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NEUROHORMONAL ALTERATION OF INTEGRATIVE PROPERTIES OF THE CARDIAC GANGLION OF THE LOBSTER *HOMARUS AMERICANUS*

BY IAN M. COOKE* AND DANIEL K. HARTLINE†
The Biological Laboratories, Harvard University, Cambridge, Massachusetts, U.S.A.

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SUMMARY

The spontaneous burst discharges of isolated lobster (*Homarus americanus*) cardiac ganglia were recorded with a spaced array of electrodes. Small regions (< 1 mm) of the ganglion were exposed to the cardioexcitor neurohormone in extracts of pericardial organs (XPO) or to 10^{-5} M 5-hydroxytryptamine (5HT). All axons were excited (increased mean firing frequency, $\bar{f}$) by both substances, but only by applications in the region between the soma (but excluding it) and proximal site of impulse initiation. Units not so exposed changed their $\bar{f}$ relatively little despite $\bar{f}$ increases of as much as threefold in exposed units and changes in burst rate and overall length. Regularity and grouping of all impulse activity into bursts was never disturbed. 5HT increases burst rate at any point of application. The increases are larger if small cells are affected than if only large cells are exposed. Burst length decreases except when the pacemaker is affected. In contrast, XPO affects neither burst rate or length unless small cells are affected. Length is increased if non-pacemaker small cells are affected; both rate and length increase if the pacemaker is affected. The pacemaker usually exhibits an $\bar{f}$ of intermediate value. Rate changes are not simply related to its $\bar{f}$. A small cell can 'burst' in the absence of impulses from any other cells. XPO may enhance endogenous 'driver potentials,' while 5HT may excite by depolarizing at limited sites.

INTRODUCTION

When a hormone acts on a nervous system, the effects can be quite complex, since the direct action on individual neurones may be compounded with indirect effects. This study represents an effort to analyse the specific, direct effects of a neurohormone on individual, identifiable neurones which are functioning integratively within a small, autonomous nervous system, and at the same time to follow the resulting indirect effects. We have exploited the small neuronal numbers and the topographical separation of the cells in the lobster (*Homarus americanus*) cardiac ganglion to study the effects of a cardioexcitor neurohormone found in extracts of the pericardial organs (Alexandrowicz, 1953; Maynard & Welsh, 1959; Cooke, 1964; Berlind & Cooke, 1970) and to compare these effects with the effects of the most potent cardioexcitor

* Present address: Laboratory of Sensory Sciences, University of Hawaii, Honolulu, Hawaii, U.S.A.
† Present address: Department of Biology, University of California at San Diego, La Jolla, California, U.S.A.

drug known for this system; 5-hydroxytryptamine (5HT) (Cooke, 1962*a*, *b*; 1966). We start with some detailed knowledge of the properties of the nine individual neurones and their interactions (Review: Hagiwara, 1961; also, e.g. Cooke, 1966; Hartline, 1967*a*), and of the functional anatomy of the ganglion (Alexandrowicz, 1932; Hartline, 1967*b*; Mayeri, 1973*a*). Using the techniques described by Hartline, it is possible to identify the site, time of initiation and course of every impulse in every cell axon. In this study, it has been possible to perturb one or a few units by localized application of the excitatory substance and to analyse how such pertubations affect the whole system and are integrated without disruption of coordinated, rhythmic bursting. A brief account of some of this work has been published (Cooke & Hartline, 1973).

METHODS

Dissection

Cardiac ganglia were dissected from lobsters (*ca.* 0·5 Kg *Homarus americanus*) obtained from a local fish store and maintained in artificial sea water at 10 °C until used. Special care was taken to dissect the posterior trunk containing the small cells to its termination at the posterior arteries. Long lengths of the efferent nerves were freed, taking care to preserve the finer branches, as these provided easily recorded single active units (see below). The dissections took up to 3 h, and care was taken to replace the perfusion fluid (Cole, 1941) frequently and to keep the preparation cool (< 20 °C). The anterior ends of the Y-shaped ganglion and a small square of the posterior aorta, which was attached to the posterior of the ganglion, were held in an array of forceps. Ten pairs (inter-electrode spacing *ca.* 1 mm) of silver wire hook electrodes were arranged at strategic points on the trunk and on terminal efferent branches, and finally the entire array was lifted from saline into mineral oil.

Recording and analysis

The differentially amplified signals from the pairs of electrodes were led to oscilloscopes, to a seven-channel FM tape recorder, and to a four-channel ink writer. The ink writer provided a continuous record of burst rate; the tape recorder records were replayed for photography, on moving film (50 cm/sec), from an oscilloscope. As a rule, four successive bursts before each application of the test substance, at the height of a response, and after recovery, were analysed in detail. Analysis consisted of identifying the site and time of impulse initiation for each independent unit of interest. A diagram of the functional anatomy is given in Fig. 1. The functional regions of each cell are: a soma and proximal inactive segment which are not invaded by the action potential, a trigger zone where impulses originate, and active segments both proximal and distal to the trigger zone. The small cells show, in addition, distal trigger zones near the anterior bifurcation. The techniques and criteria for identification of impulses of each cell are detailed by Hartline (1967*b*). When the analysed records for several consecutive bursts were aligned, the firing times of the individual units fell into consistent patterns (see Fig. 2*B*).

