Mechanisms Underlying Pattern Generation in Lobster Stomatogastric Ganglion as Determined by Selective Inactivation of Identified Neurons. I. Pyloric System

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SUMMARY AND CONCLUSIONS

1. Four factors contribute to pattern generation in the pyloric network of the lobster stomatogastric ganglion. These are: 
   a) endogenously oscillating neurons; 
   b) synaptic network properties; 
   c) nonlinear cellular properties, including the generation of plateau potentials; and 
   d) excitatory input from the commissural ganglia. The roles and relative importance of these factors were investigated with a new technique for inactivating single specific identified neurons.

2. In stomatogastric ganglia in which the excitatory input is left intact, 
   a) pattern generation continues when any cell or pair of cells other than the endogenous bursters are inactivated, 
   b) pattern generation also continues when the endogenous bursters are inactivated, 
   c) pattern generation ceases when the endogenous bursters plus one other particular cell are inactivated. This cell, although not an endogenous burster, displays a strong tendency to generate plateau potentials.

3. In stomatogastric ganglia that have been isolated from excitatory input, 
   a) pattern generation continues when any cell or pair of cells other than the endogenous bursters are inactivated, 
   b) pattern generation ceases when the endogenous bursters are inactivated.

4. Some of the inputs to the stomatogastric ganglion normally fire in bursts. However, their potentiation and acceleration of the output pattern are also produced by tonic stimulation of the nerve. The effect of one of those inputs is mimicked by bath application of dopamine to the stomatogastric ganglion.

5. The roles and importance of the three most important factors were qualitatively summarized in a chart specifying the activity of the network as a function of its intactness.

INTRODUCTION

The neural basis for the production of rhythmic movements such as heartbeat, breathing, locomotion, and mastication have been studied in many phyla, from mollusks to man. In many instances where such rhythmic phenomena are observed, the generation of the rhythm can be shown to operate without peripheral sensory feedback (7, 17, 36). The stomatogastric ganglion of the lobster fits the criteria for such an automatic "central pattern generator" (10, 31). When totally isolated from the rest of the nervous system, this ganglion generates a regular and highly ordered motor-output pattern, similar in all essential respects to the pattern observed in intact animals (24, 31).

The lobster stomatogastric ganglion has been described in great detail with respect to its circuitry (31), cellular properties (12, 29), and neurotransmitter biochemistry (20). Of the 30 neurons in this ganglion, 14 neurons control the peristaltic filtering and transport of food particles in the pylorus of the foregut. These "pyloric" neurons produce a rhythmic motor-output pattern with a repetition frequency of about 2 Hz.
Models for the generation of such rhythmic patterns fall into two distinct classes. The first class requires one or more cells in the neuronal network to be "endogenous oscillators." According to this model, the existence and frequency of the output pattern derive from the cellular properties of these specialized "bursting" neurons. The phasing of the remaining cells is determined by the synaptic interactions among all the cells in the network. The second class of models does not require any of the neurons in a network to be endogenously "bursty." Rather, the synaptic connectivity of the network as a whole results in an oscillatory or "resonant" mode of activity. Thus, the existence, frequency, and phasing of the output pattern are said to be emergent properties of the network, as opposed to intrinsic properties of single neurons.

The pyloric system has been considered a classic example of a central pattern generator of the first class, i.e., one driven by an endogenous oscillator neuron (21). Although the synaptic connectivities of neurons in this and several other invertebrate pattern generators have been worked out in considerable detail (31, 33), the complexity of these systems has confounded attempts to understand and explain their operation at a mechanistic level. In a series of reports beginning here, we describe investigations of the mechanism underlying pattern generation in the lobster stomatogastric ganglion. In these studies, we have used a new technique to inactivate functionally specific identified neurons during ongoing physiological experiments. Our rationale has been that the role and importance of a particular cell can be judged by the qualitative and quantitative effects its inactivation has on the output pattern. Using this technique, we have obtained several unexpected results, necessitating a reevaluation of pattern generation in this system.

On the basis of earlier experimental evidence, the pyloric rhythm was thought to be generated by a group of three endogenously bursting neurons. Surprisingly, although the endogenous bursters do play an important role, the patterned output will continue when the bursters are inactivated. The excitatory input to the ganglion, the network interactions, and nonlinear proper-

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**FIG. 1.** Schematic diagram of dissected stomatogastric nervous system and the spontaneous pyloric rhythm in the blocked and unblocked condition. Abbreviations: CG, commissural ganglion; R, recording electrodes; S, stimulating electrodes; STN, stomatogastric nerve; STG, stomatogastric ganglion; MVN, median ventricular nerve; PDN, pyloric dilator nerve; LVN, lateral ventricular nerve. The position of the sucrose block is indicated. The extracellular recordings show units contained in each nerve. The MVN contains the VD neuron (large spike) and the IC neuron (small spike). The PDN contains axons of the two PD neurons. Note the decrease in frequency of the rhythm during the STN block.

