SHORT COMMUNICATION

RECORDINGS FROM THE STOMATOGASTRIC NERVOUS SYSTEM IN INTACT LOBSTERS*

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Abstract—1. The neural output of the stomatogastric ganglion was recorded from intact lobsters with implanted electrodes.

2. The recorded discharge was rhythmic and patterned, and was similar to that recorded previously from isolated stomatogastric preparations.

3. Variations in the period and form of the output during quiescence and following feeding suggest that although rhythmic patterns probably originate in the stomatogastric ganglion, they are also under control of modulating interneurons from the central nervous system.

INTRODUCTION

THE STOMATOGASTRIC system of lobsters and crabs has been studied in isolated preparations and in semi-intact animals (Maynard, 1966, 1967; Maynard & Burke, 1966). Under these conditions motor neurons in the stomatogastric ganglion discharge in rhythmic patterns. While this activity can occur spontaneously, presumably in the normal animal it is modulated and controlled by interneuron input from the central nervous system. This paper describes initial experiments documenting such control by recording neural output from the ganglion with implanted electrodes in the essentially intact lobster. The basic motor neuron discharge patterns observed in isolated preparations were present, but varied extensively with time and the behavioral state of the lobster, particularly during and immediately following feeding.

MATERIALS AND METHODS

Lobsters (*Homarus americanus* Milne-Edwards) weighing about 1·25 lb were used. The stomatogastric ganglion in these animals is located above the anterior–dorsal wall of the cardiac stomach just beneath and behind the base of the rostrum. Most of the efferent fibers leaving the ganglion and passing to the gastric mill or pyloric stomach muscles run posteriorly in the dorsal median ventricular nerve. Approximately 1 to 2 cm posterior to the ganglion this nerve branches bilaterally into the right and left lateral ventricular nerves (*LVN*) which go to their respective sides of the stomach. Since the *LVN* is just beneath

the carapace it is easily exposed. Because it contains most of the nerve fibers (18–20) whose activity is known from isolated preparations, it was chosen as the locus for initial electrode implantations.

Electrode construction

The implanted electrode assembly forms an insulating sleeve around the *LVN* with two platinum wires spaced in the center to record nervous activity. The sleeve is a 6-mm long polyethylene trough (made from Intramedic tubing, i.d. 0·011 in. × o.d. 0·024 in.) containing two platinum alloy wire electrodes (90 per cent Pt, 10 per cent Irid., wire dia. 0·033 in., Teflon coated, coated dia. 0·044 in., made by Medwire Corp., Mt. Vernon, N.Y.) with bared ends spaced about 1·5 mm apart. The nerve was held in the trough and the open side of the trough filled with IBC-2 Ethicon tissue adhesive (isobutyl 2-cyano-acrylate monomer, made by Ethicon, Inc., Somerville, N.Y.). The wires emerging from the poly-ethylene trough were secured by heat-fusing additional polyethylene around the exit holes of the trough. Fiberglass sealant was used to further insulate the wires and solder joints (Fig. 1A).
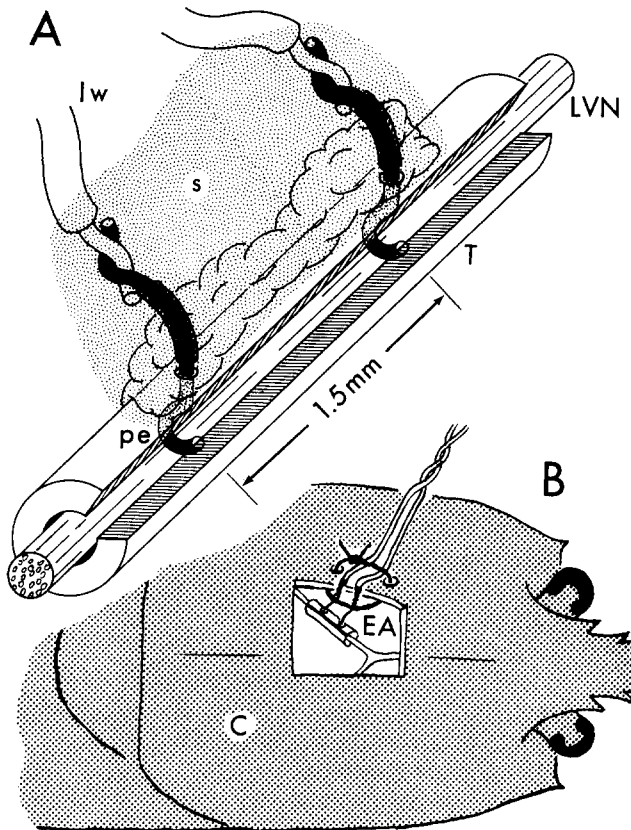

FIG. 1. A. Diagram of electrode assembly. l.w., Lead wires to oscilloscope; *LVN*, lateral ventricular nerve; p.e., platinum alloy electrodes; s, sealant; T, central portion of polyethylene trough. B. Diagram of electrode assembly *in situ*. C, Carapace; E.A., electrode assembly.

