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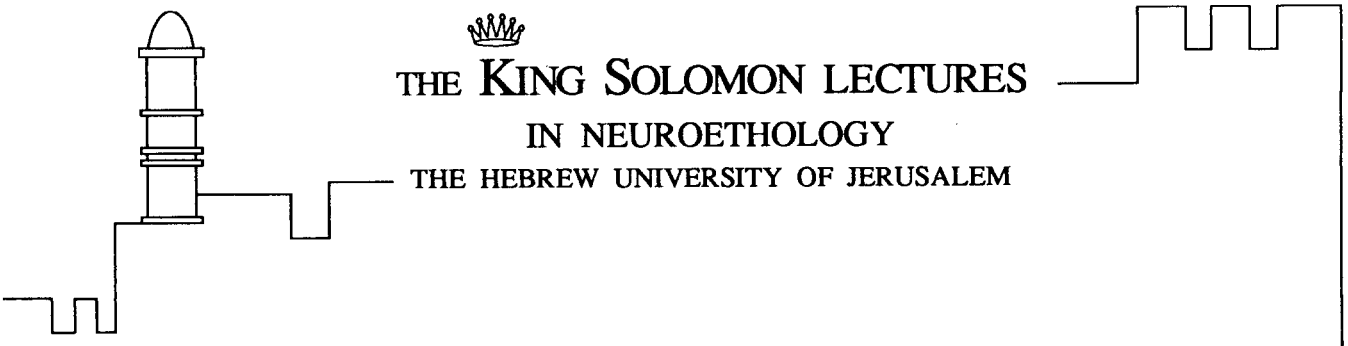
REVIEW




A. Selverston

# Modulation of circuits underlying rhythmic behaviors

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A fundamental question in neuroethology is how systems of neurons interact with one another to produce behavior. The underlying premise is that behavior results from the patterns of impulses in motor nerves causing the sequential contraction of muscles. To be effective, the impulses must occur in the correct nerves and at the appropriate time. Such "spatio-temporal" motor patterns were long thought to be generated by "hard-wired" circuits in the central nervous system, continuously modified by sensory feedback so as to be adaptive to the environment. However, it has been known for some time that behaviors could be triggered or modulated by chemical substances (neuromodulators) acting as hormones. New immunohistochemical methods have shown that there is a wide distribution of neurons containing putative neuromodulatory substances located within the central nervous system itself (Beltz et al. 1984), suggesting they may have local as well as distributed actions. Such substances are responsible for very complex fixed action patterns, such as egg-laying in *Aplysia*, but they also play a role in biasing specific behaviors so that they are accentuated or supressed (Kravitz 1988). The mechanisms involved in their action on neural circuits generating such behaviors are not well understood because in most cases the detailed circuitry is unknown. This general problem is overcome by one very common and easily studied class of behaviors – rhythmic movements such as feeding, locomotion and respiration.

The generation of rhythmic motor patterns can be shown to be produced by a collection of neurons called a central pattern generator (CPG) (Delcomyn 1980). A CPG produces the basic rhythmicity and the approximate phase relationships between motor neuron bursts (the central pattern). Initially, it was assumed that during development, the CPG was wired up according to a fixed plan. In an intact animal the CPG does not work in isolation, but is affected by sensory feedback of all kinds, as well as by descending influences from the brain – all of these interactions leading to a final motor program (Fig. 1). Although the outputs of CPGs are in some ways simple, there is enormous variation in the fine details of the motor patterns. Some must be extremely reliable (e.g., heartbeat), while others must be more flexible (e.g., scratching).

As research on CPGs progressed, it became clear that the neuronal circuits comprising them were not strictly hard-wired as originally presumed, but instead could be functionally rearranged in a way which allowed considerable behavioral flexibility (Harris-Warrick 1988; Harris-Warrick and Marder 1991). For example, consider tetrapod locomotion as a typical rhythmic behavior. It can obviously occur in different modes – walking, galloping, trotting, etc. No matter which mode is operating, the CPG is still responsive to the environment, i.e. the visual, tactile and auditory sensory streams feeding into the CNS. The question then is not only how one CPG produces different modes but also:

- Is there a distinct CPG for each mode?
- How does the linkage between different CPGs change within modes?
- How is sensory information adapted to each mode?
- What mechanisms are responsible for switching from one mode to another?

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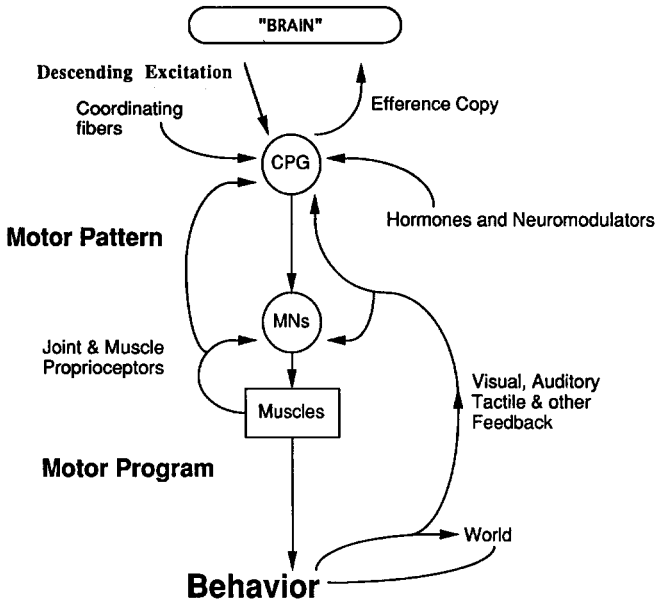