**METHODS**

Experiments were performed on the California spiny lobster, *Panulirus interruptus*, obtained locally and kept in running seawater aquaria. Procedures for removing the stomatogastric nervous system from the lobster stomach are described elsewhere (25). All experiments were done with the commissural ganglia left attached to the stomatogastric ganglion via the stomatogastric nerve. The dissected preparations were pinned out in Sylgard-lined petri dishes and kept at a constant temperature of 18°C. Extracellular pin electrodes were used to monitor pyloric activity and to stimulate the stomatogastric nerve (STN). The entire output pattern of a preparation can be monitored by recording only two nerves: the lateral ventricular nerve (LVN) and the median ventricular nerve (MVN), as diagrammed in Fig. 1. The LVN contains axons from the pyloric dilator (PD) cells, the lateral pyloric (LP) cell, and the pyloric (PY) cells. The MVN contains axons from the ventral dilator (VD) cell and the inferior cardiac (IC) cell.
Fig. 2. Schematic diagram of illuminator. The light from a 100-W mercury arc lamp is condensed and passed through heat filters consisting of a water chamber and two dichroic mirrors. The light is then passed through a blue filter (Zeiss BG 12) and an interference filter with peak transmission at 426 nm. This blue light is reflected up through a long working-distance condenser onto the tissue. The intensity of the light at the sample plane is $2.5 \times 10^7$ erg/cm$^2$ s, as measured with a YSI-Kettering 65 radiometer. Fluorescence is viewed through a yellow-barrier filter (590-nm shoulder) attached to the objective of a stereoscopic dissecting microscope. The reflector and condenser can be adjusted by means of a rack and pinion or moved out of the way entirely to allow normal white-light transillumination of the ganglion.

Because the PD cells' action potentials were usually very small on the LVN recordings, we also routinely recorded their activity on the pyloric dilator nerve (PDN).

Three standard experimental techniques were employed in these studies: 1) A sucrose block of the stomatogastric nerve was used in several experiments to block temporarily and reversibly traffic in the stomatogastric nerve. This was accomplished by building a small Vaseline dam around the nerve and filling the pool with isotonic sucrose. In such cases, activity through the nerve was blocked within a minute. Replacing the sucrose with normal saline restored the activity just as rapidly. The position of the Vaseline dam and the effect of the sucrose block on normal activity are shown in Fig. 1. 2) Passage of current through intracellular microelectrodes in cell bodies was used to depolarize or hyperpolarize neurons, activating or suppressing neuronal activity for brief periods of time. Intracellular recording and current passing was carried out with 3 M KCl microelectrodes having resistances of 20–60 MΩ. 3) The stomatogastric nerve could be stimulated with extracellular pin electrodes, proximal to the sucrose block point, to mimic the activity of excitatory input.

A newly developed technique was used to inactivate single identified neurons functionally. This technique for dye-sensitized photoinactivation of single cells is described in detail elsewhere (23). To summarize, the cell to be inactivated was first filled with the dye lucifer yellow (32), and the tissue containing that cell was then illuminated with intense blue light for 5 min. Only that cell containing the dye was inactivated. Three criteria were used to judge when the neuron had become completely inactivated: 1) the resting potential depolarized to zero, 2) action potentials ceased in the cell's peripheral axon, and 3) postsynaptic potentials (PSPs) onto follower cells were no longer observable. Preparations were monitored for up to 6 h after the cell inactivations. Over this period, no recovery of function was ever seen in an inactivated cell. Neither illumination nor dye filling alone had any observable effects on cell function. For the purpose of this paper we use the terms killed and inactivated interchangeably.

A neuron was dye filled by passing 3–5 nA of hyperpolarizing current into the cell for 15–30 min through a beveled electrode containing 2% lucifer yellow. An adequately filled cell will appear light yellow with normal white-
light transillumination. To inactivate a dye-filled neuron, the ganglion was illuminated with the apparatus diagrammed in Fig. 2. A substage sliding reflector permitted switching between normal diffuse white light and the intense, focused blue light for cell inactivation without disturbing the extracellular recording electrodes. Lucifer yellow fluoresces brilliantly when illuminated with this blue light, allowing clear visualization of the dye-filled neuron through a conventional stereoscopic dissecting microscope, as shown in Fig. 3. This permitted us to judge whether or not a neuron had been completely dye filled before we proceeded with the experiments.

Our purpose in conducting the experiments described below was to investigate the role and relative importance of specific identified neurons in this network. The cell-inactivation technique offered a unique tool for this purpose. It is possible to shut off a cell temporarily by passing hyperpolarizing current into its cell body. However, inactivating an entire cell quickly, without damaging any other cells, is more effective than hyperpolarization for three reasons: 1) With hyperpolarization it is difficult to keep cells shut off for more than a few seconds without having to pass prohibitively large amounts of current. 2) It can never be known with certainty that the level of hyperpolarization that suppresses spiking is completely blocking transmitter release at the most distal terminals, especially in systems where subthreshold voltage fluctuations can modulate synaptic transmitter release. 3) The electrotonic connections in the pyloric system allow injected current to spread between several neurons. Thus, hyperpolarization of one neuron functionally inhibits all neurons with which it is electrically coupled. The cell-inactivation technique eliminates this problem, since only the dye-filled and irradiated cell is affected. Lucifer yellow is, however, freely diffusible across gap junctions. When one cell is filled with dye and irradiated, any other electrically coupled cell is damaged to an extent dependent on the amount of dye it has accumulated. However, when one cell in an electrically coupled pool of neurons is rapidly filled and immediately irradiated, no signs of damage are observable in the coupled cells. Rather, as discussed previously (23), the inactivated cell uncouples from the other cells in the electrically coupled pool. Thus, the subsequent depolarization of the inactivated cell does not affect other cells in that pool.

All experiments reported here were repeated with similar results at least 3 times.

RESULTS

Inactivation of all endogenous bursters

The synaptic connectivity of neurons in the pyloric subset of the stomatogastric ganglion is diagrammed in Fig. 4. The pyloric system contains three endogenous

![FIG. 3. Appearance of the stomatogastric ganglion with a VD cell filled with lucifer yellow as it is viewed in the dissecting microscope during an experiment. Lucifer yellow, as well as fluorescing brilliantly under the blue light, sensitizes the photodynamic inactivation of the dye-filled neurons.](image-url)
bursters: the anterior burster (AB) and two pyloric dilator (PD) cells. These three cells are electrically coupled to one another, and fire synchronously.