When an efferent branch contained a single active unit, or one whose recorded impulse was much larger than any others, it was possible to generate a 'dot' pattern electronically. The amplified impulses of the unit were led to the 'Z-axis' of an oscilloscope. The dimmed beam, with its horizontal sweep triggered by the first

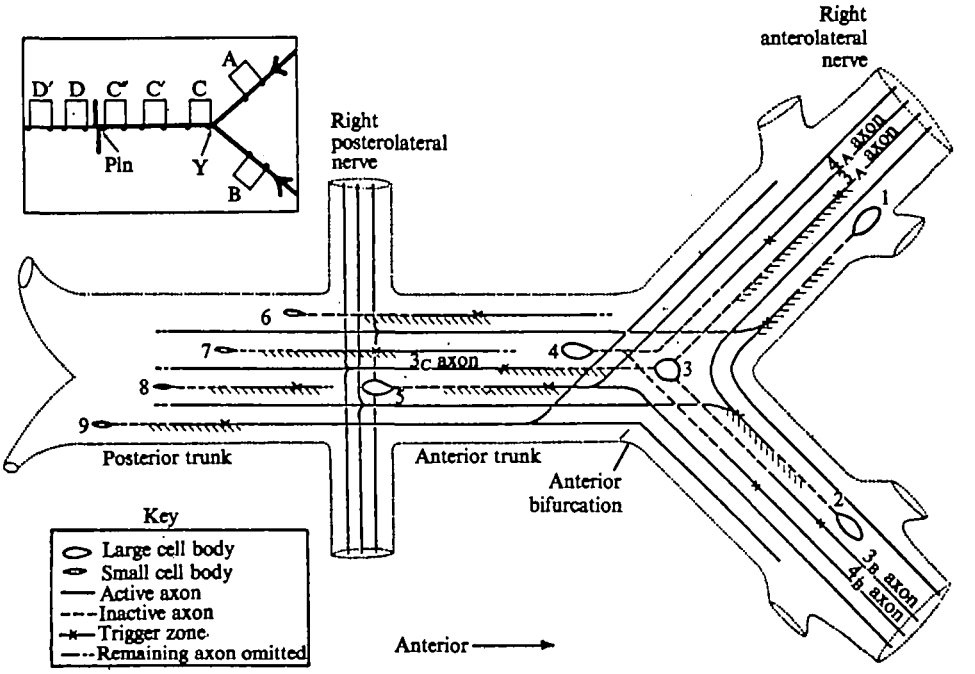

Fig. 1. Functional anatomy of *Homarus* cardiac ganglion. Diagram of soma locations, axon courses, and locations of anatomical regions for the nine intrinsic neurones. Active axon (refer to key) is axon carrying a regenerative impulse. In each cell, impulses arise at the trigger zones (proximal ones only shown) and propagate both distally and proximally. The impulse in the proximal (relative to trigger zone) active segment terminates on reaching inactive axon still at some distance (1–2 mm) from the soma. The shaded portion between soma and trigger zone represents a region of pharmacological sensitivity found in our experiments. Note that Cells 3 and 4 have more than one independent axon. The anatomy is idealized here; it shows variation in detail from ganglion to ganglion. Width of ganglion exaggerated relative to length (total length about 1 cm in 0·5 kg animals). In part after Hartline (1967*a*). Inset: Designations given to locations along the ganglion used in identifying electrode and drug placements.

impulse, was intensified by each impulse, leaving a series of bright dots as it swept across the screen. A slow vertical sawtooth caused the next burst to be registered immediately below the preceding train (see Figs. 4, 5).

Measurements

Burst rate (bursts/min). The number of bursts occurring during a 1/2 min or longer period, when the response had stabilized (2–5 min after application), were counted from the penwriter record. At the lowest burst rates (e.g. 20/min) this procedure would not reliably detect less than a 10 % change in rate.

Burst length (msec). Four successive bursts were measured from photographic records and averaged. Due to variability of burst length, we consider changes of less than 5 % insignificant.

Mean frequency of individual units: $\bar{f}$ (impulses/sec). The number of impulses per burst (averaged for four bursts) were multiplied by the number of bursts per second. Since integral numbers of impulses were used, the number of observations was limited and, especially in large cells, the number of impulses/burst was low, a change

Table 1. *Percentage change of burst rate and length, and of average firing frequency and pattern of individual units for all experimental applications analysed*

				XPO:									
#	APPL	ΔR%	ΔL%	3_A	1	2	3_C	5	6	7	8	9	CODE
PACEMAKER NOT AFFECTED													
Large Cells Only Affected													
1	Y	↓9	↓2						[↓50 C4]	↓20 C9	↓9 C4	↓9 0	D1/6
2	Y	↓3	↓4				↓15 C7	↑2 0	↓15 C7	↓6 C9	↓3 C<5	↓9 0	D3/3
3	Y	0	↑7				↓18 C18	0 0			0 C4	0 C6	D6/9
4	C′	↑3	↓4		↓11 C5	↑7 0	↓21 C15		↑5 C5	↓4 C5	↓3 0	0 0	D6/6
Small Cells Affected													
5	C′C″	0	↑17							↓16 C5	↓17 C10	↓10 C7	D3/1
6	C″	↑22	↑55	↑6 C17	↓16 C14	↑5 C34	↓2 C27	↓4 C18		↓4 C8	0 C14	↓2 C11	D2/7
7	Pin	↑45	↑233	↑29 0	↑27 C15	↑6 C17	0 C19				↑87 C27	↓6 C11	D3/9
8	D'_p	↑5	↑96			↓12		↑2 C5	↓22 0	↓12 0			D1/2
FINAL PACEMAKER AFFECTED													
9	C′C″	↓13	[↑23]		↓10 X5					↓8 C13	↓23 C21	↓13 C15	D4/11
10	Pin	↓17	↓11		0X59	↓12 X23	↓11 X22	↓30 C20	↓52 C13	↓43 C12	↓25 C6	↓4 X23	D6/13
11	Pin	↓64	↓9		↓38 X74	↓36 X49	↓62 X51	↓106 C17	↓113 C15	↓127 C20	↓71 C7	↓37 X35	D6/20
12	Pin	↓29	↓14		[↓28]	[↓33]				↓19	[↓76]		D3/2
13	D D′	↓12	↓20		↑2	↑7	↓11 X21	↓13 X24	↓36 C6	↓33 C5		↓9 X11	D6/10
14	D'_p	0	↓38		↓10 X32	↓16 X39	↓9 X31	↓9 X29	↓21 0	↓23 X8	↓34 C8		D6/2