**Fig. 1** Overall scheme for the role of CPGs in the formation of motor programs. Note that the pattern produced by the CPG is under the control of higher centers in the nervous system as well as neuromodulators. The final inputs to the muscles are the result of factors illustrated, which shape the raw CPG pattern into a final motor program

It appears that for all of these, neuromodulation plays a key role. Neuromodulation is a response to chemical factors which does not rely on ligand-gated channels, but rather G-protein mediated receptors. Instead of short-acting changes in membrane potential due to transient changes in ionic conductance, the cascade of events triggered by neuromodulators (metabotropic effects) can last from minutes to hours and involve fundamental changes in neuronal and synaptic properties (Kaczmaarek and Levitan 1987).

The importance of neuromodulatory substances to invertebrate behavior had been demonstrated previously in groundbreaking papers by O'Shea (1985), Kravitz (1988) and others. Substances such as peptides and amines could be shown to act hormonally – producing major changes in animal behavior which could be analyzed at the cellular level. However, with the advent of immunological and molecular techniques it became possible to localize specific modulatory substances to *identified cells*, or to specific regions of the nervous system, and the opportunity for studying the role of neuromodulation in CPG function became widely available.

As a result of analyzing two particularly well-studied CPGs, contained within the stomatogastric ganglion of the lobster, it has become clear that the concept of fixed, hard-wired circuits is no longer applicable, and that CPG circuits can be *functionally* rewired by exposing them to specific neuromodulatory agents. It also seems likely that the results obtained from the study of "simple" circuits may have significance for understanding the microcircuitry of more complex brains, where similar effects have been reported (Dowling 1989).

Invertebrate nervous systems have played a key role in the analysis of rhythmic behaviors at the circuit level, because they contain large, identifiable neurons. Many such neural circuits have been described – leech heartbeat (Calabrese and Arbas 1989), snail feeding (Benjamin and Elliot 1989), *Tritonia* swimming (Getting and Dekin 1985) and *Clione* swimming (Arshavsky et al. 1985), to name a few. All CPGs produce stereotyped rhythmic patterns without rhythmic input (Delcomyn 1980). That is, if a CPG is activated non-rhythmically, the machinery for producing the spatio-temporal motor pattern can be shown to be intrinsic to the CPG itself. The "fictive" motor pattern, (i.e., the pattern produced in the neurons which would result in real movements if the nerves were connected to muscles). The fictive pattern is somewhat different from the motor pattern responsible for the behavior in the intact animal, because of the lack of sensory feedback and chemical modulation normally present. It was generally assumed that a complete description of a circuit meant that the generation of the motor patterns and the subsequent behavior was now physiologically understood (Selverston 1980). We know now that a description of the circuit is only the first step towards understanding.

One characteristic of invertebrate CPG circuits is that each produces remarkably similar and rather simple motor output patterns – bursts of impulses with varying phase relationships between the bursts. The dynamics of each burst and the details of the phase relationships between bursts in different motor neurons vary from animal to animal just as the behaviors they produce do. It is clear, however, that the fundamental cellular and synaptic "building blocks" of CPGs, as Getting referred to them, are present in all CPGs (Getting 1989). Each identified neuron posesses a complement of receptors, transmitters and channels which help to give it a unique identity – a distinct set of biophysical characteristics. Some of the most important, in terms of pattern generation, are: bursting pacemaker potentials, plateau properties, accommodation, postinhibitory rebound, excitatory and inhibitory synaptic potentials. Each CPG system arranges these neuronal building blocks in a unique way in order to generate specific motor patterns for a particular set of evolved behaviors.

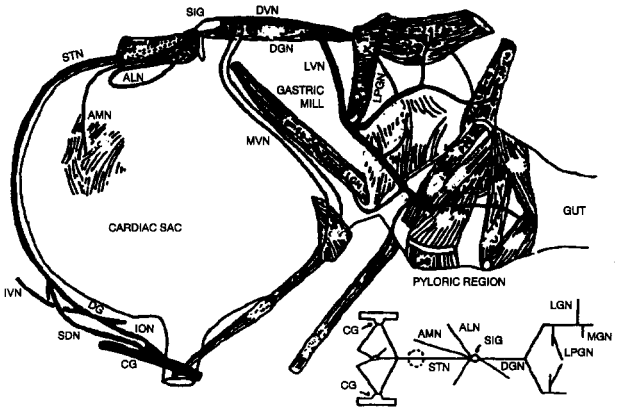
At the moment, there does not appear to be a general set of rules for CPG circuit construction, but instead there are a large number of combinations and permutations which can be used to assemble the building blocks into a functional network, i.e. many solutions to the problem. Taking a reductionistic approach suggests that because of all the parameters which can be measured, CPGs are really complex systems of a rather high dimension. If this is true, why do the emergent patterns appear simple? Is it reasonable to ask if there are any general principles which can be recognized. Can we, for example, determine whether a pattern arises from intrinsic cellular properties (like bursting pacemaker potentials) or from the collective computational properties of the circuit? Or, when the same muscles are used for different

behaviors (say, scratching or running), are they controlled by different circuits or by changing the properties of a single circuit, i.e., are the circuits dedicated to one behavior or can they be functionally rearranged to perform different behaviors? Are there limits to how wide a range of behaviors can be produced by a collection of neurons? Do different behaviors represent variations of what is otherwise a dominant behavior, as in the walking, trotting and galloping modes of quadruped locomotion? The answers to these questions have been given a new dimension as a result of recent studies of neuromodulation in the crustacean stomatogastric system.