Electrode implantation technique

During implantation lobsters were placed on paper toweling under a dissecting micro-scope. Perfusion solution (Cole, 1941) was used intermittently throughout to rinse the preparation. A small piece (about 1 cm²) of the carapace was removed about half-way between the rostrum and the cephalic groove (Fig. 1B) and the underlying hypodermis opened and laid back above the *LVN*. Bleeding was extensive at first but could be controlled with cotton swabs. The *LVN* was freed from the surrounding tissue with fine glass probes. The dry electrode assembly was tied in place with ligatures through two small holes in the carapace. The *LVN* was placed inside the trough of the electrode assembly with glass probes, taking care to avoid excessive stretching of the nerve, and fastened to the electrode with the aid of tissue adhesive. The electrode assembly was similarly glued to the dorsal surface of the stomach and the piece of carapace that had been removed was replaced.

RESULTS

Satisfactory records were obtained from four of the seven lobsters implanted. These differed somewhat in detail, but certain features of the discharge patterns were common to all. Two patterns of rhythmic activity were recorded from the intact, quiescent lobster.

1. *Pyloric cycle* (Fig. 2A)

The pyloric rhythm was the most obvious and was the pattern usually observed. It is known from isolated preparations and involves neurons innervating pyloric muscles (Maynard, 1966, 1967; Maynard & Burke, 1966). A total of nine to ten neurons belonging to three functional groups discharge in an ordered cycle of bursts. A group of two neurons innervating the pyloric dilator muscles forms the pacemaker of the cycle and initiates the sequence with a burst of impulses. Then, after a silent period of about 50 to 200 msec, a single neuron innervating one of the lateral pyloric muscles discharges. As it stops, the third and final group of several neurons supplying more posterior pyloric muscles discharge and then cease as the dilator elements begin again (Fig. 2A). In three of the four lobsters the pyloric rhythm was very regular over prolonged periods as has also been observed in isolated preparations (Maynard, 1966). The mean frequency and standard deviation of the rhythm recorded at 30-min intervals from one lobster are plotted in Fig. 3. The large variations in mean frequency at the spaced intervals contrast with the small variance over briefer periods. The results suggest that the pyloric cycle may be under the control of tonic input from modulating interneurons entering the ganglion from the CNS whose function is to produce changes in activity which persist over many cycles. Similar effects have been produced in isolated preparations by repetitive stimulation of interneuron axons in the stomato-gastric nerve (Maynard, 1966). In the fourth lobster the pyloric rhythm was almost absent. Dilator bursts occurred irregularly at 6 to 30-sec intervals, and though they were usually followed by activity in lateral and posterior pyloric elements in the appropriate sequence, the regularity of the usual pattern was lacking. Pyloric elements also discharged alone in irregular bursts in the long intervals between dilator bursts (mean period $2 \cdot 90 \pm 2 \cdot 26$ sec; mean number of

spikes/burst $15\cdot6 \pm 16\cdot9$). As described below, such irregularity disappeared after feeding and a regular pyloric rhythm appeared (Fig. 2E).

2. *Gastric mill cycle* (Fig. 2B)

The second rhythmic pattern did not occur in all animals, nor was it present at all times in any one animal. It involved activity in neurons that presumably innervate muscles directly associated with the gastric mill. The rhythm was much less regular and the period was usually considerably longer than the pyloric rhythm. For example, in one lobster (Fig. 2B) a gastric mill rhythmic period ranging between 15 and 25 sec occurred simultaneously with a pyloric rhythm having a period of $1\cdot33 \pm 0\cdot18$ sec.