Each of the 14 applications of XPO and of 5HT is given a number for reference in the text (first column). In the next column the point of application is specified with reference to the inset diagram of Fig. 1; a pair of letters signifies that application was between the two pairs of electrodes specified; a subscript 'p' indicates the application was to the posterior of the two wires in an electrode pair. Burst rate changes (ΔR %), and overall burst length changes (ΔL %) are tabulated, followed by percent changes of average firing frequency, $\Delta\bar{f}$ %, and change of firing pattern (C, contraction; X, expansion, %) of each analysed unit. The columns follow the usual anterior to posterior topographical sequence of the units' sensitive regions, as shown in Fig. 1. The code identifies the ganglion and application in authors' protocols. Fully shaded boxes identify units whose trigger zone or proximal active axon were exposed to the test substance; half shaded boxes identify units whose inactive proximal axon is exposed. The pacemaker during the response is indicated by the symbol ◆; a shift of pacemaker is indicated by ◇ beneath the former pacemaker and a connecting arrow. Numbers in brackets are excluded from averages and other numerical analyses because controls were inadequate. XPO No. 11 is exceptional in that rate increased abruptly at the beginning of the application; the response is probably due to mechanical stimulation of a dendrite. 5HT No. 8, application of 10^{-6} M, instead of 10^{-5} M as in others. 5HT No. 10, the extreme values are unexplained; possibly an error in dilution to give 10^{-4} M. Heaviest type is used for values > 50 %, intermediate type for values > 25–49 %. Note that the majority of values so emphasized occur in shaded or half-shaded boxes.

of more than 10 % was required to be considered significant. Changes of $\bar{f}$ are presented as percentages (e.g. Table 1). To the limited extent examined, the qualitative conclusions are not changed if absolute $\Delta\bar{f}$ values are used.

Pattern expansion. When the intraburst frequency of a unit decreases in response to an experimental manipulation, the distance between successive dots in a row (i.e. interspike interval in the train) increases. Percentage expansion was estimated by

Table 1 (*cont.*)

5HT:													
#	APPL	ΔR%	ΔL%	3_A	1	2	3_C	5	6	7	8	9	CODE
PACEMAKER NOT AFFECTED													
Large Cells Only Affected													
1	Y	↓14	0	[↓13 X5]				↓5 X6	↓3 X7		↓9 X4	↓15 X6	D2/4
2	Y	↓12	↓7	[↓38 C32]				[↓25 C12]	[↓11 C3]	[0]	[↓28 C8]	[↓6 C8]	D2/11
3	Y	↓27	↓18	[↓42 C10]			[↓23 C5]	↓33 X15	↓9X15	↓10 X6	↓6 X7	↓5 X10	D3/5
4	Y	↓24	↓18	[↓20 0]			[↓30 0]	↓4 X11	↓19 X8	↓12 0	↓2 X22	↓24 X4	D4/6
5	C C′	↓12	↓12		↓3 X22	↓10 X17			↓7 X20	↓5 X9	↓10 X5	↓7 X13	D6/4
Small Cells Affected													
6	C″	↓40	↓3	[↓55 X13]	[↓22 X25]		[↓66 X21]	[↓40 X10]		↓67 X50	[↓67 X5]	[↓68 X41]	D2/6
7	C′	↓62	↓16		↓5 X81	↓2 X85	↓22 X56				↓16 X15	↓4 X28	D6/3
8	Pln	↓21	↓9		↓4 0	↓10 0	↓2 0	[↓20 0]	[↓45 C18]		↓35 C<5	↓10 X17	D3/8
9	D′	[↓13]	[↓18]		↓1 C12	↓1 0	↓8 C18	↓15 C23	[↓14 C3]	↓13 C2	[↓20 0]		D6/5
FINAL PACEMAKER AFFECTED													
10	C′C″	↓100	↓1		↓35 X36	↓23 X18				↓23 C11	↓24 C23	↓100 X17	D4/12
11	Pln	↓42	↓21		↓9 X37	↓13 X28	↓21 X16	[↓42 X33]	[↓75 C5]		↓54 X5	↓58 0	D3/4
12	Pln	↓28	↓23		↓6 0		↓6 X18	[↓50 X<5]	[↓38 C<5]			↓28 0	D4/5
13	Pln	↓55	↓12		↓9X65			↓31 X25	↓33 X5	↓37 X12	↓25 X19	↓16 X31	D6/11
14	D D′	↓23	↓6		↓9 X42	↓7 X32	↓15 X30	↓10 X54	↓30 0	↓23 X9		↓25 X4	D6/7

comparing in control and experimental records the distance from the initial impulse to the last impulse having a stable position. In Table 1, expansion is denoted by an 'X' followed by the percentage.