## The lobster stomatogastric system

The lobster stomatogastric (STG) system contains 4 CPGs, each producing a unique rhythmic motor pattern. The patterns are used to operate muscles of the foregut comprised of the esophagus, cardiac sac, gastric mill and pylorus. The general features of each pattern are similar to the patterns produced by many other CPGs, both vertebrate and invertebrate. While the actual behaviors are often not thought of in the same way as neuroethologists usually view behavior, in fact there is no physiological distinction between foregut movements and the movements of externalized appendages. And the fact that only 30 neurons are involved puts them in a class by themselves in terms of working out neural circuits. The entire stomatogastric nervous system can be dissected off of the stomach wall and placed in a saline filled Petri dish, where it will remain alive for many hours. All of the CPG patterns can be observed by recording their "fictive" activity in the motor nerves (Fig. 2), while the synaptic integrative activity is recorded intracellularly from the cell bodies in the stomatogastric ganglion.

Four different patterns can be observed. The *esophageal* rhythm has the lowest frequency and is the most irregular of all the patterns, occurring at intervals of 2 to 20 s (Spirito 1975). The cardiac sac pattern has a period of approximately 7 s and is also somewhat irregular (Dickinson and Marder 1989). The pyloric and gastric mill patterns were the first to be described in detail (Mulloney and Selverston 1974a, b; Selverston and Mulloney 1974; Maynard and Selverston 1975) and their synaptic circuitry has been completely determined (Selverston et al. 1976). The pyloric rhythm has a frequency of approximately 2 Hz while that of the gastric mill is slower, approximately 0.33 Hz.



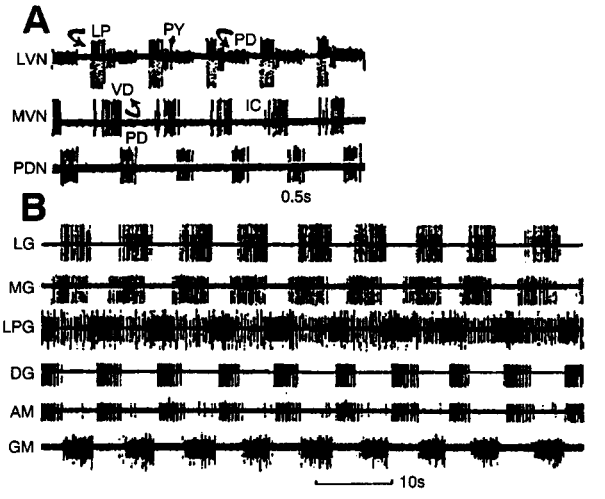




**Fig. 2A, B** A schematic side view of the lobster foregut showing the 4 principle parts, the esophagus just below the cardiac sac, the gastric mill and the phyloric region. The principle input nerve to the stomatogastric ganglion (*STG*) is the stomatogastric nerve (*STN*). This in turn is made up of the inferior and superior oesophageal nerves (*ION* and *SON*), which leave the paired commissural ganglio (*CG*). A small, unpaired oesophageal ganglion (*OG*) lies at the confluence of the two IONs and the IVN nerve from the brain. All of the other nerves shown in the diagram are motor and can be dissected from the stomach and pinned out as shown in the inset. Extracellular recordings from these nerves show the fast pyloric rhythm (**A**) and the slower gastric mill rhythm (**B**). The *dotted circle* around the STN (*inset*), is the location of a vaseline well which can be used to apply a sucrose block to the nerve. This removes descending inputs from the commissural ganglia reversibly and can be used to temporarily stop the rhythmic activity

## Behavior

Experimental neuroethology usually begins by analyzing a behavior in, or close to, conditions as natural as possible, and then working from the peripheral nervous system inward to the CNS, the so-called "top down" approach. For example, behaviors are generally plotted as an "ethogram" over time. Myograms monitor the timing of muscle contractions and therefore the output of the central nervous system. The bursts of electrical activity in the muscles can be correlated with the behavior, and how each individual muscle is involved in producing a specific movement can be determined. For the lobster, the stomatogastric ganglion and its associated nerves are removed from the stomach wall and electrodes placed on the motor nerves. Bursts of impulses are recorded which can be shown to be almost identical to bursts in the intact animal (Rezer and Moulins 1983; Turrigiano and Se-

