VISCERAL AFFERENT SIGNALS IN THE CRAYFISH STOMATOGASTRIC GANGLION

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(Received 29 October 1965)

INTRODUCTION

With the striking exception of the cardiac ganglion of decapod crustacea, almost nothing is known about the neurophysiology of 'autonomic' regulation in arthropods. The crustacean stomatogastric system provides a promising target for such analysis; the stomatogastric ganglion (SG) supplies the motor output to a complex system of striated muscles that control the anterior digestive tract, and presumably receives afferent signals from as-yet-undescribed chemo- or mechanoreceptor elements associated with visceral organs. Previous studies (Allen, 1894; Orlov, 1926, 1927), as well as our own, indicate that the SG contains less than thirty cells. As suggested by Bullock & Horridge (1965), it may thus be intermediate in complexity between the cardiac ganglion and the larger segmental central ganglia. Compared with the cardiac ganglion, it offers the potential advantages of two-way traffic and of a somewhat more distributed and accessible efferent target. Preliminary reports (Maynard, 1962, 1966) indicate that motoneurones from SG are capable of producing a complex, patterned output in response to preganglionic efferent stimuli. Here we present studies on the afferent impulse traffic in the SG system, in particular on a single unusual mechanosensory element exhibiting autogenic activity. A preliminary report has appeared elsewhere (Larimer & Kennedy, 1965).

METHODS

Histological sections of the stomatogastric ganglion, and of its distal and proximal branches (the lateral nerves and the superior oesophageal nerves, respectively) were cut at 10 μ from material fixed in alcoholic Bouin's and stained with the silver method of Fitzgerald (1964).

Several different sorts of preparations were employed in physiological experiments, all of which were performed on Procambarus clarkii Girard. In the initial series of experiments, carried out at Stanford during the summer of 1964, the SG was exposed by a ventral dissection that involved removal of all the anterior digestive tract ventral to the anterior stomach and cardiac ossicles. Fine silver-wire hooks were used to record from the cut central ends of the two superior oesophageal nerves (see Fig. 1). Alternatively, recordings were made from the cut central end of a single superior oesophageal nerve in an otherwise intact, minimally dissected preparation. This permitted uninterrupted motor outflow to the stomach through the other superior oesophageal nerve, so that the afferent response to 'normal' contractions could be
recorded. The standard preparation employed in the later experiments, performed by one of us (J. L.) at the University of Texas during 1964–65, involved isolation of the SG with the superior oesophageal nerves cut but with the lateral and dorsal ventricular nerves intact and in their normal relationship to the gut and ossicles. In such preparations, activity in the two lateral nerves could be recorded *en passant* or from cut distal or proximal ends; it was also possible to record simultaneously from one of the superior oesophageal nerves and a lateral nerve.

![Diagram of the stomatogastric system of *Procamus clarkii*.](image)

Electrical recording was accomplished by monopolar or bipolar silver hooks carried on low-power micromanipulators. The ends of the various nerves—or, in the case of *en passant* recording, a central loop—were drawn up into an oil or air layer above the physiological solution (van Harreveld, 1936) used to bathe the preparation. The electrodes fed capacitance-coupled amplifiers and a dual-trace oscilloscope, the stationary traces of which were recorded on moving film. Stimuli (isolated square pulses of 0.5 msec. duration or less) were delivered through similar pairs of fine silver or platinum wire electrodes. Mechanical stimulation was performed by manual probing of the left or right portions of the cardiac ossicles, or by a probe held in a rack-and-pinion micromanipulator coupled to a potentiometric circuit which provided a signal for the second oscilloscope channel.
RESULTS

When the central end of one superior oesophageal nerve was cut and arranged for recording in an otherwise intact preparation, a sequence of activity such as that shown in Fig. 2 resulted. Characteristically, there was a complex but rather stereotyped sequence of discharges in about three axons, superimposed on a background of activity in several others. This pattern was repeated at a frequency identical to that of the stomach contractions, so it is clear that the afferent signals shown in Fig. 2 represent

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a centrally directed report of the complex movements of the anterior digestive tract. The most prominent element in this discharge pattern characteristically is a fibre that fires rhythmically at low frequency in preparations in which only the peripheral connexions to SG have been left intact. Further experiments were concentrated on the analysis of this single unit.