Pattern contraction. When the intraburst frequency of a unit increases, the distance between successive dots contracts. The percentage change was estimated as in the case of expansions. In Table 1 this is denoted by a 'C' followed by the percentage.

Test substances

Unless noted otherwise, 5HT was applied as a 10^{-5} M solution in perfusion fluid. The solution was made up freshly for each experiment from a stock solution of 10^{-5} M 5-hydroxytryptamine creatine sulphate (Regis Chemical Co.) in distilled water. The stock solution was kept refrigerated for not longer than a month.

The extracts of pericardial organs (XPO) were prepared from freshly dissected pericardial organs of single individuals of the crab *Libinia emarginata*, average wet weight of tissue about 2·5 mg. The tissue was homogenized in 0·2 ml of distilled water, heated for 1½ min in a boiling water bath and then 0·8 ml of lobster perfusion fluid added. This stock solution was kept refrigerated throughout the experiment. For application to the ganglion, unless noted, a 1/10 dilution in perfusion fluid was used. In a single experiment (D–2), freeze-dried organs of two *Cancer borealis* (wet weight about 2·7 mg, dry weight 0·5 mg) were homogenized in 0·2 ml water and heated as before, but the stock not diluted.

Crab pericardial organs were used rather than those of the lobster because they are much more easily dissected. Cross specific tests on isolated, perfused hearts (lobster XPO on crab and lobster hearts, crab XPO on crab and lobster hearts) have shown little quantitative and no detectable qualitative differences.

Application of test substances

The solution of drug or XPO was taken up in a fine glass micropipette and a small droplet deposited under mineral oil on the trunk of the ganglion, where it adhered well. Such a droplet covered up to 1 mm of the trunk. After recording the response (a minimum of 2 min following application), and usually within less than 10 min, the droplet was removed by aspiration and that portion of the trunk 'rinsed' by successive application and removal of droplets of perfusion fluid.

It was essential for our purposes to define the location of an application relative to certain topographical features of each cell. For this purpose the trigger zone has been defined by the place (*ca.* 1 mm) between electrodes which record the unit's impulse in opposite polarity (× 's in Fig. 2A); the inactive axon segment is taken to include the position occupied by the electrode pair beyond the most proximal pair in which the propagating impulse is visible. Since the electrodes were spaced at approximately 1 mm intervals, there is an uncertainty of up to 1 mm in these topographical definitions. If the impulse propagated to within 1 mm of the soma (as for example in unit 3_A of several ganglia), this definition resulted in classification of an application to the soma (e.g. for unit 3_A, an application at the anterior bifurcation) as also to the 'inactive segment.'

Ganglion variations

Every ganglion showed individual anatomical and functional variations from the general norm, which are noted in presentation of the results where appropriate. Cell bodies of the large neurones were located either visually during the experiments, or with methylene blue staining following the experiments, but small cell somata could not be consistently located.

RESULTS

Excitation by localized XPO and 5HT application

The excitatory effects of XPO and 5HT previously observed on isolated and perfused hearts and cardiac ganglia are here shown to have their bases in excitatory responses of each active unit of the ganglion to these substances. We have taken as a criterion of an excitatory response for an individual unit an increase in mean impulse frequency, $\bar{f}$, of at least 25 % above the control level. A unit showing such a response will henceforth be referred to as 'affected.'

Localization of sensitivity. Application of a droplet of XPO or 5HT to a circumscribed portion of the isolated cardiac ganglion results in increases in $\bar{f}$ of a limited number of the units, and at times, depending on its location, to changes in the burst rate and overall burst length of the ganglion. Figure 2 shows an example of an application of XPO to the mid portion of the trunk (arrow) of the ganglion. The burst rate slowed from 60 to 47/min. Figure 2B shows the complete analysis of both control and experimental burst patterns, from which the percentage changes of rate, length, mean frequency and pattern were calculated. These values are entered in Table 1 as

XPO entry No. 6. Note that the extract was applied to the trigger zone of Cell 6, which responds with a large increase in $\bar{f}$, a contraction of impulse pattern, and addition of impulses. Other cells change their $\bar{f}$ very little and generally show smaller pattern contractions.

Analyses of a total of 14 applications each of XPO and 5HT to five ganglia are presented in Table 1. The generalization which covers the largest number of observations is that each cell is responsive to these substances in a restricted region bounded by the soma, but not including it, and the site of impulse initiation (trigger zone, see Fig. 1), an area which we will term the topographically defined 'sensitive region' of the cell. A unit to which a test substance has been applied within this region will be termed 'exposed.' In Table 1 we have superimposed shading over all entries corresponding to topographically 'exposed' units. Applications covering the proximal 1–2 mm of axon usually affect a cell, though, on the average, less than those to the trigger zone (Fig. 3), so these are only half-shaded. Also, in Table 1, all changes of $\bar{f}$ of 50 % or greater are printed in heavy bold type, and those of > 25 % up to 50 % in less bold type. Most of the numbers so emphasized will be seen to fall within the shaded areas. A majority of those not within the shading have topographical sensitive regions immediately adjacent to the point of application. Note that all of the cases in which $\Delta\bar{f} < 25$ % in the 'exposed' category consist of applications at the inactive proximal segment (half-shaded) which, as mentioned above, is less sensitive on the average. Note also that all but two of these cases do nonetheless have significant (> 15 %) increases in $\bar{f}$. Exceptions in the 'unexposed' group are interpretable as units having physiological sensitive regions extending a small distance (1 mm) distal of their trigger zone.