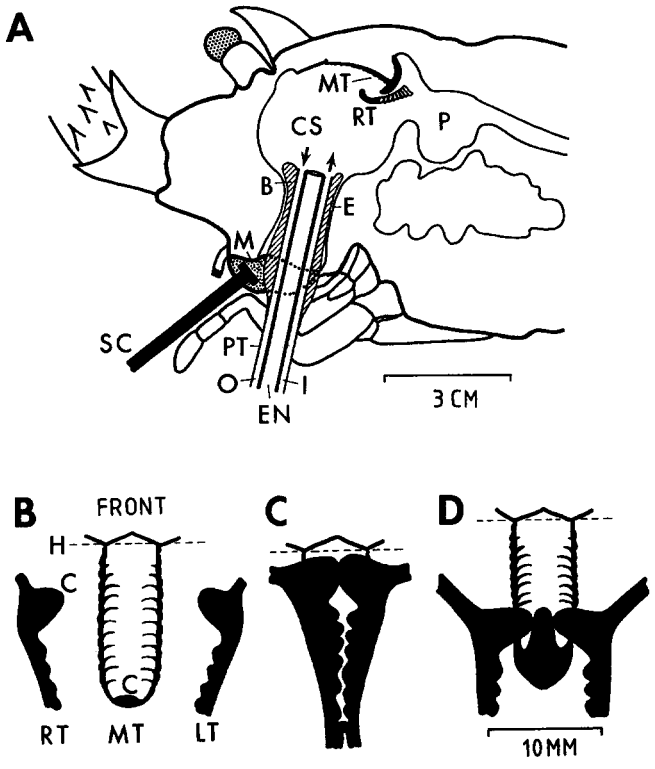



**Fig. 3A–D** Method for viewing the teeth of the gastric mill was developed by George Heinzel in the author's laboratory. A rigid endoscope (*EN*) is inserted into the mouth and the stomach is filled with sea water to inflate it and to carry away gastric juices (**A**). A television camera can be attached to the endoscope to record the movements for quantitative analysis. The teeth appear as shown in the open position (**B**), in the closed position before the medial tooth is pulled forward (**C**), and after the 3 cusps of the teeth come together (**D**). *Abbreviations*: *MT* – Medial tooth; *RT* – Right lateral tooth; *P* – Pylorus; *CS* – Cardiac sac; *B* – Balloon; *E* – Esophagus; *M* – Mandible; *SC* – Screw; *PT* – Protective tube; *O* – Outlet; *I* – Inlet; *EN* – Endoscope

lverston 1990). The most complex lobster CPG operates the gastric mill, a set of teeth which macerate coarse pieces of food torn off by the mandibles and stored in the cardiac sac portion of the stomach. A second CPG is responsible for movements of the pylorus, basically alternate constriction and dilation movements which filter the macerated food. The mechanisms which generate the stomatogastric motor patterns are similar to those which generate motor patterns for the legs or other external appendages. The underlying assumption is that stomatogastric circuitry uses the same building blocks and similar basic architectures as are used by all other CPGs.

The esophagus and cardiac sac regions have movements which also consist of dilations and contractions, and each movement has its own CPG. The muscles controlling these behaviors are arranged antagonistically, much like the muscles of the pylorus. Within the gastric mill are two lateral and one medial tooth. Each of the three teeth has its own set of antagonistic muscles, but unlike the other regions, all three teeth perform coordinated chewing movements roughly similar to the chewing movements of our own teeth. The movements of the teeth can be observed by placing an endoscope into the stomach (Fig. 3). In the intact animal the spontaneous movements of the teeth are irregular, but have a typical pattern (Heinzel 1988). The two lateral teeth close on a food particle like the jaws of a vise. The medial tooth pulls forward, grinding over the food, and then resets to its original position. As the medial tooth is reset, the lateral teeth are opened to complete the cycle. While the rhythmic activity is almost clock-like in the in vitro preparation, recordings made from intact animals show considerable variability (Rezer and Moulins 1983). The irregularity of the movements is not surprising in the intact animal, since both sensory feedback loops and neuromodulatory substances are present. By videotaping the movements, the trajectories of the teeth can be plotted as an ethogram, and the different movement patterns quantified.

## The STG neural circuits

All of the motor neurons participating in the gastric and pyloric rhythms were identified by correlating the impulses in the motor nerves with spiking activity recorded from cell bodies within the ganglion. Then, by recording from each neuron while stimulating each of the others, all of the synaptic and electrical connections could be definitively established. There are 10 motor neurons in the gastric mill CPG and 13 in the pyloric CPG. In addition, each of the CPGs has one interneuron. As seen in Fig. 4, most of the connections are inhibitory, despite the fact that they are also motor neurons and excitatory at the neuromuscular junction. There are extensive electronic connections between neurons, but very few depolarizing synapses.

How can we interpret such a complex set of interconnected neurons in terms of the spatio-temporal patterns they generate? One can describe the circuits simplistically, cell by cell and synapse by synapse, describing the output of the different neurons in relation to the connectivity. For example in the gastric mill, we see that the LG and MG neurons which close the lateral teeth are electrically coupled and are connected by reciprocal inhibition with the two LPGs which open the lateral teeth. The electrical coupling insures that the LG and MG fire at the same time, while the inhibitory connections insure that they are out of phase with the LPGs. Such reciprocal inhibition had been postulated for years as the basic mechanism for the alternate contraction of antagonistic muscles (Brown 1911). Similarly, the DG and AM are electrically coupled, insuring they fire together, and inhibitory connections from DG and AM to the GMs insure that they do not fire at the same time as the GMs. But as we can see from the circuit diagram, there are many more connections than these, and all of the connections are operating in parallel, not serially, as I have described them.