Previous experiments have shown that if all of the synapses in the ganglion are blocked with high Mg²⁺-low Ca²⁺ saline, only the AB and PD cells continue to fire rhythmically. All of the other neurons go into continuous firing or become inactive (30). When the AB and PD cells are simultaneously hyperpolarized below their firing threshold, the other cells in the pyloric network fire tonically. Hyperpolarizing the other cells, however, has little effect on the activity of the AB and PD cells (30).

Based on such experimental evidence, the pyloric motor pattern was thought to be driven by the endogenous bursters. However, the experiments had been done on isolated stomatogastric ganglia, cut off from the excitatory input of the commissural ganglia. In the experiments discussed below, the same questions were approached using a more intact preparation and a more effective technique for functionally removing a neuron from the network. These two technical improvements, discussed in the METHODS section, had significant effects on the experimental results.

To test whether or not the endogenous bursters are necessary for the production of patterned motor output, we asked the following question: Will pattern generation cease when the endogenous bursters are inactivated? Figure 5 shows the results of inactivating the AB and PD cells. Contrary to our prediction, inactivation of all three endogenous bursters does not result in the cessation of the patterned motor output. The activities of the remaining cells are still patterned into bursts with constant durations, phase relationships, and numbers of spikes per burst. In comparing parts A
and B of this figure, three significant effects of the inactivation are observed: 1) the pattern repetition frequency decreases by 33%, 2) the activity of the IC cell increases (small unit on the second trace), 3) the relative phases of bursts in the remaining cells shift by as much as 180°. These effects can be understood through a careful examination of the synaptic circuitry and will be considered in the discussion. The important conclusion remains, however, that the endogenous bursters are not necessary for production or maintenance of the rhythmic pattern in an otherwise intact preparation.

Sucrose block of inputs to STG

Several excitatory inputs, most of which originate in the paired commissural ganglia, enter the stomatogastric ganglion via the stomatogastric nerve (STN). It has been shown that blocking this nerve has significant effects on the pyloric motor-output patterns of intact preparations (28). Two major effects are: 1) the bursting frequency is decreased by as much as 50% of the rate in the normal, unblocked preparation, as shown in Fig. 1; and 2) the slow waves that produce the endogenous bursting in the AB and PD cells are reduced to about one-half their normal amplitude. The inputs appear to be responsible for increasing the overall activity of several neurons via direct excitation and by unmasking the ability of several cells to generate plateau potentials (29). The role of each different input fiber has not been determined, but it is clear that their combined activity contributes to the maintenance of a vigorous pyloric rhythm in an intact preparation.

Since neither 1) inactivation of the endogenous bursters, nor 2) blockage of the input nerve alone will terminate the pattern

**FIG. 5.** AB and PD cells inactivated. A: normal motor pattern in the intact, combined preparation. B: activity after the PD and AB cells have been inactivated. Note the vigorous cycling of the VD cell (intracellular recording). C: all cyclic activity is absent during sucrose blockade of the STN. Some cells become silent, while others fire tonically. D: stimulation of the STN on the ganglion side of the block with a short volley. The stimulus interval can be seen on the PD trace. In this and subsequent figures, we have presented schematic circuit diagrams along with the physiological recordings to represent the functional state of the network. Each cell type is represented by double concentric circles enclosing the abbreviation of the cell name. Inhibitory chemical synapses are represented by black dots, excitatory synapses by triangles. The electrical connection between VD and the PD-AB group is represented by a resistor symbol. In the following figures, inactivated or killed cells are indicated by 1) darkening the circle around the cell symbol, and 2) erasing the synaptic interactions for the inactive cell. The input from the commissural ganglia, including the two P-fibers, is represented by an arrow. P-cell synapses onto the pyloric cells are indicated explicitly as triangles with dotted "axons." Sucrose block of this input is represented by a flag across the arrow.
generation, we asked the following question: Will pattern generation continue in the absence of both the input and the bursters? Figure 5C shows the results of blocking the STN after the endogenous bursters have been inactivated. The rhythmic pattern ceased immediately, with the VD cell firing tonically and all other pyloric cells becoming silent. A short period of electrical stimulation to the STN proximal to the block restored the rhythm for a period of about a minute, as shown in Fig. 5D. After the sucrose block was removed, the activity returned to a pattern similar to Fig. 5B.

The conclusions are: 1) with the inputs intact, the endogenous bursters are not necessary for maintenance of the rhythmic pattern; 2) with the inputs blocked, the bursters are necessary for the pattern generation; and 3) the activity of the input nerve does not have to be phasic in order to drive the rhythm in the absence of the bursters. In fact, the pattern is maintained for a time after a short stimulus train to the nerve.

Inactivation of AB cell

The three endogenous bursters (PDs and AB) are electrically coupled to one another and fire at the same time. However, the PD cells have different functions than the AB cells.

The two PD cells are motor neurons that, in addition to playing an important role in the generation of the pyloric rhythm, innervate pyloric dilator muscles. The AB cell, on the other hand, is an interneuron. Although the AB cell has a set of synaptic interactions within the stomatogastric ganglion that are identical to those of the PD cells, the targets of the AB axon branches are two neurons in the commissural ganglia (28). These two neurons, called P-cells, are rhythmically inhibited by AB cell bursts and, therefore, are entrained to fire bursts in antiphase to the AB/PD cell group, i.e., the AB acts like an efference copy neuron (18). The P-cells send axons back down the stomatogastric nerve, where they make excitatory synapses onto all of the pyloric neurons except the endogenous bursters (Fig. 4). In order to characterize the roles of these two cell types in greater detail, experiments were performed in which only one or two of these endogenous bursters were inactivated. In each experiment, the effects of blocking the STN were also observed.