Several neurons are obviously involved in the gastric mill rhythm and they are clearly different from those active in the pyloric cycle. In isolated preparations sequential discharges of individual groups of elements have been identified (Maynard & Hartline, unpublished observations), but this was not possible in the intact animal because of the multi-unit recording method.
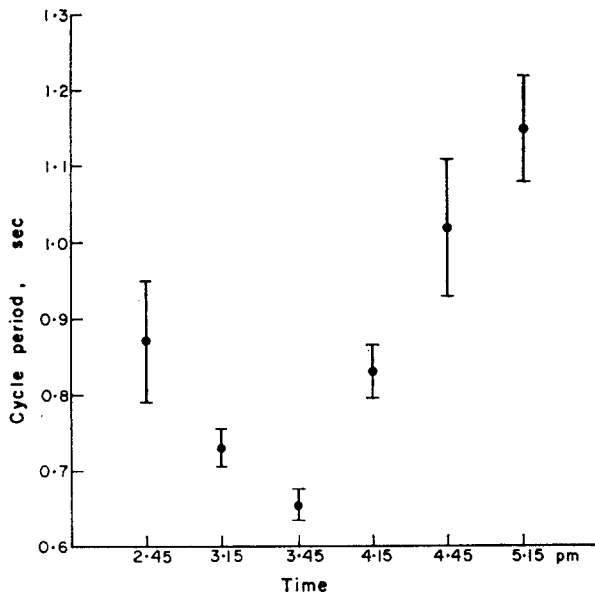

FIG. 3. Mean period of pyloric cycle measured at various intervals after implantation of electrodes. Each point represents the mean of 100 cycles taken at the time indicated. Vertical line represents standard deviation.

Activity associated with feeding

Small pieces of fish were presented to three of the implanted lobsters. One did not accept or ingest the food and no changes in stomatogastric activity occurred. The other two ingested the pieces of fish and marked changes in the gastric
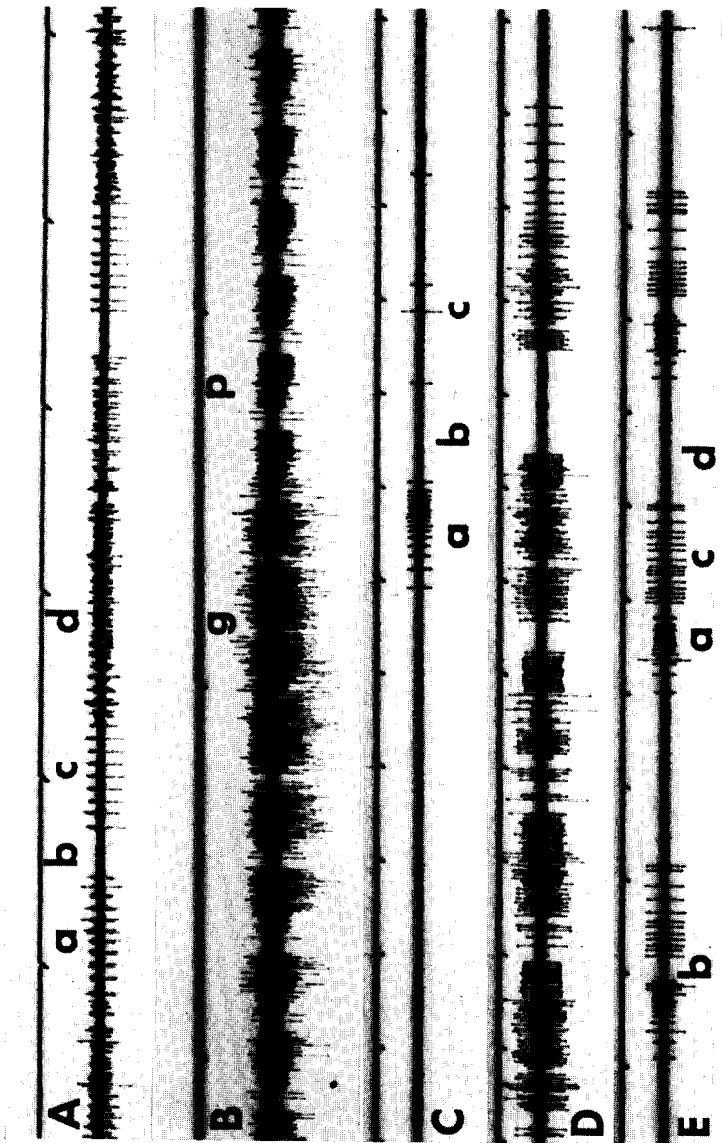
FIG. 2. Activity recorded *in vivo* from lateral ventricular nerve. A. Pyloric rhythm in a quiescent lobster. a, Discharge of dilator neurons; b, silent period; c, discharge of lateral pyloric neuron; d, discharge of more posterior neurons. Time calibration, 0·5 sec. B. Pyloric rhythm and gastric mill discharge. p, Pyloric cycle; g, discharge of gastric mill elements during active phase of cycle. Time calibration, 5·0 sec. C. Pyloric discharge before feeding. Posterior pyloric neurons inactive. D. Pyloric and gastric mill activity 30 sec after feeding. E. Pyloric rhythm 3 min after feeding. Posterior pyloric elements are now active, though their action potentials are very small. Records C, D and E are taken from one animal during a single feeding experiment. Time calibration, 0·5 sec, is the same for all three.

