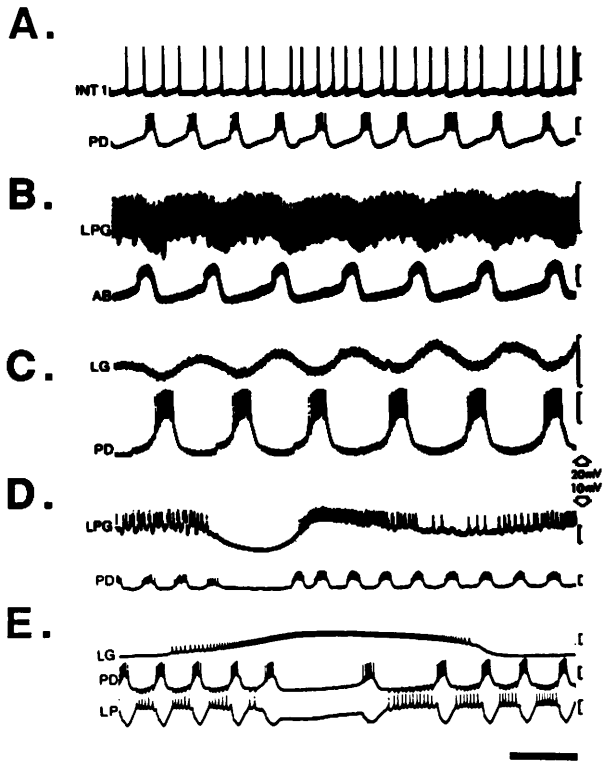

Figure 1.9 Functional interactions between the gastric and pyloric networks. (A) Inhibition of Int1 by the PD neuron. (B) Inhibition of the LPG neuron by the AB neuron. (C) Inhibition of the LG neuron by the PD neuron. (D) Inhibition of PD activity in concert with LPG inhibition. (E) Inhibition of PD and LP activity by the LG neuron. Horizontal calibration bar: 2 sec for (A), (B), and (E) and 1 sec for (C) and (D). Reprinted with permission from Selverston et al., The stomatogastric nervous system: Structure and function of a small neural network. *Prog. Neurobiol.* 7:237. Copyright 1976, Pergamon Press plc.

cillator) could be considered part of the in vivo network. These neurons send excitatory input to pyloric neurons and receive inhibitory feedback from the AB neuron and can entrain the pyloric rhythm under some conditions (chapter 3).

Interconnections between the Gastric and Pyloric Networks

In *P. interruptus,* the gastric and pyloric pattern generators generally function, at least in in vitro preparations, as separate neural networks. However, there are interconnections between these STG circuits that allow "cross-talk" (Selverston et al., 1976; Mulloney, 1987). Figure 1.9 shows examples of interactions between gastric and pyloric neurons of *P. interruptus.* It is not known if all the interconnections are monosynaptic. Such interactions provide a substrate for the integration of these two motor patterns. In addition, CoG neurons that are higher order to pyloric neurons make excitatory connections to several gastric neurons.

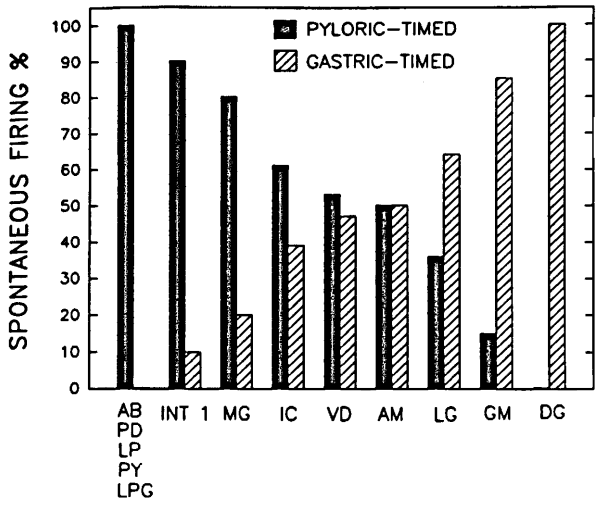

Figure 1.10 Distribution of spontaneous activity patterns of gastric and pyloric network neurons from the crab *C. borealis*. Neurons were scored for the percent of time they displayed pyloric or gastric activity. From Weimann et al. (1991).