Averaged topographical sensitivity. As another way to illustrate the topographical sensitivity of ganglion cells, we have plotted in Fig. 3 a bar graph of the average firing frequency change of all units, grouped according to the topographical point of application of XPO and 5HT, with a horizontal axis representing the cell topography. The plot shows that there is, on the average, about a five-fold greater change of $\% \Delta\bar{f}$ in response to these substances when applied near the trigger zone than on the soma or far distal to the trigger zone. The proximal region in this plot includes the entire region between trigger zone and soma, including the inactive segment as defined above. The proximal region appears to have less sensitivity than the trigger zone. We have included as a distinct section in the graph, averages from units for which applications were made to the position occupied by the electrode pair next distal to the trigger zone. Large variability of individual responses makes it uncertain whether the apparent greater $\bar{f}$ change of units exposed at this point to 5HT compared to XPO is significant. The standard deviation of the groups other than those we have classed as 'unexposed' is large, however. In the case of XPO, all of the significant responses to applications in the region distal to the trigger zone represent responses of Cell 8 in applications to two ganglia; about half of those to 5HT in this category represent responses of these same cells. This may reflect a variation of functional anatomy equivalent to the many variations from the generalized pattern observed in gross anatomy of ganglia.

Lack of soma sensitivity. In light of the demonstrated chemical sensitivity of molluscan ganglion neurone somata (e.g. Tauc & Gerschenfeld, 1962), it seems important to examine more closely evidence suggesting that the soma of cardiac ganglion cells is relatively insensitive to the active substances. The observations consistently show

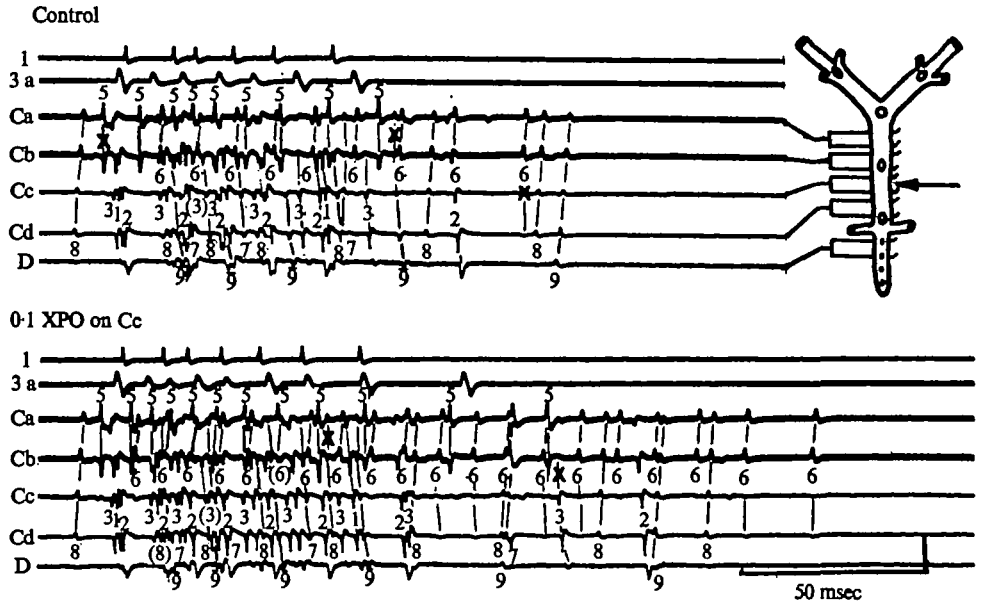

Fig. 2. Analysis of a pericardial organ extract (XPO) application. This figure illustrates the manner in which data were obtained and analysed for a drug application. See text for details. Application XPO No. 6 (D2/7). (A) (above) A control burst (top) and one experimental burst (bottom). Diagram at right indicates electrode placements on ganglion and large cell soma positions (small cell positions not accurate, for diagrammatic purposes only). Arrow indicates position of application of XPO droplet. Numbers identify impulses of corresponding cell axons; $3 = 3_C$. × 's indicate sites of impulse initiation. A bar over the cell number indicates impulses triggered distally. Brackets indicate uncertainty in identification or timing. (B) (opposite) 'Dot pattern' analysis of the records. For each of eight bursts (four control; four experimental), including the two from (A), firing times for each unit are indicated as a horizontal line of dots. Beneath the first burst pattern for a unit, the succeeding three burst patterns for that unit are positioned relative to the pacemaker impulse in Cell 8. Note the typical consistency in firing pattern of a unit within the four bursts. The arrow indicates the change of firing position of the last stable Cell 6 impulse during the response. The effect of the XPO application has been to increase the frequency and number of impulses of Cell 6 selectively and to contract its firing pattern. The data are given in Table 1 (XPO No. 6).

relative insensitivity of the somata to XPO. The evidence does not conclusively exclude the possibility of some sensitivity of the soma to 5HT. A typical example is the application illustrated in Fig. 2, in which XPO applied to the soma of Cell 5 resulted in a relatively minor increase in the $\bar{f}$ of that cell (Table 1, No. 6). Applications of XPO to the soma of Cell 3 (Table 1, Nos. 2, 3) show sharp differences (about four-fold) between the $\bar{f}$ changes of large cells whose trigger zones are exposed (Cells 1 and 2) and that of the Cell 3_C axon. Marked though smaller differences are also seen in similarly placed 5HT applications (Table 1, Nos. 2, 3, 4).