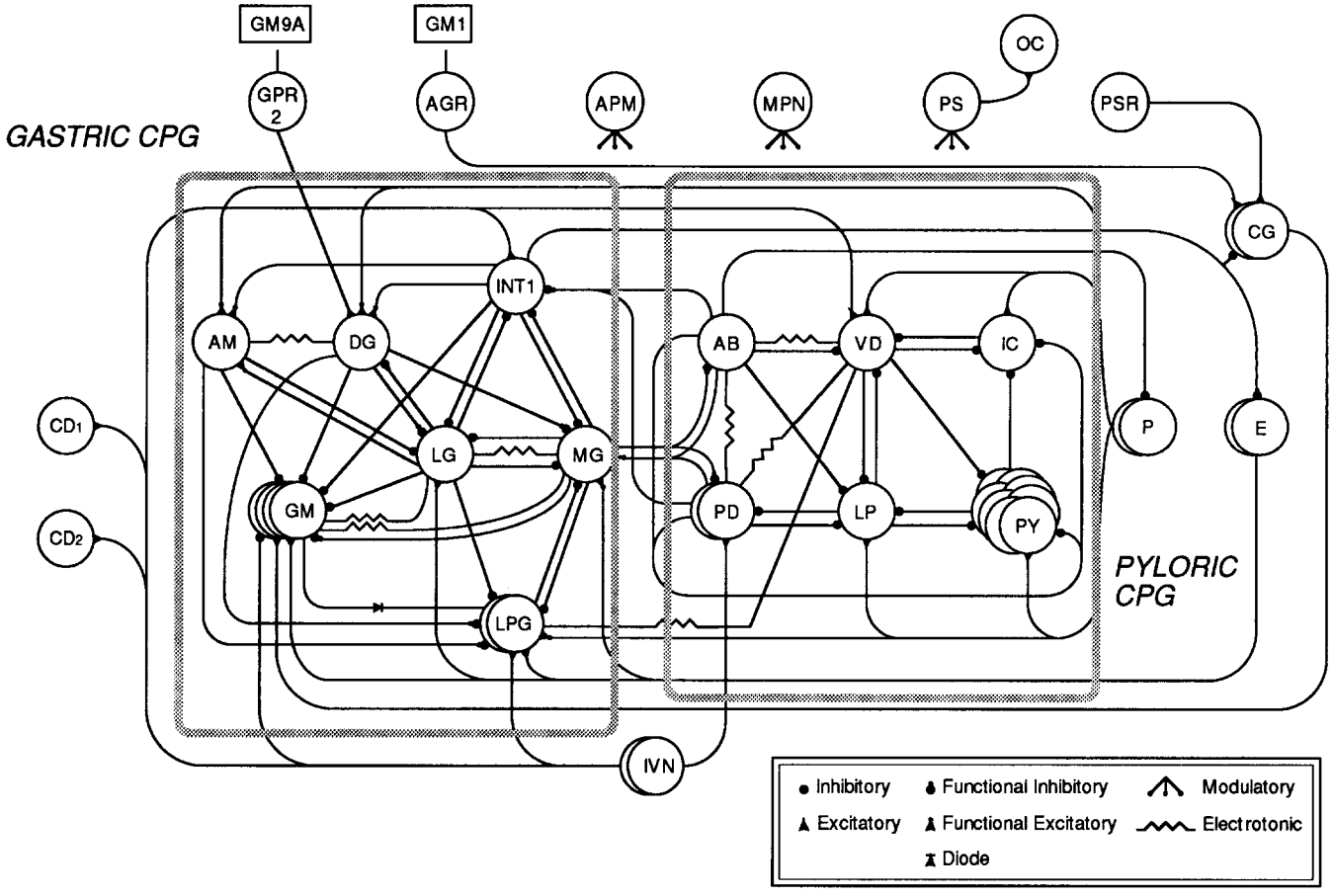



**Fig. 4** Summary of the known synaptic connections between neurons of the stomatogastric network. Data are a composite derived from several crustacean species. The *two hatched boxes* enclose neurons of the gastric mill and pyloric CPG. $CD_1$ and $CD_2$ are identified neurons of the cardiac sac CPG. *GPR* and *AGR* are stretch receptors of muscles *GM9A* and *GM1*, respectively. *APM, MPN* and *PS* are identified neuromodulatory cells. *P* and *E* neurons are phasic drivers of the pyloric and gastric rhythm located in the commissural ganglia (note that they receive a copy of the CPG pattern via the AB and Int1 neurons). The two *IVN* neurons are located in the brain. The synaptic connections are predominantly inhibitory and there is linkage between the gastric and pyloric CPGs. The PS neuron inhibits OC, one of the esophageal motor neurons and the PSR (posterior stomach receptor) inhibits the CG (Commissural gastric) neurons, two identified neurons in the commissural ganglia

## Modulation of the STG circuits

One of the biggest surprises that emerged from STG research was the finding that the circuits were not fixed "hard-wired" entities as originally assumed, but chemically modifiable in terms of their synaptic and cellular properties. Reports from a large number of laboratories began to indicate that not only were there many putative neuromodulatory pathways leading to the stomatogastric circuits, but that stimulating identifiable neurons containing these substances led to profound changes in the motor output patterns (see: Harris-Warrick et al. 1992). Similar changes could be produced simply by superfusing the ganglion with certain peptides or amines, suggesting the neuromodulators could be released in a paracrine fashion into the ganglionic neuropil.

Furthermore, immunohistochemical staining for particular modulatory substances showed a large number of immunoreactive axons descending via the stomatogastric nerve into the stomatogastric ganglion, where they branched profusely within the neuropil (Marder et al. 1987). Using radioimmunoassay (RIA), it was possible to show that some neuromodulators were released from distant sites such as neurohemal organs (Turrigiano and Selverston 1991), and reached the STG neuropil via the blood stream. This was consistent with behavioral results observed following the injection into the blood of such substances as serotonin (Kravitz 1988). While it is probable, based on latency of action, that substances released from axons entering the ganglionic neuropil are released in paracrine fashion, there is no proof yet that this is acutally the case. The terminals of these axons may in fact make conventional synaptic contact with postsynaptic neurons. An additional possibility is that the chemical substances which are considered to be conventional neurotransmitters in the ganglion (glutamate and acetylcholine) may also have metabotropic effects.