In the ‘isolated’ preparations described above, constant-frequency firing of an apparently identical unit could be observed in either superior oesophageal nerve, or in the main stomatogastric nerve before it branches. Simultaneous recordings made from both superior oesophageal nerves revealed that these discharges were nearly synchronous (cf. Fig. 4), strongly indicating that each nerve contained a branch of the same cell.

Fig. 3 illustrates the regularity of discharge of this unit, and shows the characteristics of its response to natural stimulation. The sweeps at the right were taken on continuously moving film and were themselves continuous; the duration of each is 1 sec. As can be seen from the record, the discharge frequency of this unit in such semi-isolated preparations was extremely constant in the absence of any deliberate stimulation. In some such units the range of interval variation over 10 cycles was of the order of 2–3%. In different preparations, however, the spontaneous frequency varied from 2 to 8 impulses per sec. On the left are continuous records showing responses to pressure exerted (in a dorsal direction) on the cardiac ossicle. Characteristically, the high-frequency discharge resulting from such a stimulus was followed by a post-excitatory depression of the spontaneous discharge; the duration of this depression depended upon the frequency and duration of the preceding impulse burst. When such stimuli were repeated (lower record on left), the discharge pattern broke up into a series of bursts, without intervening activity.

The spontaneous activity characteristic of this unit in the semi-isolated preparation was unaffected by cutting the dorsal ventricular nerve. Severing either lateral nerve, however, produced transient increases in frequency. If both lateral nerves were cut, the results were variable. Often there was an eventual cessation of activity, but on some occasions discharge was maintained at an altered frequency for upwards of an hour,
suggesting that the cell was capable of maintaining autogenic activity with the ganglion totally isolated. It was, however, clear that the lateral nerves contained the elements responsible for the mechanical response of the cell, since cutting either one eliminated the discharge that could be evoked by manipulation of the ossicle on that side.

In subsequent experiments simultaneous recording was carried out involving the two lateral nerves and the two superior oesophageal nerves in various combinations. The lateral nerves were left connected in these experiments, and recording from them was en passant. In all possible paired combinations from among these four nerves,

![Image](image_url)

Fig. 4. Effect of electrical stimulation upon the spontaneous activity pattern. Upper traces, recording from an intact lateral nerve; lower traces, from the centrally cut superior oesophageal nerve. A, Stimuli applied to other superior oesophageal nerve; B, to other lateral nerve. C, Response to the last of a series of stimuli (at 1–2/sec.) to the other lateral nerve.

there was matching of impulses from the spontaneously active SG unit. Fig. 4 illustrates an experiment confirming this synchrony. In each record the upper trace is an en passant record from one of the lateral nerves and the lower is from a superior oesophageal nerve. Brief-pulse stimuli were then applied to the other superior oesophageal nerve (A) or to the other lateral nerve (B). In either case the interpolated response (visible in A but lost in the stimulus artifacts in B) reset the spontaneous discharge rhythm, with an added compensatory increase in the succeeding intervals. The magnitude of this increase was greater the earlier in the spontaneous cycle the interpolated response occurred. This behaviour, which presumably is related to accumulated refractoriness in the pacemaker region, is entirely analogous to that observed in other autogenic crustacean neurones (Preston & Kennedy, 1962; Kennedy, 1963). Resetting of the spontaneous discharge rhythm was also utilized in an attempt
to trace the central course of the branches to the superior oesophageal nerves. Stimuli applied to the circumoesophageal connectives or to the ventral nerve cord did not produce resetting of the discharge rhythm at any intensity; it must be concluded that the central branches terminate in the commissural ganglion. In Fig. 4C repeated low-frequency electrical stimulation (1–2/sec.) of the lateral nerve, the last pulse of which is shown, produced a sustained after-discharge of the cell, in addition to a shorter repetitive response in a second unit in the lateral nerve. Such behaviour is similar to the ‘triggering’ of pacemaker activity in other systems (Kennedy & Preston, 1963). Similar results were obtained in a number of completely isolated preparations when one of the cut lateral nerves was stimulated electrically, even after spontaneous activity had stopped completely.