This provides another mechanism for integration of the two networks (Selverston et al., 1976).

In other decapod crustaceans, the gastric and pyloric networks may have so many interconnections that it becomes difficult to distinguish some neurons as belonging to either one network or the other. In the crab *Cancer borealis*, for example, there are extensive functional interactions between gastric and pyloric neurons that make the gastric and pyloric networks less distinct than in lobsters (Weimann et al., 1991a; figure 1.10). Neurons considered to belong to the gastric network in crabs may fire entirely or mainly in pyloric time. Some neurons fire strictly in either pyloric or gastric time as they do in lobsters, whereas others can switch back and forth to spontaneously fire at different times in both pyloric and gastric rhythms (figure 1.10).

CELLULAR AND SYNAPTIC PROPERTIES OF THE STNS NEURONS

The ability of the STNS to produce rhythmic motor outputs stems from a dynamic interaction among (1) the intrinsic membrane properties of the neurons, (2) the synapses among them, and (3) the synapses at the neuromuscular junctions and the biomechanics of the stomach muscles. Although we shall present these three topics separately, it is important to stress that this separation is an artifice; the behavioral output of the STNS depends on the synthesis of all three, and seldom can any aspect of STNS activity as a whole be understood solely on the basis of individual cellular, synaptic, or muscular characteristics. A more detailed discussion of some of these topics is found in chapter 2.

Cellular Properties of STNS Neurons

Anatomy All STNS neurons are monopolar, i.e., a single process leaves the cell body, extends for various distances to branch extensively in a neuropil region or regions, and then continues to innervate distant postsynaptic targets. The cell body is usually electrically passive, and receives no synaptic input. Spikes recorded from the cell bodies are very attenuated, demonstrating that the cell bodies are electrically far from the spike initiation zones. Synaptic interactions occur and active membrane properties (postinhibitory rebound, oscillatory and plateau properties) are expressed in the neuropilar regions; spike initiation zones are located close enough (in electrical terms) to the neuropilar regions to respond to their summed membrane potential. Action potentials propagate from spike initiation zones to distant targets in the normal fashion, but it is not known whether they propagate actively into the neuropilar regions themselves.

These anatomical considerations allow STNS neurons to have complicated morphologies with profound functional consequences. Primary among these is that single neurons (OD1, CD1, CD2) can have several different neuropil/spike initiation zones (located centimeters apart and in different ganglia), each of which receives synaptic input and fires spikes independently (Vedel and Moulins, 1978; Moulins and Nagy, 1981a,b; Nagy et al., 1981b). Such neurons could theoretically be active with more than one STNS rhythm, firing with one rhythm from one spike initiation zone and another rhythm from another zone. This could provide another means by which different rhythms can be coordinated. It is perhaps significant that these complicated neurons are present in those networks that are distributed over several different ganglia (the esophageal and cardiac sac networks), whereas the neurons of the pyloric and gastric networks, in which the majority of the cell bodies are in the STG, typically have only one neuropil and spike initiation zone.

Active Membrane Properties Some combination of active membrane properties, such as bursting pacemaker potentials, plateau properties, postinhibitory rebound, and frequency adaptation, are generally observed in the neurons of networks that produce rhythmic outputs (Selverston and Moulins, 1985). The systems of the STNS are no exception to this rule. As was noted above, the neuropilar regions (where these properties are expressed) of the known neurons of the esophageal and cardiac sac networks are very distant electrically from the neuronal cell bodies, and thus nothing is known about the active membrane properties of these neurons. The cell bodies of the gastric and pyloric network neurons, however, are electrically close enough to their neuropilar regions to observe the active membrane properties from soma recordings.

Of the four active properties mentioned above, only plateau properties are sufficiently unusual to require a definition. A neuron capable of plateau potentials has two quasistable membrane potentials (a more hyperpolarized "rest" potential and a depolarized "plateau" potential). The neuron can make a transition between the two states in response to brief synaptic input, postinhibitory rebound, or current injection. The transitions themselves are regenerative, i.e., a depolarization above a certain threshold voltage from the rest state will activate voltage-dependent depolarizing conductances that then drive the neuron to the fully depolarized plateau, and relatively small hyperpolarizations from the plateau will induce an active repolarization to the rest state (Russell and Hartline, 1978, 1982).