Figure 6 shows the results of an experiment in which the role of the AB cell was investigated. We asked, How is pattern generation affected by inactivation of the AB cell? In this experiment, the AB and P-cells were monitored indirectly, since their axons in the STN are too small to be recorded by extracellular electrodes. Their activities were monitored by an intracellular electrode. Their activities were monitored by an intracellular recording from another cell in the stomatogastric ganglion, the anterior median (AM) cell. Its membrane potential is shown in the bottom trace of each panel. The AM cell received input from the P-cell, clearly visible in Fig. 6A as bursts of excitatory postsynaptic potentials (EPSPs) in antiphase to the PD cell bursts.

The effects of inactivating the AB cell are shown in Fig. 6B. With the AB cell inactivated, the P-cells receive no rhythmic inhibition and fire tonically. This can be seen in the AM cell intracellular recording, where the P-cell EPSPs have been demodulated from phasic bursts into a steady tonic barrage. Although the P-cell input has been demodulated, the total number of EPSPs per unit time remains approximately the same.

Two changes in the pattern were observed: 1) the overall pattern frequency was reduced by 33%, and 2) the VD cell bursts shifted in phase with respect to the remaining cells.

The most general conclusion of these results is that the integrity of all three bursters is not necessary for production of patterned output. A more detailed interpretation of these results is complicated by the fact that AB cell inactivation changes the system in three distinct ways: 1) the synaptic interactions of the AB cell within the stomatogastric ganglion are eliminated, 2) the P-cell input to the stomatogastric ganglion is demodulated, and 3) any other commissural input that affects the stomatogastric ganglion via a connection to the AB cell would also be eliminated by the AB cell inactivation. The frequency decrease and VD cell phase shift could conceivably have been
FIG. 6. Inactivation of the AB cell to show both its role and the role of the P-cells. The AM cell, part of the gastric mill subnet, receives excitatory input from the P-cells. A: AB-P-cell loop is intact. Bursts of EPSPs can be seen in the AM trace (arrow). B: when the AB cell is inactivated, the P-cell is not modulated and the EPSPs from the P-cell become tonic (arrow). C: result of blocking P-cell input but leaving the AB connections within the ganglion intact. Note the disappearance of the EPSPs from the AM cell (arrow). D: AB cell removed from the circuit and the P-cell blocked. Under these conditions, the two active PD cells are insufficient to modulate VD cell activity. All data is from a single experiment. The extracellular traces in B and D were inverted during figure preparation.
caused by a combination of these changes to the system, and the above experiment could not distinguish between the different possibilities.

However, the overall contribution of the commissural inputs to pattern generation in this case could be assessed. Sections C and D of Fig. 6 show the results of a sucrose block of the stomatogastric nerve, before and after AB inactivation; C shows the block before the AB cell had been inactivated, and duplicates the results of Russell (28). Two changes in the pattern were observed: 1) the overall pattern frequency decreased by 35%, and 2) the VD cell bursts were prolonged to span the whole interval between the PD/AB cell group bursts. Note that the VD cell's spike frequency slightly decreased during the LP cell burst. The P-cell EPSPs totally disappeared from the intracellular AM cell recording.

With all commissural input blocked, inactivation of the AB cell yielded the results shown in Fig. 6D. The burst frequency of the PD cells decreased by another 44%, and the VD cell went into a tonic-firing mode. Activity in the PY cells stopped altogether.

The conclusion is that with the commissural inputs blocked, a complete pattern cannot be generated without the AB cell. The AB cell functions to accelerate the overall burst frequency of the PD/AB group and to increase the frequency of action potentials within the PD/AB cell bursts. The contribution of the AB cell to pattern generation thus seems to be additive with the contributions of the PD cells in these respects.

The conclusions from a comparison of sections B and D of Fig. 6 are: with the AB cell inactivated, a complete pattern cannot be generated without input from the commissural ganglia. The function of the inputs are quantitatively similar to the function of the AB cell, causing a general excitation of the whole pyloric system. The P-cell inputs need not be phasic to exert their acceleratory effects on the pattern rhythm. These experiments could not, however, differentiate between the effects of different input fibers nor determine the mechanisms by which those effects were produced.

**Inactivation of both PD cells**

The previous experiment demonstrated that the AB cell was not necessary for the production of patterned output if the PD cells and commissural inputs were left intact. The following experiment asked whether or not the AB cell was sufficient to drive the pattern generation in the absence of PD cells. In this experiment, shown in Fig. 7, the two PD cells were inactivated and the AB and all other cells were
left intact. The results were: 1) the PY cells went silent, and 2) the number of spikes per burst in the VD and IC cells diminished. Note that the burst frequency was not altered significantly when the two PD cells were inactivated. Thus, with the commissural inputs intact, the influence of the endogenously bursting AB cell was strong enough to drive all the cells in the pattern except for the PY cells. This is consistent with the experiment in which patterned activity was generated even when all three endogenous bursters had been inactivated.

A more crucial question was addressed by blocking the STN in this case, i.e., Can the AB alone drive the rhythm in the absence of commissural input? When the input nerve was blocked, all cells but the AB went tonic or silent even though the AB cell kept bursting. Thus, in the absence of input and with both PD cells inactivated, the AB cell is not sufficient to drive the pattern.