activity resulted. The most striking response is illustrated in Fig. 2C–E and in Fig. 4. Two things occurred: (1) The pyloric cycle increased in frequency and quantitative aspects were altered within the cycle. For example, the neuron innervating the lateral pyloric muscles discharged more frequently during the cycle and reached higher maximum rates than during non-feeding periods. (2) New elements, presumably associated with the gastric mill, became active. However, the complexity of the activity and the difficulty in identifying individual units with certitude preclude a more detailed analysis at this time.
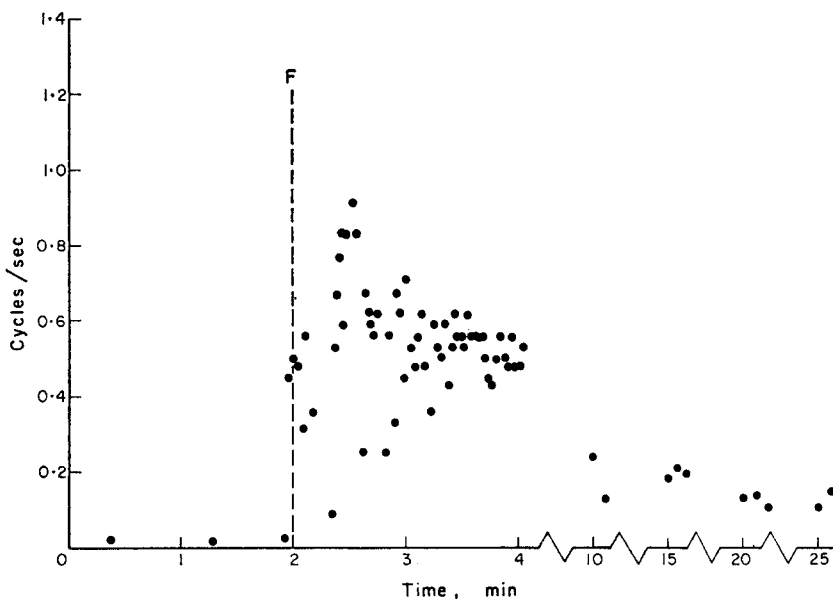

FIG. 4. Effect of feeding on the rate of pyloric rhythm discharge. Food taken by lobster at 2 min mark (F).

Major activity resulting from feeding small pieces of fish was complete within a few minutes of ingestion (Figs. 2 and 4) but increased pyloric activity continued for a much longer time, as long as 25 min or more.

DISCUSSION AND CONCLUSIONS

The preliminary observations reported here leave little doubt that the rhythmic patterns observed in isolated preparations of the stomatogastric ganglion also occur in the intact animal. They also indicate that quantitative aspects of these patterns alter appreciably over time or with changes in the behavioral state of the animal. Such alterations do not occur spontaneously in the isolated preparation, though they can be evoked by repetitive stimulation of the stomatogastric nerve axons which terminate in the ganglion (Maynard, 1966). Most likely these changes in the intact lobster result from activity in modulating interneurons acting on the

intrinsic pacemaker system in the ganglion. The nature of many of the changes suggests that at least some of these modulators are tonic elements, normally producing effects over many cycles. Although there is evidence that specific components of a rhythmic cycle may be stimulated or inhibited independently (Maynard, 1966), the qualitative characteristics of the intrinsic, cyclic pattern remain unaltered over a broad range of quantitative variation. Unfortunately the data are not complete enough to say whether this must always be so, or whether it is possible through appropriate interneuron activity to shift an element from one intrinsic pattern to another qualitatively different pattern.

Recent studies have shown that input from sensory elements in the stomach may affect the rhythmic output of the stomatogastric ganglion (Dando & Laverack, 1969). Direct connections between sensory fibers and stomatogastric elements within the ganglion have not yet been demonstrated, however (see Orlov, 1927). Although the changes described here in stomatogastric output associated with feeding could be explained in terms of a peripheral reflex involving only sensory elements and stomatogastric neurons, we feel it is more likely that interneurons from the central nervous system are involved. If so their modulating discharge to the stomatogastric ganglion would then represent an integrated function of sensory input from the stomach and central nervous system commands.

Further observations with implanted electrodes should allow us to catalogue the activity repertoire of the stomatogastric ganglion and, in conjunction with isolated preparations, determine the relative roles of interneurons, stomatogastric ganglion elements and possibly sensory feedback in creating the patterns observed. The mechanisms of such patterned output may well have relevance bearing on the genesis of rhythmic activity in other systems such as those producing insect locomotion (Wilson, 1966) or respiratory movements in a number of animals.

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