It follows that functionally rewiring the CPG circuits has profound behavioral consequences. When a particular neuromodulator is injected into the intact animal, it produces a characteristic pattern of activity. For the teeth

in the gastric mill this may be observed both endoscopically and electrophysiologically. These substances produce modification of ongoing patterns, or in the case where a CPG was inactive, the initiation of a characteristic form of movement. Similar effects are observed in vitro by superfusion of the neuromodulator directly over the ganglon or by direct depolarization of a neuron known to contain a neuromodulatory substance (Nagy and Dickinson 1983). The action is quite similar to the distinct behaviors previously observed when a "command" fiber was stimulated, except that in the present case the behavior far outlasts the period of stimulation.

What cellular mechanisms are involved the initiation or change in behavior? From studies using amines on the pyloric CPG, Harris-Warrick and his colleagues showed that octopamine, serotonin and dopamine all produced different rhythms by binding selectively to different neurons and changing their biophysical properties in a characteristic way (Flamm and Harris Warrick 1986). Some cells became bursters while other fired tonically or not at all. The strengths of chemical and electrical synapses were weakened or strengthened selectively. Many of the conductances responsible for the "building blocks" of the neural circuits were altered; for example the duration of the plateau phase could be lengthened or shortened. When the modulated circuit was compared with the unmodulated one, it was clear that the circuit had been had been *functionally* reorganized. That is, the CPG is in essence an anatomical concept even though the connections between neurons were determined electrophysiologically. Neural circuits in invertebrates are determined by paired stimulation and recording from identified neurons. This was adequate for mapping out the connectivity; however, it provided little information regarding the functional properties of the system. We now know these properties are determined by the chemical "soup" which is bathing a CPG at any given time.

Preliminary studies indicate that the cellular mechanisms are the same as those which have been described for other neuromodulatory systems. The particular neuromodulatory substance binds to those neurons which have receptors for it. Each neuron in a CPG appears to have a unique constellation of receptors. Once bound, the substances exert a metabotropic effect, i.e., a G-protein in the membrane is activated after the neuromodulator has bound to a receptor on the outside of the cell. This raises the concentration of a second messenger inside the cell which, in turn, leads to an increased concentration of a protein kinase, again with different neurons appearing to use different kinases. This cascade of events leads to the phosphorylation of particular membrane proteins in each neuron – a specific ion channel, for example – and the result is a change in ionic conductance. Depending on which proteins are phosphorylated, the neuron changes its biophysical properties, or synapses change their strengths. As a consequence, the output of the network as a whole is changed. We still have a lot to learn about the cellular mechanisms responsible for these changes, but it is clearly an experimentally tractable problem.

At the present time, about 15 neuromodulators are known for the STG system. Most have been studied independently of one another, so possible synergistic or antagonistic effects between modulators have not yet been described. There are some data which suggests the responses follow complex dose-response curves, and that different concentrations may not lead to simple gradations in response, but to entirely different states altogether (Flamm and Harris-Warrick 1986). Another important problem about which little is known in this system is determining the mechanisms involved in the termination of modulatory effects. Dephosphorylation by phosphatases are likely to play a role here (Kaczmarek and Levitan 1987).

Many of the modulators for the STG have been localized within identified neurons – the APM cell (Nagy and Dickinson 1983), the IVN neurons (Claiborne and Selverston 1984), the GPRs (Katz and Harris-Warrick 1989). MPN (Nusbaum and Marder 1989). The GPRs are mechanosensory neurons with both fast and slow effects on STG circuitry and which are activated by movements of the gastric mill. The mechanisms which activate most of the other identified neuromodulatory cells are unknown. It remains to be discovered whether such neurons are activated internally or externally, and how they regulate the amount of modulator secreted. Since most conventional transmitters can also have metabotropic effects, the possibility exists that the release of glutamate and acetylcholine within the ganglion may have both fast, ligand-gated effects as well as slow, second-messenger gated effects. This would be an action different from the already demonstrated co-transmitter release by the GPRs. Here, Katz has demonstrated that activation of the gastropyloric receptors releases both Ach, which has a fast nicotinic effect, and serotonin, which has a slower metabotropic effect (Katz et al. 1990).

## Behavioral role of neuromodulation

The only substance which has been examined with a view towards linking neuromodulation to behavior is the lobster version of the mammalian gut peptide cholecystokinin. Cholecystokinin-like peptides (CCKLP) have been found in many species of vertebrates and invertebrates (Larson and Vigna 1983). In mammals, CCKLPs are released from the gut and enter the bloodstream where they affect gut motility and induce a feeling of satiety. Lobster CCKLPs appear to play an important role in modulating the gastric mill. CCKLP can be shown by immunohistochemistry to be contained in fibers of the stomatogastric nerve that terminate in the stomatogastric ganglion. Electrical stimulation of these input nerves causes release of CCKLP into the ganglion (Turrigiano and Selverston 1989). Application of mammalian CCK to the in vitro preparation can initiate activity in a nonbursting ganglion, or intensify ongoing activity. These two observations suggest that lobster CCKLP can act as a endogenous modulator of the gastric rhythm.