While the observations are consistent with lack of sensitivity of small cell somata, we do not know the small cell soma positions accurately, and we thus cannot reach a firm conclusion about the extent to which small cell somata are insensitive to the test substances.

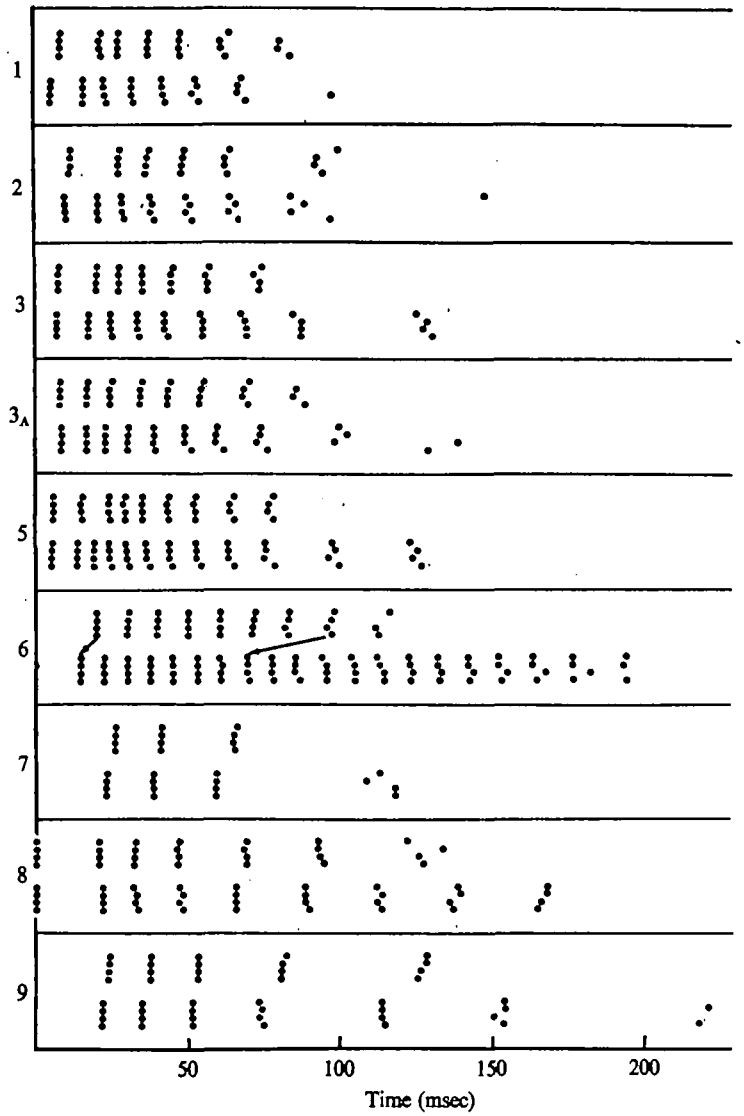

Fig. 2B. For legend see opposite.

Consequences of increased individual neurone activity

One of the most striking observations of this study is that we never produced a disruption of the spontaneous rhythmic bursting and of the tight grouping of all impulses within the burst, in spite of very large increases in the activity of one or a few of the units. Examination of the firing pattern changes of individual units during response to localized applications of the excitatory substances shows how the increased activity of the affected units is held within the overall burst pattern of the ganglion.

Impulse firing pattern changes. Extremely stable timing of the impulse firing of each unit within a burst is a consistent observation. Changes resulting from application of active substances to the ganglion are therefore easily detectable. Units whose $\% \Delta f$ is

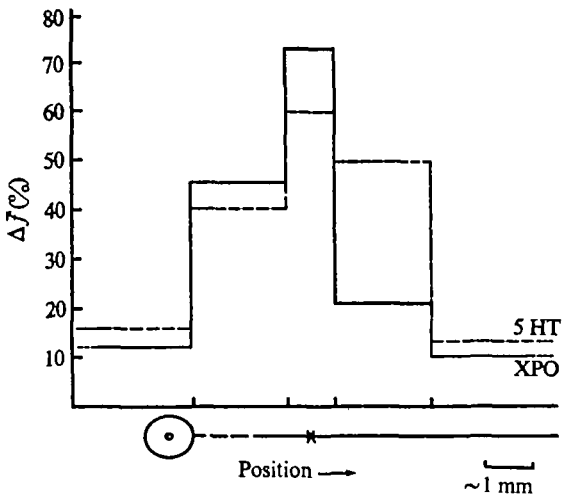

Fig. 3. Distribution of drug sensitivity along neurones. Plot of mean $\%\Delta\bar{f}$ value for all applications of XPO (solid line), and 5HT (dashed line) listed in Table 1 as a function of positions relative to each cell. Five segments represented by the horizontal levels of the graph were analysed. Location of these relative to anatomical features of the cell is indicated by reference to the diagrammatic neurone below. Variation is too large to give more than qualitative significance to the figure.

greater than the percentage rate change maintain their firing within the burst by an addition of impulses, usually accompanied by a pattern contraction. An example of this behaviour is seen in the response of Cell 6 in Fig. 2B.

The pattern changes of cells unaffected by an application reflect accommodations which maintain their $\bar{f}$ constant. Referring again to the example shown in Fig. 2B, all of the units are seen to exhibit contraction of their pattern and addition of impulses to the burst; thus the $\bar{f}$'s show little change despite the significant burst rate decrease. The increased burst length is seen to involve not just the affected cell, but all units of the ganglion.