This result introduced confusion as to what mechanism was actually driving the pattern, in the presence of input, when both PD cells had been activated. The following possibilities were recognized: 1) the central input was unmasking or turning on nonlinear properties in cells other than the AB, which acted to produce the rhythm; 2) phasic bursts of excitation in the input nerve, presumably from the P-cells, were the actual "drivers" of the pattern; 3) the input caused a general increase in activity or excitability in all cells, making the rhythmic activity from the remaining endogenous burster (AB) more effective.

Further experiments, shown in Fig. 8, indicated that the third possibility is the most likely. To eliminate the first possibility listed above, the VD cell was inactivated in addition to the PD cells. Russell and Hartline (29) have shown that commissural input unmasks nonlinear properties in several pyloric cells, giving them the ability to "plateau" or flip-flop between two stable polarization levels. These properties are the strongest in the VD cell. We therefore inactivated the VD cell to minimize the contribution from these effects. The behavior of the system with the PD and VD cells inactivated is identical to the behavior when only the PD cells are inactivated. The

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**FIG. 8.** PD and VD cells inactivated, STN cut. In this preparation, both PD cells and the VD cell were inactivated and the STN was cut. The FY cell recording is intracellular. A: with only the AB cells present and no excitatory input, the motor output was reduced to almost zero. Periodic inhibition of the PY cell by the AB cell can be seen. B: stimulation of the STN could restore the alternating activity of the remaining cells. C: same recordings after 10^{-4} M DA added to the bath.
rhythm proceeds while the inputs are left intact but stops when the input is blocked by cutting the nerve, as shown in Fig. 8A. Thus, unmasking the plateauing properties of the VD cell is not responsible for pattern production in the absence of the PD cells.

To discriminate between the second and third possibilities listed above, the experiments shown in sections B and C of Fig. 8 were performed. As shown in section B, when the STN was tonically stimulated proximal to the cut, the rhythm was generated during and for a short period after the stimulation. Thus, the input need not be phasic for the rhythm to be produced, eliminating the second possibility.

The third possibility still remains: that the commissural inputs cause a general excitation of one or several pyloric neurons. The effects of one such input have been reported elsewhere by Anderson (2) and were further examined here. Dopamine (DA) has been shown by Anderson to increase the amplitude and frequency of AB endogenous bursting. The existence of dopaminergic neurons in the commissural ganglia has also been demonstrated (8, 19). These neurons send axons down the STN and terminate in the STG. The result of adding small concentrations of DA to the ganglion with VD and PD cells inactivated is shown in Fig. 8C. DA increases the activity of the AB cell, as seen by the increase in amplitude of the AB cell's inhibitory postsynaptic potential (IPSP) volley onto the PY cell. The PY cells, as well as the other remaining pyloric cells, began to fire in bursts. The pattern was maintained for as long as DA was left in the bath. This excitation of pyloric activity could operate via two mechanisms. First, DA could directly affect membrane properties of several or all pyloric cells, increasing their activities or excitabilities. DA-induced changes in the current-voltage relations of several pyloric cells have been demonstrated by J. Raper (unpublished observations). Second, the activity of all pyloric neurons would be expected to increase when the AB cell activity increases, due to the enhancement of postinhibitory rebound from the AB cells' IPSP volleys. The contribution from this second mechanism is substantial because addition of DA to ganglia in which the PD and AB cells have
been inactivated does not restore the rhythm (data not shown). It is interesting that DA produces an effect identical to STN stimulation in restoring the rhythm, yet it is not DA that unmasks the plateau potentials. This is further evidence that the unmasking of plateauing properties in the nonendogenously bursting cells is not necessary for rhythm initiation and maintenance.

**Inactivation of nonendogenously bursting cells**

According to the notions of how the pyloric pattern is generated, inactivation of any cell other than an endogenous burster was expected to have little effect on the ongoing activity. To test this prediction, experiments were performed in which single neurons other than the AB or PD cells were inactivated. For example, the results of inactivating an LP cell are shown in Fig. 9. Section A shows the normal activity before the LP was inactivated. All units were active except the IC cell. After inactivation the LP cell, Fig. 9B, the LP spike was no longer observable in the peripheral nerve. The only measurable effects on the rhythm were: 1) a 15% increase in frequency, 2) a slight prolongation of the PY bursts, and 3) the introduction of IC burst activity. These effects will be considered in the Discussion.

Regardless of these three effects, patterned output was obtained with the LP cell inactivated. However, when the STN was blocked in this case, an unanticipated result was obtained. As shown in Fig. 9C, activity in the IC and PY cells cease and the PD-AB group. Thus, in the absence of STN input, inactivation of the LP cell had an effect similar to the inactivation of all three endogenous bursters. In other words, with the LP cell inactivated and the input nerve blocked, the endogenous bursters are not sufficient to drive bursting in the remaining cells of the network.

**PDs-AB-LP KILLED**

**BLOCK**

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**FIG. 10.** PD, AB, and LP cells inactivated. A: PD continues to burst as long as the STN inputs are present. Units shown on the top extracellular trace are tonically firing PY cells. The IC neuron is inactive. B: blocking inputs causes the PD to fire tonically and somewhat irregularly. A short and spontaneous burst of the IC neuron (arrow) produces some inhibition of the PD activity.
Inactivation of a PY or IC cell alone had little effect on the pattern of the remaining cells with or without the input nerve blocked.