We can easily list which of the above active membrane properties are present in which neurons: Every single pyloric network neuron is known to be capable of expressing each of these properties (Russell and Hartline, 1978, 1982; Hartline and Gassie, 1979; Miller and Selverston, 1982a; Bal et al., 1988). Of the gastric network neurons, the DG neuron is capable of endogenous oscillation (Hartline and Russell, 1984; Heinzel and Selverston, 1988), all the neurons except the GM neurons can produce plateau potentials (Russell and Hartline, 1984; Dickinson et al., 1988), all of the neurons can produce postinhibitory rebounds (Selverston et al., 1976), and many of them show spike frequency adaptation. Initially, this may lead one to think, particularly for the pyloric network, that the neurons are essentially identical, and that their different activities in the network output patterns stem solely from the specifics of the network synaptic connectivity. In fact, however, the different neuronal types are intrinsically different, in two general ways. First, the expression of these active membrane properties is conditional, i.e., they depend on the influence of extrinsic inputs to the circuits, and the extent and type of response to a particular extrinsic input vary from neuron to neuron (Russell and Hartline, 1982, 1984; Dickinson and Nagy, 1983; Bal et al., 1988; see chapter 3). Second, even when turned on, the characteristic time course and voltage dependence of these properties differ from neuron to neuron. As an example of how these differences between neurons help give rise to their unique identities, consider why the AB neuron is identified as the pyloric pacemaker. First, of all the pyloric network neurons it is the least dependent on extrinsic activation, and so under conditions in which the other neuron types are largely passive, the AB neuron continues to oscillate and thus to drive a pyloric rhythm. Second, under conditions in which all the pyloric neurons are oscillators, the AB neuron has the fastest inherent oscillation frequency, and thus entrains the other neurons to near its frequency (Bal et al., 1988).

The ionic basis of these properties is not well understood due to the fact that it is technically difficult to voltage clamp the electrically distant active membrane from the cell body. However, these neurons possess

all the typical channels (voltage-dependent Na^+, Ca^{2+}, K^+, and Ca^{2+}-activated K^+ currents with various inactivation kinetics and voltage dependences) required for both action potential generation and slow active properties such as endogenous oscillation and plateaus (Gola and Selverston, 1981; Hartline et al., 1988a; Harris-Warrick, 1989; Graubard and Hartline, 1991; Golowasch and Marder, 1992; Kiehn and Harris-Warrick, 1992a,b). A detailed study of the LP neuron has been used to construct a computer model of the neuron, and the model can be made to express plateau properties or oscillate by appropriate modifications of various currents (Golowasch et al., 1992); experimental verification of the ionic basis of these active cellular properties awaits further work.

Synaptic Properties of STNS Neurons

As with the cellular properties mentioned above, synaptic properties in the STNS have been investigated in detail only for the pyloric and gastric network neurons. However, the neurons of the esophageal and cardiac sac networks have gross anatomies (inexcitable cell bodies, distant neuropil and spike initiation zones) and activity patterns similar to those of the pyloric and gastric neurons; this suggests that the synaptic properties of all these neurons are also similar.

Anatomy A detailed electron microscopic analysis by King (1976a,b) showed that each physiologically observed chemical synapse is actually the result of the concerted action of hundreds to thousands of anatomical synaptic contacts. These synaptic contacts are spread diffusely across all of the fine branches of the neuronal arborization in the neuropil and it is thus unlikely that any specific synaptic contact is more "important" than another in generating the physiologically observed postsynaptic potential. Input and output synapses are present on all branches, and thus the neurons are not divided into specific pre- and postsynaptic regions. This close proximity of input and output synapses also suggests that the relationship between neuronal input and output observed physiologically may result from local integrative processes occurring in the individual branches, as opposed to a whole cell integration (e.g., action potential generation) as is typical in polarized neurons such as vertebrate motor neurons. A recent study by Hall et al. (1991) in the crab showed that gap junctions are similarly distributed diffusely on the fine neuronal branches.