In other applications, large increases in rate were accompanied by pattern expansion and reduction of the number of impulses produced in each burst by unaffected cells. Again, this resulted in the conservation of the unaffected units' $\bar{f}$'s.

An interesting exception to the observation that unexposed cells tend to maintain their $\bar{f}$ constant was Cell 9. In several of the experiments this cell initiated the majority of its impulses from a distal trigger zone and held the number of distally triggered impulses per burst constant in spite of changes in burst rate. Thus in those cases the change of burst rate was paralleled by change in $\Delta\bar{f}$.

Pattern change differences between XPO and 5HT. Impulse pattern changes could be monitored more continuously when single unit recordings were used to produce a graphic display electronically (see Methods). Examples are given in Figs. 4 and 5. Figure 4 shows the electronically generated impulse firing pattern of Cell 5 in about 75 consecutive bursts. The change in pattern is the result of an application of XPO to this cell's trigger zone (Table 1, No. 4). It was the only cell affected ($\bar{f}$ increased 38 %); there was no change in burst rate or length. The figure shows the development of increased firing frequency and how integration of this increased activity of a single cell

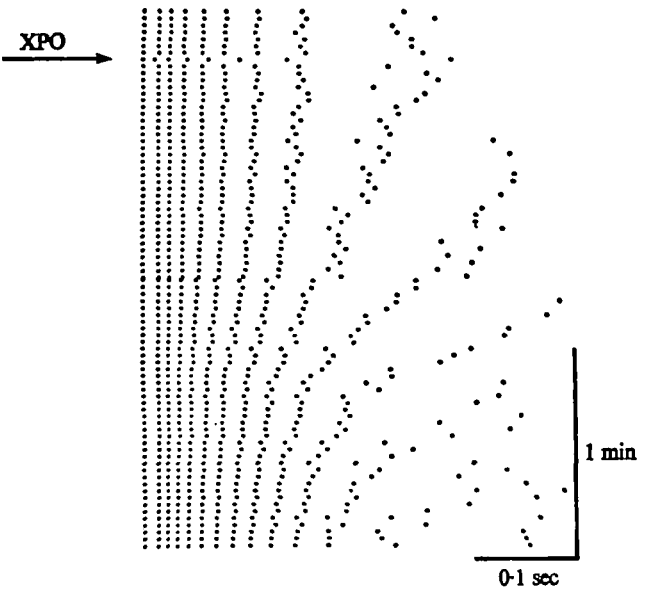

Fig. 4. Effect on impulse firing pattern of direct application of XPO to a large cell. This figure shows the electronically produced 'dot pattern' (see Methods) for a large cell, Cell 5, to which an application of XPO had been made on its proximal active segment. The application was made after the seventh burst (arrow and electrical artifact). Midway down the illustration the near superposition of two bursts indicates where a few bursts were omitted while the film was repositioned for the second half. (XPO No. 4, D6/6, P19). The response, $\Delta\bar{f}$ 38 %, is coordinated without change of burst rate (dot rows remain evenly spaced), by increasing the intraburst frequency and number of impulses, seen here as a contraction (42 %) of the pattern.

is accomplished: note the 'closing in' of the late-firing impulses and addition of others as the response develops. Affected cells typically show pattern contractions, the extent of which depends on the excitatory substance (see below) and whether or not burst rate has been increased by the application.

The effect on pattern of combined interaction between change of $\bar{f}$, burst rate, and specific responses to excitatory substances is illustrated in Fig. 5 (the same cell and ganglion shown in Fig. 4). Figure 5A shows response to 5HT, and Fig. 5B shows response to XPO applied to a sensitive region (inactive proximal segment of the axon, i.e. at the Pln) (Table 1, 5HT No. 13; XPO No. 10). The responses were equivalent, in that $\bar{f}$ of Cell 5 increased about 30 % in both cases. Cells 6, 7, and 8 were also comparably affected in each application. However, the XPO application resulted in small rate (17 %) and length increases (11 %); while 5HT produced a very large rate increase (55 %) accompanied by a small length decrease (− 12 %). The differences in the Cell 5 impulse patterns reflect both an integrative accommodation of the cell and specific responses to the two substances. In the XPO application it (together with all other affected cells) contracts its pattern. In the 5HT application all of the cells exhibit expansion of their pattern (i.e. decreased intraburst frequency) in accommodation to the large burst rate increase.

The differences in these responses to 5HT and XPO applications to the same point are representative of the entire experimental series and seem to reflect a difference in the mode of action of the excitatory substances.