The relative importance of nonendogenously bursting cells was also investigated in another set of experiments. These experiments were actually continuations of the experiment shown in Fig. 5. In addition to inactivating the AB and PD cells, one more of the remaining pyloric cells could be inactivated. When the additional cell was an IC, LP, or PY cell, patterned activity was still maintained in the presence of central input (see Fig. 10). However, the VD cell played a more crucial role than did the other cells. The results shown in Fig. 1A and B demonstrate again that, when the AB and PD cells were removed, the pattern continued. However, when the VD was subsequently inactivated, the pattern was totally disrupted, even with the STN input intact. Thus, under certain conditions the VD cell must be considered a component of the pattern-generating mechanism.

This conclusion was strengthened by several other experiments. A neuron can be considered part of a central pattern gener-

tor if altering that neuron's activity disrupts or resets the ongoing pattern (9). Figure 12 shows the results of altering VD cell activity in a preparation where the endogenous bursters had been inactivated (as in Fig. 1A). When the VD cell was shut off by hyperpolarization, the remaining cells began firing tonically. When the VD cell was depolarized so that it fired continuously, the remaining cells were inhibited and the pattern stopped. The VD cell was unique in this respect. The patterned output could not be totally disrupted by polarizing any other cell of the pyloric system. The uniqueness of the VD cell and the possible role of plateauing potentials to that cell's function will be considered below.

DISCUSSION

The lobster stomatogastric ganglion has long been thought of as a simple, isolated, central pattern generator. We now realize that it is more complex and more integrated with the rest of the central nervous system than we had thought. In these experiments
we have tried to judge the role and importance of several functional components of the pyloric pattern generator. These functional components, identified over the last decade by several researchers, are: 1) endogenously oscillating neurons (the AB and two PD cells) (12), 2) the synaptic connections acting as a resonant network, 3) neurons generating plateau potentials (especially the VD neuron), and 4) excitatory inputs from the commissural ganglia.

The complex interrelationships of these components in producing the pyloric pattern, as determined in the present experiments, are summarized in Fig. 13. These tables qualitatively specify the activity states of the pyloric system under different conditions. Two plus signs indicate the normal pattern, one plus sign indicates a slowed or altered pattern, and a zero indicates no pattern. We have limited the variables to those that show the strongest effects on pattern generation: the endogenous bursters, the commissural inputs, and the VD cell.

Relative importance of endogenous bursters versus network properties

It is no surprise that the endogenous oscillator cells play a central role in pattern generation. In a stomatogastric ganglion totally isolated from the rest of the nervous system, the endogenous bursters are necessary for pattern production and maintenance. Because our previous experimental results indicated that the endogenous bursters were crucial to the generation of the pyloric rhythm, we expected their inactivation would stop all of the cyclic activity. However, when the commissural ganglia are left attached to the stomatogastric ganglion, the pattern continues even when the endogenous bursters are inactivated. In retrospect, this result was anticipated in a study by Warshaw and Hartline (34). In that study, a subset of the pyloric

![Diagram](image-url)

FIG. 12. Hyperpolarization-depolarization of VD. Effects of passing direct current into the VD cell soma following removal of the endogenous bursters. A: hyperpolarizing current shuts off the VD cell. This removes inhibition from the LP cell, allowing it to fire continuously. No signs of LP cell bursting were apparent in this preparation during the time the VD cell was silent. B: depolarizing the VD cell does not cause an increase in its burst frequency, but instead causes it to fire continuously. During this period, the LP cell escaped from VD cell inhibition occasionally, but showed no sign of bursting activity. Inputs from the commissural ganglia were present in both cases.
network was modeled on a computer, and
the model predicted output similar to the
experimentally observed pattern. However,
the model did not incorporate the endoge-
nous oscillatory properties into the AB or
PD cells nor the ability to produce plateau
potentials into any of the remaining cells.
Thus, the cyclic motor pattern could derive
from the synaptic connections of the net-
work itself. The significance of those results
is still equivocal, however, due to the in-
completeness of the model in other im-
portant respects. Moreover, the experiment
"performed" in the modeling study was not
identical to the one discussed in this re-
port. Whereas the modeling study incor-
porated these modified nonbursting AB and
PD cells into the network, we inactivated
these cells altogether. The similarity of the
results is still striking and significant, how-
ever. It will be of great interest to recheck
the computer model by programming these
physiological complexities into the model
neurons and duplicating the present experi-
ments explicitly.

The results of the present experiments
can easily be understood if the endogenous
burster cells are considered as phase and
frequency controllers for the other neurons
in the pyloric system. In other words, the
synaptic connectivity of the network as a
whole results in an oscillatory mode of
activity, and the endogenous bursters func-
tion to "tune" that rhythmic activity to the
behaviorally "correct" pattern and
frequency.

Two experiments in particular support
this characterization of the endogenous
bursters as phase and frequency control-
ners. First, when the AB and PD cells were
inactivated in an otherwise intact prepara-
tion, three effects were observed (see Fig.
5); 1) the cycle frequency in the remaining
cells decreased, 2) the relative phases of
bursting in the remaining cells shifted, and
3) activity in the IC cell increased. The
decrease in pattern frequency indicates that
the intrinsic or "characteristic" frequency
of the endogenous bursters is greater than
that of the system as a whole. Consistent
with the general theories of oscillatory
systems (3), the components of this network
are entrained to the element with the
greatest characteristic frequency, which in
this case is the AB/PD cell group. Note that
this frequency decrease cannot be due to a
general loss of excitation since the synapses
made by the bursters onto all other cells are
inhibitory.