Synaptic Transfer Function As might be expected from the synaptic anatomy, synaptic transmission in both the pyloric and gastric networks is observed to be a graded function of presynaptic membrane potential that does not depend on action potentials (Graubard, 1978; Graubard et al., 1980, 1983; Selverston et al., 1983a). In fact, action potentials seem to play a relatively small role under some conditions in the generation of the pyloric network output. For example, a pyloric pattern

initiated with dopamine will continue with normal cell oscillation sequence, phasing, and frequency even when action potential generation is blocked with TTX (Raper, 1979b; Anderson and Barker, 1981). Action potentials may be important for rhythm generation at low environmental temperatures where graded synaptic transmission is weak compared to action potential-evoked transmission (Johnson et al., 1991). Action potentials are, of course, important for the distributed networks of the esophageal and cardiac sac networks, where they are required for communication among neurons separated by large distances. However, even for these neurons, if the ultimate synaptic output sites are located in branched neuropil unable to support action potentials, the actual transmitter release will be a function of (1) the amplitude and temporal filtering expected to occur as the action potential passively invades the branched neuropil and (2) any active membrane responses of the neuropil triggered by this depolarization.

Neurotransmitter Substances Where known, the neurons of the pyloric and gastric networks use, at both their peripheral and central synapses, either glutamate or acetylcholine (ACh). Motor neurons that innervate extrinsic muscles use ACh (PD, VD, LPG, GM, and DG); those that innervate intrinsic muscles and the interneurons use glutamate (LP, PY, IC, MG, LG, AM, AB, and Int1) (Marder, 1974, 1976; Selverston et al., 1976; Marder and Eisen, 1984b). Anatomically, ACh has been associated with clear irregular synaptic vesicles and glutamate with larger clear round vesicles (King, 1976a). The *ivn* fibers of the cardiac sac network use histamine (Claiborne and Selverston, 1984a) and possibly another neurotransmitter, inasmuch as exogenously applied histamine does not reproduce all the effects of *ivn* fiber stimulation. The neurotransmitters used by the other esophageal and cardiac sac network neurons are unknown. Many other neurotransmitters (such as dopamine, serotonin, octopamine, proctolin, and GABA) that function as modulators of pyloric and gastric network output have also been putatively identified as present in the STNS (see chapter 3).

Pharmacology and Postsynaptic Responses The cholinergic ipsps in the pyloric network neurons are blocked by high concentrations of atropine, are due to an increase in K^+ conductance, and are relatively slow. Those of glutamate are blocked by picrotoxin, are due to an increase in both Cl^- and K^+ conductance, and are fast (Bidaut, 1980; Eisen and Marder, 1982; Eisen and Marder, 1984). Pharmacological studies also indicate nicotinic and muscarinic type excitatory ACh receptors and two different GABA receptors exist on various neurons in the STG (Marder and Paupardin-Tritsch, 1978), but their functional significance is as yet unclear. There are, of course, presumably many other receptors present on these neurons given the large numbers of neuromodulatory

substances believed to impinge on the pyloric and gastric networks, but these are as yet mostly unidentified.

Muscles of the STNS

Innervation Pattern, Muscle Characteristics, and Synaptic Transfer Function The different muscles of the STNS are evolutionarily well conserved in different species, allowing apparent homologues to be identified in crabs, lobsters, and shrimps. Perhaps even more remarkable, the motor neuron innervation of specific muscles is also highly conserved. In fact, innervation pattern is the only completely reliable method of identifying neurons, given that it is now known that individual neurons can switch between multiple neural networks depending on the state of the STNS. The nerve-induced junctional potentials in different muscles vary widely in initial amplitude and facilitory/defacilitory characteristics (even in muscle groups innervated by the same neuron) (Govind et al., 1975; Hooper et al., 1986); the contractile properties of the muscle fibers are similar to the slow fibers of crustacean limb muscles (Jahromi and Govind, 1976). In crabs and lobsters, the strength of foregut muscle contractions induced by nerve stimulation can be enhanced by dopamine, serotonin, octopamine and proctolin, and some muscles will show spontaneous rhythmic activity after application of some of these modulators (Lingle, 1981; Govind and Lingle, 1987). In vivo, modulatory compounds could be delivered to neuromuscular sites through the circulatory system (Govind and Lingle, 1987) or as a cotransmitter in the nerve terminal. Electron microscopy indicates that at least in one gastric mill muscle of the blue crab, *Callinectes sapidus*, both clear and dense core vesicles are located near the neuromuscular synapses (Atwood et al., 1977a). In the shrimp, one of the pyloric muscles can also produce rhythmic myogenic contractions; myogenicity can be induced by the application of either dopamine or FMRFamide (Meyrand and Moulins, 1986; Meyrand and Marder, 1991).