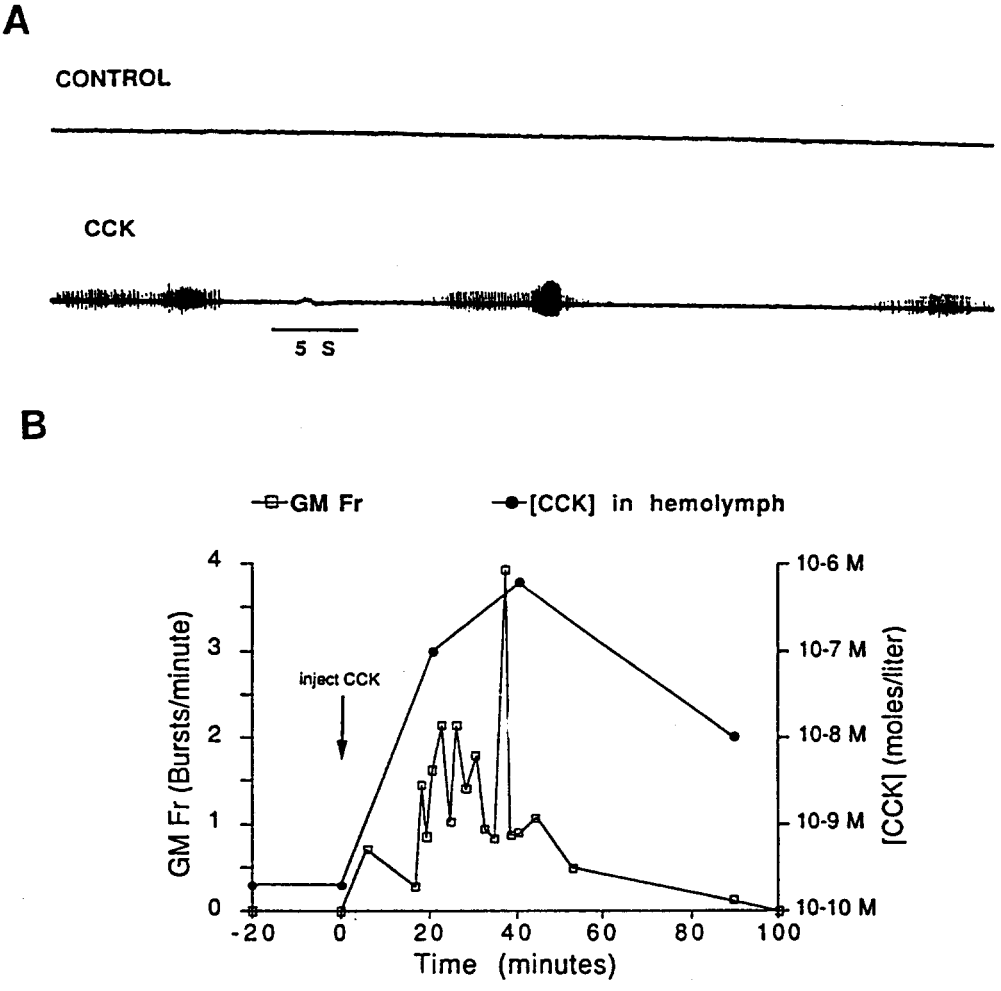



**Fig. 5A, B** Behavioral effects of the neuromodulator cholecystokinin (*CCK*) can be seen by monitoring the gastric mill muscle GM1. In the control record of **A**, the muscle is inactive indicating the gastric mill is not chewing. After injection of mammalian CCK-8 into the hemolymph, the gastric mill turns on as indicated by the long bursts. **B** The increase in burst frequency of muscle GM1, is correlated with a gradual increase in hemolymph concentration (measured by R.I.A.)

In addition to local release, CCKLP also can have a neurohormonal effect in controlling the gastric mill. CCKLP is present in high concentrations in paired neurohemal pericardial organs. Measurable quanties of CCKLI can be found in the hemolymph of lobsters, using RIA and antibodies directed against mammalian CCK8. Levels of CCKL peptides can be shown to increase following feeding (Turrigiano and Selverston 1989). It has also been shown that this increase can be correlated with the onset of EMG activity in gastric mill muscles. Injection of mammalian CCK8 also can be shown to cause activation of the gastric mill (Fig. 5) (Turrigiano and Selverston 1990). In both cases the activation can be stopped by the selective CCK antagonist proglumide, suggesting a CCKL peptide is actually used by the animal during feeding.

## Interactions between related circuits

The idea that neural circuits are under continuous modulation, and therefore are dynamical as opposed to fixed systems, has had a revolutionary effect on our thinking about the neural control of behavior. What is clear from recent data is that the concept of the CPG is changing drastically. It is now clear that many of the most fundamental questions regarding the cellular mechanisms of rhythmic behaviors, and possibly more complex episodic behaviors as well, may be answered by the analysis of neuromodulators. For example, most of the muscles producing rhythmic movements, especially those involving the appendages, participate in many different behaviors. It has been tacitly assumed that the most likely basis for this was for there to be a separate CPG for each distinct behavioral mode, say scratching, walking, running etc. There is a growing body of evidence, however, that the concept of even a single CPG is arcane and that CPG circuits can be carved out of the CNS by the action of neuromodulators. Presently, there appear to be 4 distinct classes of modulation which imply this is the case (see: Dickinson and Moulins 1993).