It was, in fact, the loss of these strong
inhibitory synapses that caused the relative
phase shifts in the activity of the remaining
cells. Before the inactivation of the bursters,
all other cells received strong volleys of in-
hibitory postsynaptic potentials, one volley
per cycle. This constrained the activity of
these other cells to the intervals between
the AB/PD cell bursts. In fact, even though
the LP and VD cells are coupled by recipro-
cally inhibitory chemical synapses, they
were constrained to fire nearly in syn-
chrony. When the sources of these strong
IPSPs were inactivated, that constraint was
lifted from the remaining cells, allowing
them to reestablish phase relationships
based on their remaining synaptic inter-
connections. The reciprocally inhibitory
connections of the LP and VD cells then
causd them to fire in antiphase.

The third result noted in this experi-
ment was an overall increase in IC cell activity.
This can be explained by its release from
strong inhibition by the AB and PD cells.

The second experiment that supports the
characterization of the endogenous bursters as phase and frequency controllers was the LP cell inactivation (Fig. 9). When the LP cell was inactivated in an otherwise intact ganglion, three changes in the pattern were noted: 1) a 15% increase in burst frequency, 2) a prolongation of PY cell bursts, and 3) the introduction of IC cell bursts. The increase in pattern frequency was due to disinhibition of the endogenous bursters. The LP cell is the only source of inhibitory input onto the AB and PD cells. The elimination of that rhythmic inhibition allows the bursters to oscillate at their characteristic frequency, free from any feedback from the other neurons in the network. (The electrical connections between the bursters and the VD cell is very weak.) This 15% increase in frequency again demonstrates that the characteristic frequency of the bursters is greater than that of the network as a whole, fitting with the requirement for a frequency controller.

The prolongation of PY cell bursts is, similarly, a result of their release from LP cell inhibition. The increase in IC cell activity is more difficult to interpret and may necessitate modeling studies for a complete understanding.

An important point to note, however, is that no significant phase shifts resulted from inactivation of the LP cell or from inactivation of any other nonendogenously bursting cell alone. This is further evidence that cell activity-phase relationships are determined to a large degree by the endogenous bursters.

Relative importance of nonlinear cellular properties

The ability of the network to resonate must certainly be enhanced by the effects of nonlinear properties in several pyloric cells. These properties include postinhibitory rebound, nonspiking inhibition, and plateau potential generation. Postinhibitory rebound (PIR) is the overshooting depolarization of neurons beyond their normal resting potential on release from strong inhibition. As discussed by Perkel and Mulloney (26), the PIR of a cell can be of sufficient strength to bring the membrane above spike threshold. PIR thus mimics the effects of an EPSP barrage and would strengthen a cell’s tendency to alternate its firing with any reciprocally inhibitory neuron.

Graubard, Hartline, and Raper (14, 15, 27) have demonstrated the strength and ubiquity of nonspiking synaptic interactions in the pyloric system. Every pyloric neuron releases transmitter as a continuous function of its membrane potential and need not fire an action potential to modulate its follower cells. Thus, when considering the physiological effect of synaptic interactions, subthreshold polarizations must be considered along with action-potential generation. Any factors that affect subthreshold activity can therefore affect the overall functioning of the system. Though the effects on pattern generation due specifically to nonspiking interactions are difficult to isolate and characterize, effects of this magnitude are expected to contribute significantly to the shaping of the output pattern.

The effects on pattern generation due to the production of plateau potentials are more easily interpreted and must play a more important role than either PIR or nonspiking interactions. The generation of a plateau potential can act as a PSP amplifier. In other words, the small voltage transients caused by PSPs can trigger large-amplitude, sustained polarizations. These plateaus of polarization modulate the synaptic and spike initiation regions of plateau-generating neurons in a way very similar to large bursts of PSPs. In the extreme case, one PSP onto a cell capable of producing plateau potentials could have the same effect as tens of PSPs on a nonplateauing cell. Thus, a network containing such plateauing cells could sustain its activity pattern with some of its external excitatory input diminished or removed.

This seems to be the case with the pyloric network. The VD cell, in particular, is a plateau-generating cell that contributes to pattern generation in some circumstances. The VD cell can be inactivated in an otherwise intact preparation, and rhythmic motor output will still be produced. However, with the endogenous oscillators inactivated, the VD cell is necessary for maintenance of the pattern. Figure 12 shows that the VD does not act like an endogenous oscillator. Although it can be “triggered” to flip into either of two stable polarization levels, it
does not flip-flop rhythmically on its own, as do the AB and PD cells. When true endogenous bursters are depolarized, they do not fire tonically as the VD did in Fig. 13. Rather, their frequency of bursting increases. A true endogenous oscillator can operate totally isolated from synaptic input (1, 4).

Although the VD cell does not oscillate spontaneously, it is triggered to oscillate between two polarization levels. The trigger for positive steps is presumably a combination of 1) EPSPs from the commissural ganglia P-cells, and 2) postinhibitory rebound from the LP and IC cell bursts. The trigger for downward steps may normally be the inhibition from LP and IC cell synapses, as those two cells escape from VD cell inhibition. The termination of a plateau potential may, on the other hand, be an inherent property of the plateau-generating neuron itself (5, 22). This must be the case in Fig. 5 where the VD cell is shown to be oscillating between two states at a fairly regular interval in the absence of any phasic input. The only active input in this case is the tonic excitation from P-cells. This could serve as the upward trigger, but no external downward trigger is observable. One possible source of inhibition is by nonspiking transmitter release from the IC cell. Even though no action potentials are observable in the IC cell axon, the membrane potential of the IC cell may still be oscillating due to input from the P- and PY cells.