Anatomy and Pharmacology Electron microscopic studies of STNS neuromuscular junctions have been made only in *C. sapidus*. Synaptic contacts are widely distributed over the muscle; synaptic contact areas range from 0.2 to 10 μm^2. It is likely that only the larger synapses are functional (Atwood et al., 1977a,b).

Pharmacological studies of the channels present on STNS muscles have been performed only on two gastric mill muscles, gm1, which receives cholinergic innervation, and gm6, which receives glutamatergic innervation. The channel opened by ACh on gm1 is selectively permeable to Na^+, is activated by nicotine, and is blocked by picrotoxin, chlorisondamine, hexamethonium, and trimetaphan (Marder and Paupardin-Tritsch, 1980a,b; Lingle, 1983a,b). The glutamate gated channel on gm6 is also selectively permeable to Na^+, and is also blocked by

chlorisondamine (Lingle et al., 1981). A comparison of the ACh and glutamate gated currents shows that the ACh gated channels have longer open times and are more voltage sensitive than the glutamate gated channels (Lingle and Auerbach, 1983a,b). Surprisingly, both these muscles also have channels sensitive to substances that are not the transmitters in the nerve terminals. A glutamate gated Cl^- channel is found in gm1 (Lingle and Marder, 1981) and both a picrotoxin-insensitive GABA gated Cl^- channel and an ACh gated channel are found in gm6 (Albert et al., 1986). The functional significance of these "extra" channels, if any, is completely unknown.

HOW ARE THE RHYTHMIC MOTOR PATTERNS PRODUCED?

It was once widely assumed that if one knew the pattern of synaptic connectivity within a network and the cellular and synaptic properties of its neurons, it would be evident how the network produced its rhythmic output. This is clearly not the case. No one, given just the information we have presented above, would be able to predict the motor patterns produced by the gastric or pyloric networks. This early optimism, we believe, stemmed from two tacit assumptions: (1) networks would be built in a serial and hierarchical fashion and (2) network rhythmicity would, for a given network, derive from a single mechanism, e.g., endogenously oscillating pacemaker neurons, half center oscillators. It is becoming increasingly clear that biological networks (not just in the STNS, but, as other systems are described on a cellular level, in general) violate these rules. Biological networks are characterized by massive synaptic interconnectivity (in the pyloric network, over 50% of the possible synaptic connections are in fact made) and the existence within the same network of several reinforcing rhythm-producing mechanisms. For example, the pyloric network has endogenous oscillators, plateau properties, postinhibitory rebound, spike frequency adaptation, and patterns of mutual synaptic inhibition. Each of these alone could possibly support the rhythm. This does not mean that understanding these systems is impossible, and in fact pyloric network pattern generation is understood on a fairly deep level. What it does mean is that reductionist analysis that associates specific neurons, synaptic connections, or cellular properties with specific characteristics of the network's output is suspect. Pattern generation in such networks is likely to be an emergent property of the network as a whole. Thus responsibility for any specific characteristic of the pattern (cycle period, phasing, interburst firing frequency) is distributed across many, if not all, the network's neurons and synaptic connections and, conversely, each neuron and connection contributes to several of the pattern's characteristics. New approaches that do not rely on a static analysis of synaptic and cellular properties are needed to explain pattern generation. Advances in computer modelling will be important to track the

complex, nonlinear interactions that produce the outputs of the STNS networks (see chapter 5).

The esophageal and cardiac sac networks are insufficiently described to explain the basis for their rhythmicity and patterning, and we shall therefore only deal with the gastric and pyloric networks.