These experiments led to the conclusion that the unit under analysis was a four-branched cell with its soma in SG and processes in each lateral and each superior oesophageal nerve, that it was capable of autogenic discharge and possibly also synaptic activation in SG, and that it responded to mechanical stimulation of the anterior stomach. The remainder of the experiments were designed to elucidate the function of the distal branches and the nature of the ‘spontaneous’ discharge in preparations with intact lateral nerve innervation.

The lateral nerves were cut at their SG terminations, and responses to movement of the cardiac ossicles were recorded. Fig. 5 is an example of the discharge observed in such a preparation in response to pressure applied to the cardiac ossicle of that side. The stomach was already lightly stretched, producing an accelerated ‘resting’ discharge of the large spikes. When the ossicle was depressed the frequency increased. The response typically showed a dynamic and a static component, resembling in general form that from a variety of other deformation-sensitive receptors. Only a single such unit appeared in each lateral nerve. This latter finding, in particular, seemed difficult to reconcile with the fact that only a single large unit responded to stomach movement in en passant records from the lateral nerves. Indeed, when simultaneous recordings were made from the two intact lateral nerves, spikes of only a single amplitude were present in each channel, and their discharges were synchronized (Fig. 6A). When one of the two lateral nerves was then cut centrally it still contained a single, spontaneously active unit, the discharges of which were now desynchronized with those in the other lateral nerve; they were sometimes reversed in polarity but usually only slightly altered in frequency (Fig. 6B). This result suggested that the
Fig. 6. Lateral nerve responses in intact and centrally cut preparations. Upper traces, left lateral nerve; lower traces, right lateral nerve. A, Both nerves intact, *en passant* recording. B, After cutting right nerve centrally, near its entrance to SG.

Fig. 7. Lateral nerve responses to stimulation of right and left sides in an intact preparation. *En passant* records from left (upper traces) and right (lower traces) lateral nerves. A, Stimulation of left side; B, of right side. Insert: records from a different experiment on a 5 × expanded time-base; the left side is on the lower trace, and the right side was stimulated. The reversal of timing relationships shown in B is more evident on the faster time-base (see text). Time-mark, 1 sec. for A and B, 0.2 sec. for insert.
lateral nerve branches of the SG unit were the stretch-sensitive elements themselves, i.e. that each was a dendrite capable of transmitting impulses to all other branches of the cell and thus of controlling its overall frequency. Such a situation would explain the apparent absence, in methylene blue-stained preparations, of peripheral sensory cell bodies in the mechanically sensitive region of the stomach.

This view was supported by an analysis of the responses in the two intact lateral nerves to localized mechanical stimulation, in particular with respect to the timing of impulses in the two branches (Fig. 7). Even under symmetrical \textit{en passant} recording conditions, the impulse in one lateral nerve led that in the other by a few msec. Mechanical stimulation of the ossicle on the leading side (Fig. 7A) produced matched high-frequency discharges on both sides, without changes in waveform or interval between pairs of impulses. Stimulation on the follow-side, however, reliably altered the waveform of the impulses on the two sides, usually reversing their polarity, and \textit{changed the time relation between them such that the stimulated side led during the high-frequency response} (Fig. 7B, insert). This is perfectly understandable on the hypothesis that the spontaneous discharge in such intact preparations originates in one of the two dendritic terminals, whichever has the higher inherent excitability or is under the greater mechanical stress. Under such conditions the impulse would always be expected to appear first in the 'leading' lateral nerve. This situation would be unchanged with mechanical stimulation of that side, but should reverse if impulses suddenly begin to arise on the other side at higher frequency.

A model of the SG mechanoreceptor cell consistent with the data would resemble the drawing in Fig. 8. Its basically bipolar configuration is consistent with the indication that it is a primary sensory cell. Details of the dendritic terminations are unknown, but they are presumably associated with muscular or connective-tissue elements in the vicinity of the cardiac ossicle.