Thus, the ability of the VD to maintain the rhythm in the absence of endogenous bursters is dependent on two factors of equal importance: 1) the reciprocally inhibitory network interactions of the VD cell with the other pyloric cells, and 2) the ability of the VD cell to plateau when commissural inputs are active.

Although the VD cell displays the strongest tendency to generate plateau potentials of all the pyloric cells, all of the other cells have plateau-generating ability to some degree. This plateauing must contribute to the maintenance and stability of the pattern in the absence of endogenous bursters. However, the role and importance of plateau potentials must not be overemphasized. The inactivation of the endogenous bursters in the experiments discussed above is a totally artificial situation. The AB and PD cells are always active in an intact preparation. When the plateauing abilities of the VD and other cells are eliminated by blocking the input nerve, the complete pyloric motor pattern is still generated. This latter condition—blockade of the input nerve—on the other hand, could have physiological relevance in freely behaving animals.

The significance of plateau potentials to pyloric pattern generation is still undetermined. Their function must be somewhere between 1) "fine tuning" of the pattern generator, and 2) reinforcing or "backing up" the other mechanisms.

Role of commissural input to ganglion

Experiments by Dando and Selverston (6) and Russell (28) have demonstrated the importance of the input into the stomatogastric ganglion from the commissural ganglia. The magnitude of that importance was emphasized in the present experiments and is summarized in Fig. 13. The mechanisms by which these inputs influence pyloric activity have been investigated in several studies. Russell and Hartline (29) have shown that the P-cells enhance pyloric activity in two ways. Not only do the P-cells unmask the ability of some cells to generate plateau potentials, as discussed above, but they rhythmically excite all pyloric cells other than the endogenous bursters. This rhythmic excitation acts to reinforce the tendency of the other neurons to fire in the intervals between the AB/PD cell group bursts.

Other results obtained in these experiments indicate the involvement of additional input fibers, some of which may be tonically active. The effects of DA on the pyloric activity mimic the effects of the inputs identified and discussed by Kushner and Maynard (19), Friend (8), and Anderson (2). Regardless of whether or not these other inputs fire tonically or phasically in an intact preparation, our results indicate that their effects may be duplicated by tonic stimulation of the stomatogastric nerve.

Interganglionic feedback loops, such as the AB–P-cell loop in this preparation,
have been shown to exist in the *Pleurobranchia* feeding system (11) and in the cat locomotor system (16). They may also exist in leech (35) and *Helisoma* (13). The overall function of the inputs is to increase excitability and activity of the pyloric neurons, both by depolarizing them directly and by unmasking plateauing abilities. Whether or not these inputs are labeled command fibers, they do exert a strong influence over the activity of the pyloric pattern generator. These input neurons in the commissural ganglia are, themselves, likely targets for modulatory synapses from even more central levels of the nervous system. Instead of modulatory synapses going directly to the motor neurons, the excitatory drive could itself be modulated, and this in turn would effectively alter the rhythm.

**Pyloric pattern generation: a reexamination**

An evaluation of the results presented here have led us to a new overall conception of how the pyloric pattern is generated. The points of major importance are: 1) The synaptic connectivity of the network as a whole results in an oscillatory mode of activity. Reciprocal inhibitory connections between individual neurons in the network are the basis for this oscillatory activity. 2) The endogenous bursters set the frequency and phase relationships of the other pyloric cells, acting through two mechanisms: a) the strong inhibitory synapses from the endogenous bursters onto the other cells constrain those other cells to fire during the AB/PD group interburst intervals, and b) this constraint is reinforced by the activity of the P-cells. Due to the P-cells' rhythmic inhibition from the AB burster cell, the P-cells rhythmically excite the nonendogenously bursting cells during the AB/PD group interburst intervals. 3) The frequency and amplitude of the activity in the pyloric system is influenced by input from the commissural ganglia. This influence is mediated via several mechanisms, three of which are: a) rhythmic excitation from P-cells, discussed above; b) unmasking of the ability to generate plateau potentials, induced by P-cell synapses. (This function seems to be independent of their firing pattern and is of undetermined significance.) c) enhancement of AB cell activity by the DA input, which causes an overall increase in activity of the pyloric system.

In reviewing the data now available concerning the stomatogastric ganglion, several striking points present themselves. The first is the extreme complexity of the system as a whole. This complexity was not fully appreciated in early studies in which the mapping of "simple" circuitry and the presence of endogenous bursters seemed to promise immediate explanations of the pattern-generating mechanisms. The experiments presented here greatly complicate that simplistic picture. The strength and ubiquity of nonspiking interneurons in the ganglion are an additional complication (27), and the complexities that will be added by a consideration of sensory feedback cannot be anticipated.

The second striking feature is the apparent robustness or redundancy built into the system. In these experiments, the inactivation of system components that had been considered "integral" to the pattern generation mechanisms did not terminate the rhythmic motor output. Is this evidence of a true redundancy, developmentally built into the system? Two alternatives to this possibility are: 1) that even the most subtle pattern alterations would be of importance at the behavioral level, or 2) that effects of these same inactivation would be qualitatively or quantitatively different if performed in an intact animal. It will be of interest to determine if similar degrees of this apparent redundancy exist in other central pattern generators.

Finally, the high degree to which this "isolated" central pattern generator interacts with other centers of the nervous system is becoming more apparent. This central pattern generator in particular, and perhaps many more in general, must be thought of as integrated components of larger distributed systems. This is emphasized by the importance and role of commissural input in these studies.

The identification of all these complexities will not, hopefully, discourage research into this or any other simple central pattern generator system. Rather, this should en-
courage the development and reexamination of such preparations, both as ends in themselves and as models for more complex nervous systems.

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