The Gastric Mill Rhythm

The gastric network was originally believed to be an example of a purely network-based oscillator because the expression of oscillatory membrane characteristics in the gastric neurons depended on the presence of inputs extrinsic to the network, and these inputs were not activated in the original work. The network's rhythmicity and phase relationships were therefore explained as arising from postinhibitory rebound, firing frequency adaptation, and synaptic depression (Mulloney and Selverston, 1974a,b; Selverston and Mulloney, 1974). A conditional oscillator neuron, the DG neuron (Selverston et al., 1976), has since been discovered, and other active cellular properties, such as plateau potentials, play an important role in this network's activity, and must be considered to understand it (Elson and Selverston, 1992). Thus this network is not a pure network-based oscillator. We shall now walk through this network and give an explanation of how the pattern shown in figure 1.7B is produced. It is essential to note, however, that this network is less well understood than the pyloric, and many aspects of this "explanation" are speculative and need further verification. Furthermore, this network and the pyloric are each capable of producing many different rhythmic patterns both in vivo and in vitro in the presence of different modulatory input (see chapters 3, 4, and 6). The explanations presented here thus refer only to the patterns shown in figures 1.7B and 1.8B.

The heart of the gastric rhythm is a mutually reinforcing endogenous neuronal oscillator (the DG neuron) and a half center oscillator (the LG/MG neurons and Int1) (see figure 1.7B, C). In the absence of endogenous oscillations in the DG neuron, Int1 fires tonically and the LG/MG neurons are silent; the half center does not operate because there is no mechanism that allows the LG/MG neurons to escape from inhibition and fire. However, when the DG neuron endogenously oscillates and fires its first long, high-frequency spike burst, the resulting inhibition of the LG/MG neurons triggers a postinhibitory rebound that makes them fire a burst. The DG neuron burst ends probably because of a combination of endogenous properties and LG/MG neuron inhibition. The LG/MG neuron burst occurs before the end of the DG neuron burst; this synapse is hypothesized to suffer depression of transmitter release or postsynaptic desensitization (Mulloney and Selverston, 1974b). The LG/MG neuron burst strongly inhibits Int1, and when the LG/MG neuron burst ends, Int1 in turn is triggered to plateau and fire a spike burst. This burst excites the DG neuron, and apparently triggers the DG neuron to burst somewhat earlier than its endogenous oscillation

period (note that Int1 firing precedes the DG neuron burst). And the cycle repeats. Thus, one possible role of the endogenous oscillator DG neuron may be to "jumpstart" the half center oscillator and prevent it from running down. The GM neurons are triggered to fire during the LG/MG neuron bursts by a combination of postinhibitory rebound from Int1 and DG/AM neuron inhibition and the electrical coupling between the GM and LG/MG neurons. The LPG neurons simply fire in antiphase to the LG/MG neurons as a result of postinhibitory rebound and their electrical coupling to the GM neurons.

The Pyloric Rhythm

The activity of the pyloric network can be explained on a coarse and superficial level quite simply. The AB neuron is usually the fastest oscillator neuron in the network; it drives the electrically coupled PD neurons to fire with it. These three neurons inhibit all the other pyloric neurons, which therefore fire out of phase with the pacemaker group. The VD, LP, and IC neurons then fire due to plateau potentials triggered by postinhibitory rebound. The VD neuron begins to fire first in part because of its intrinsic rebound properties, in part because of its electrical coupling to the PD/AB group, and because only the glutamatergic AB neuron (with its short-lasting ipsp) inhibits it. The LP and IC neurons also receive a late slow ipsp from the PD neuron. However, the VD neuron inhibition of the LP and IC neurons is insufficient to block their plateaus, and they combine to reduce VD neuron firing during their bursts. In many cases, in fact, VD neuron firing is completely inhibited during the IC and LP neuron bursts, and the VD neuron fires two bursts per pyloric cycle (figure 1.3D). Finally, the PY neurons, which have cellular properties that delay their postinhibitory responses (Hartline, 1979), fire, shutting off the IC and LP neurons and releasing the VD neuron from inhibition. The VD neuron inhibition of the PY neurons is weak, and both neurons are finally turned off by the next AB/PD neuron depolarization. And the cycle repeats.