• *Single neuron switching* – one neuron in the pyloric rhythm (VD) and one in the gastric mill rhythm (AM) can be shown to switch to the cardiac sac rhythm under the appropriate conditions (Hooper and Moulins 1989). The mechanisms for producing the VD switch are known. In a typical combined preparation, with the commissural and esophageal ganglia still attached to the stomatogastric ganglion, the VD neuron always fires in py-

loric time. Under these conditions, inputs from the IV neurons of the cardiac sac CPG are not effective in overcomming the strong plateauing shown by the VD neuron. Immediately after a burst of activity in a sensory nerve called in vpln, however, the plateau phase is shortened to such an extent that it is now strongly influenced by IVN input and the neuron now switches into cardiac sac time. Although the actual neuromodulator released by the sensory nerve stimulation has not been identified, it's action on the VD is selective, and no other pyloric neurons switch from one rhythm to the other.

This simple modification in a cellular property is reflected behaviorally. The cardiopyloric valve muscle is actually bifunctional receiving innervation from both VD and the $CD_2$ neurons, and can operate in both cardiac sac or pyloric time. The switching insures only one pattern at a time is operational.

The AM neuron, usually thought of as a member of the cardiac sac CPG, can also switch to cardiac sac time under the influence of the neuromodulator Red Pigment Concentrating Hormone (RPCH) (Dickinson and Marder 1989). The behavioral effects are to convert the cardiac sac pattern from a single phase (dilation) to a two-phased rhythm (dilation and constriction).

• *Multiple neuron switching* – In the crab stomatogastric ganglion, Weimann has found that there is so much switching of neurons between the gastric mill and cardiac sac rhythms that it its really not possible to distinguish between two separate CPGs (Weimann et al. 1991). He considers the ganglion to be made up of a single "gastro-pyloric" pool of neurons which form a continuum between the two patterns. Depending on the neuromodulators present, different neurons can move freely between gastric and pyloric rhythms by changing their intrinsic membrane or synaptic properties.

A unique form of multiple neuron switching is caused by the neuromodulator serotonin, when released from the gastropyloric sensory receptor neurons (GPRs) (Katz and Harris Warrick 1990). When active, the GPRs, cause the MG and LG neurons to fire in pyloric time by enhancing the plateau properties of the IC neuron. Since IC is strongly electrically coupled to MG and LG, these neurons are constrained to now fire in a pyloric, rather than in a gastric mill rhythm.

• *Network fusion* – In the third example cited by Dickinson, an entirely new network can be constructed from two or more other networks. It had been known for many years that a strong burst from the cardiac sac dilators caused a strong interruption of the gastric mill pattern. One can achieve a semi-permanent fusion of the two networks (gastric mill and cardiac sac) by superfusion of the peptide red pigment concentrating hormone (RPCH) over the in vitro preparation. The mechanisms are similar to those described for VD neuron switching. The IV neurons normally have only weak excitatory inputs to 3 kinds of gastric mill neurons, int 1, GM and LPG. The presence of RPCH, however, greatly strengthens these synaptic connections so that they now fire with the IVs. But because the other connections are still present, there is a blending of both rhythms into a single conjoint pattern (Dickinson et al. 1990). Unfortunately, the behavioral significance is unknown.

• *De novo networks* – Meyrand and his collegues have shown in the European lobster *Hommarus gammarus*, that when two identified neurons, the pyloric supressors (PS), are active, there is a total disruption of the gastric, pyloric and esophageal patterns; there then appears an entirely new pattern involving neurons which innervate muscles of all regions of the foregut (Meyrand et al. 1991). The new pattern appears to be synchronus with opening and closing of the cardiac sac valve, and is suggestive of a swallowing movement. The cellular mechanisms involved are mainly increases and decreases in regenerative burst properties. Some neurons no longer respond to their normal synaptic inputs, and are free to respond to the strong inputs from the PS units. Like the others, this new pattern can be achieved by perfusion of the stomatogastric ganglion with the appropriate neuromodulators. Such a drastic disassembly of several "independent" CPG circuits and the construction of an entirely new circuit raises fundamental questions about how the nervous system produces different motor patterns.

## Summary

What have we learned about behavior from neuromodulatory studies of the crustacean stomatogastric system? The emphasis of this paper has been on the analysis of one single class of behaviors (rhythmic) in terms of microcircuitry (synaptic connections between identified neurons). But in the general case, all behaviors result from the generation of spatio-temporal patterns by the central nervous system. How individual nerve cells interact with each other to produce such patterns is of fundamental interest. We know from work on simple networks that it is possible to link the circuitry of the nervous system with behavior in a precise way, and that instead of a large number of dedicated circuits, behaviors can be altered by chemically adjusting the functional properties of the neuronal elements. One circuit can be configured to perform a variety of different behaviors by activating neurons which contain neuromodulatory substances or in response to neurohormones circulating in the hemolymph. At present we know only a few of the ways neuromodulatory neurons are triggered to release their contents onto the neurons making up CPGs.

The findings described here raise many questions. What are the parameters which control the distribution of neuromodulatory substances throughout the nervous system? What happens when more than one neuromodulator is present? At the cellular level, what mechanisms are involved in transforming each neuron from one functional state to another, and then how does the entire constellation of changes give rise to a new output? It is important to answer such questions in reduced networks, because there are presently no techniques available to answer

them in the more complex networks of the brain. While there is no question that modulatory activity occurs in the brain, whether or not the principles which have been discovered by using "simple" invertebrate circuits scale up to vertebrate circuits remains an intriguing question.

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