The chief interest in this cell lies in its unusual bilateral morphology, and in the implications this has for its integrative functions. Each dendritic termination is an actual or potential pacemaker zone, either through some fully autogenic mechanism (such as a membrane leaky to sodium) or through a mechanical arrangement that allows for steady deformation under 'relaxed' conditions. Whichever terminal has the slightly higher intrinsic firing rate will completely dominate the output rhythm, since its impulses invade the other terminal and reset the latter's pacemaker cycle. If
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the two have nearly identical or slightly varying rates one might expect the lead to switch occasionally, without any very dramatic change in interval; occasional spontaneous shifts in polarity and inter-channel interval (see, for example, Fig. 7 A, first two impulses after burst) suggest that this in fact occurs. Impulse conduction along such dendrites is not surprising in view of their length, and has been directly demonstrated in other crustacean bipolar sensory neurones (Mellon & Kennedy, 1964).

Functionally, the unit serves to produce a balanced output from an unbalanced input. The output frequency delivered to each commissural ganglion is identical, and equal to that of the most active receptor terminal. Dominance of the most active input channel is secured by a mechanism quite out of the ordinary for sensory cells: resetting of the discharge rhythm of the subordinate input through antidromic dendritic invasion. Until the reflex function of the afferents of the stomatogastric system is worked out, the overall significance of such an integrative mechanism is uncertain. One may suppose, however, that the SG mechanoreceptor influences the activity of motor elements driving the anterior gastric musculature. Its nature would lead one to predict that its motor influence is bilaterally distributed, the entire reflex serving as a mixing circuit that could balance accidental asymmetries in the action of the relevant musculature. Given this presumed function, or one like it, a cell with the configuration shown is the most economical way of meeting the requirement; it thus represents another instance of the parsimony with which arthropods assign neurones.

Some aspects of the unit’s function are not clear. We have not ruled out the possibility that in addition to being a primary stretch-sensitive receptor, it receives afferent connexions within SG. Such a view is supported by the long after-discharges recorded from fully isolated preparations following a series of shocks to one of the lateral nerves. The alternative explanation would involve activation of a ganglionic pacemaker locus by repetitive activation of the cell itself.

In Fig. 8 we have reproduced the drawing made by Orlov (1927) of a cell found in methylene-blue preparations of the stomatogastric ganglion of Astacus. A similar neurone is described and figured by Allen (1894) in the embryonic lobster. Since it appears to have been unique in their anatomical analyses, and since its structure fits our findings on the SG mechanoreceptor so exactly, we propose that they are the same element.

SUMMARY

1. The crayfish stomatogastric ganglion (SG) contains about twenty-five neurone somata; it supplies motor innervation for the anterior gut, and receives afferent input from mechanoreceptors associated with the stomach. Its proximal branches respond to normal stomach contractions with a complex, patterned, centrally directed discharge involving several units.

2. Discharges from the largest of these can be recorded nearly simultaneously in the two superior oesophageal (proximal) and the two lateral (distal) nerves from SG. In preparations with these lateral nerves connected to the stomach the cell shows constant-frequency spontaneous activity, which can be reset by direct stimulation of any one of the four branches. Mechanical stimulation of the cardiac ossicles evokes a burst discharge.

3. Simultaneous recording from the two lateral nerves shows that impulses in one
of them consistently occur earlier; this temporal relationship is preserved when the leading side is stimulated, but reverses (usually accompanied by polarity changes) when the other is stimulated. Each lateral branch continues to respond independently after being cut at its point of entry into SG.

4. It is concluded that the cell is a new type of receptor neurone, with a bifurcating axon and two dendrites, each autogenically active at its receptor terminal. The higher-frequency input always determines the output rhythm. A cell with the appropriate configuration was figured by Orlov (1927) from methylene blue-stained SG preparations.

Supported by grant NB-05423 (J.L.) and by grant B-2944 (D.K.) from the U.S. Public Health Service. The authors are grateful to Mrs Philip C. Hanawalt, Mr Gary Shelton and Mrs James Larimer for technical assistance.

REFERENCES