It is extremely important to realize that this explanation understates the large number of reinforcing mechanisms that support pyloric rhythmicity. For instance, in the absence of endogenous oscillators, a half-center oscillator has been shown to be present in the network (Miller and Selverston, 1982b), but it is functional only over a tiny range of voltages, and not at all unless both cells are depolarized. In addition, computer simulation studies show that the pyloric synaptic connectivity with postinhibitory rebound can support a rhythmic pattern with the correct sequence of neuronal firing (Warshaw and Hartline, 1976; see chapter 5). Going to the other extreme, Bal et al. (1988) have shown that all of the pyloric neurons can show bistability, and presumably these other endogenous sources of rhythmicity also contribute to the network's activity in at least some cases. This explanation also understates the multiple functions of the individual synaptic connections. For

instance, the electrical coupling between the AB and PD neurons makes the PD neurons fire with the AB neuron, but also (1) allows the PD neurons to alter the AB neuron activity from what the AB neuron would do in isolation, thus serving as a source of frequency control for the network (Hooper and Marder, 1987) and (2) allows synaptic input from the rest of the ganglion access to the AB neuron using the PD neurons as intermediaries (Eisen and Marder, 1982). Similarly, when the VD neuron is deleted from the circuit, the IC neuron begins its firing with the AB/PD neuron firing. Thus the VD to IC synapse plays two roles: it serves to shut off the IC neuron during the VD neuron phase, and provides an additional help (even though the VD neuron is then silent) to the relatively weak AB/PD neuron inhibition that is required to prevent the IC neuron from firing during pacemaker activity (Hooper and Moulins, 1990). This rich diversity of mechanism and distributed, multifunctional synaptic connectivity presumably exists not only to produce the "typical" pyloric output shown in figures 1.3D and 1.8B, but also to provide the substrate that allows this single network to produce multiple output patterns (see chapter 3).

Hierarchical Multioscillator Systems

Several examples of hierarchical chains of oscillators are known to be present in the STNS. In *H. gammarus* there exists both a commissural pyloric oscillator (CPO) and a commissural gastric oscillator (CGO) in the CoGs (Robertson and Moulins, 1981a). These can entrain the STG circuits. The CPO can also drive the pyloric pacemaker in several different coordination modes (i.e., one pyloric burst for each CPO burst, 1:2, 1:3), as is expected when oscillators with very different inherent frequencies are connected. However, it seems that the IC neuron is always driven 1:1. This hierarchical chain thus not only allows for the control of the pyloric network by a distant oscillator, it also provides a mechanism whereby multiple IC neuron bursts can occur within each pyloric cycle (Robertson and Moulins, 1981b). For the CGO, the input neuron to the gastric network has been identified as the commissural gastric (CG) neuron. The CG neuron is an endogenous oscillator that fires rhythmically with the gastric network and makes excitatory synapses onto several gastric neurons. A particularly fascinating cellular property of the CG neuron is that it shows spike inactivation at depolarized membrane potentials, and is thus able to generate bursts of spikes either by depolarization or hyperpolarization (Robertson and Moulins, 1981c, 1984).

Perhaps the most surprising aspect of the STNS is the massive amount of modulatory input it receives and the dramatic changes in STNS activity this input induces (see chapter 3). Over a dozen putative neuromodulators of the various STNS networks have been identified. In *P. interruptus*, the STN contains some hundred input fibers to the STG (King, 1976a), and so it is likely that many more input pathways remain

to be described. Application of neuromodulatory substances to the STNS can start or stop rhythm production, produce changes in rhythmic frequency, interburst spiking frequency, and firing phase in the output of single networks, or alter the distinction between networks, moving neurons from one to the other or fusing networks into single large ones.

It may initially seem strange that the STNS should have evolved such complexity; after all, it generates the motions only of a stomach, not a limb. However, it is important to note that the decapod crustacean stomach performs a wide set of tasks. The function of the gastric mill is chewing, and the ability to produce many different gastric mill motor patterns presumably reflects a need to effectively masticate a range of different food types. The pyloric filter must separate food particles on the basis of size; its activity presumably also must vary depending on the amount and type of food in the stomach. There must also be programs for dislodging pieces of food inappropriately stuck in the gastric mill or pylorus ("clearing the throat"), for regurgitation of food, and for the regurgitation of the stomach lining itself during molting. The network, synaptic and cellular properties we have described in this chapter provide the substrate for the enormous variety and complexity of motor programs that can be produced from this "simple" model system.