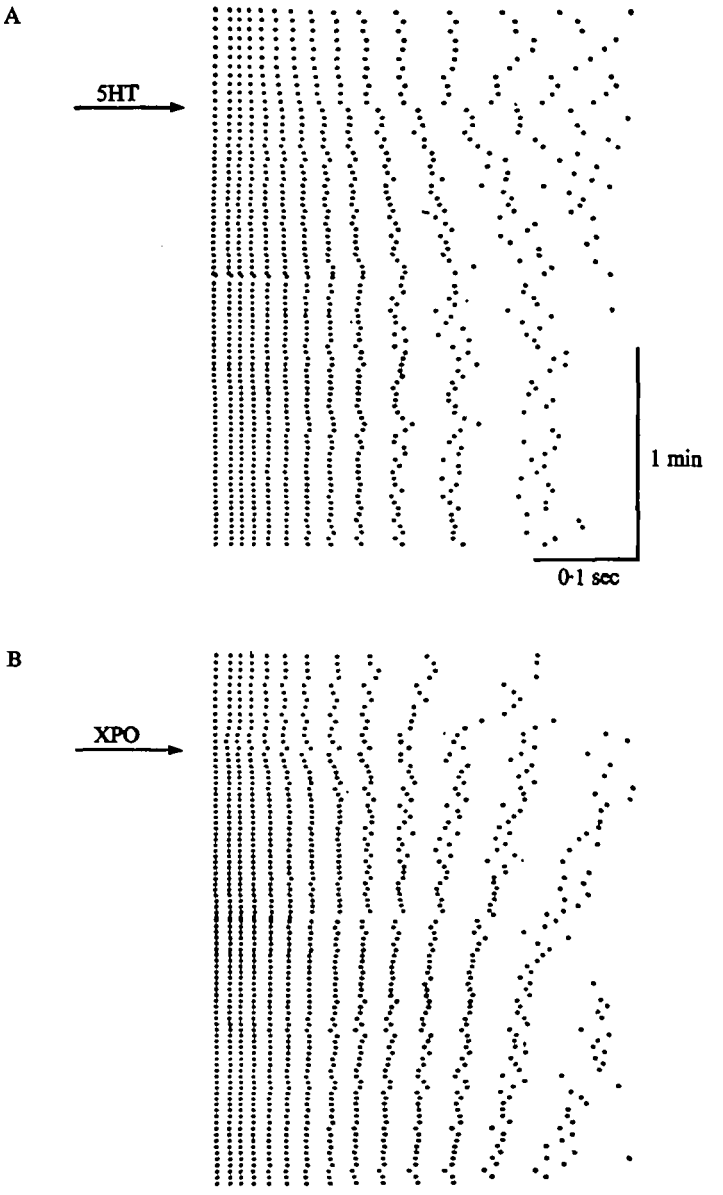

Fig. 5. Comparison of 5HT and XPO effects on a large cell impulse firing pattern. Dot patterns for Cell 5 (same ganglion) in response to applications to its inactive segment. (A) 10^{-5} M 5HT: $\bar{f}$ increased 31 %; note pattern expansion (25 %) and burst rate increase (55 %, rows of dots more closely spaced vertically) (5HT No. 13, D6/11, P23). (B) XPO: $\Delta\bar{f}$ increased 30 %; note pattern contraction (20 %); rate increased 17 % (XPO No. 10, D6/13, P25).

Examination of the 'dot patterns' over longer periods than the examples shown here, together with continuously recorded data on burst rate and length, shows that responses to applications of active substances reached a stable level within 3 min of the application; recovery after removal was slower (10 min or more).

Table 1 indicates, for most of the units analysed, changes in intraburst impulse

pattern expressed in terms of percent contraction (C) or expansion (X, see Methods). Given the observed condition that all units fire together as a 'burst' of activity, it follows that in cases where length is unchanged, units which increase their $\% \Delta \bar{f}$ more than the percent increase in burst rate should show pattern contraction and addition of impulses: those approximately matching the burst-rate change should show little pattern change, and those maintaining $\bar{f}$ constant should expand or contract their pattern if burst rate increases or decreases, respectively. If burst length changes, these tendencies should be modified by tendencies to decrease intraburst frequency when length increases. This, indeed, provides an adequate generalization of the observations.

Effects of localized applications on integrated activity

The combination of localized application of excitatory substances and limited regions of individual neuronal sensitivity to them permits a test of the contribution of individual neurones or small groups of neurones to the determination of the overall activity of the ganglion. Large cells, small cells, and the pacemaker, in ascending order of importance, are seen to influence burst rate and length. The entries in Table 1 are grouped to reflect these distinctions.

Effects of large vs. *small cell excitation.* Applications of 5HT to any point on the ganglion produced an increase in burst rate. If only large cells were involved, there was in three out of five cases a significant length decrease. The rate increase seemed to be larger if several small cells were affected.

By contrast, XPO produced no significant changes in rate or length of the burst unless small cells were affected. If small cells, but not the pacemaker, were responding, burst length increases resulted. In most cases these were very large (Table 1, Nos. 6, 7, 8), and were accompanied by rate decreases.

When an application of XPO affected the pacemaker, then, and only then, were both rate and length of the burst increased. In the case of 5HT, only applications which affected the pacemaker produced both rate and length increases.

In summary, XPO increases burst length if applied to small cells, and rate and length if applied to the pacemaker; 5HT increases rate wherever applied, and length only if applied to the pacemaker.

Correlations with pacemaker $\bar{f}$. In both XPO and 5HT applications, plots of the percentage increase of $\bar{f}$ of the pacemaker *vs.* percentage rate change show a rough proportionality. However, the absolute $\bar{f}$ of the pacemaker cell was generally intermediate among those of the small cells, both in control bursts and during a drug application. Usually the pacemaker did not change, despite large changes in $\bar{f}$ of the other small cells. In five of seven cases where the pacemaker did change (out of 19 involving small cells: ◇ → ◆ in Table 1), the new pacemaker represented a small cell which had been affected (in terms of increased $\% \Delta \bar{f}$) more than the control pacemaker, and in most cases (four) more than other small cells. However, only two of the new pacemakers had the highest absolute $\bar{f}$. Five of the seven cases of pacemaker shifts occurred in response to XPO applications. This is consistent with the suggestion that the neurohormone enhances an underlying pacemaker mechanism. Such a suggestion is also supported by observations of an 'organizing' and burst enhancing effect of XPO (see below).

The process controlling the spontaneous rhythmicity of the ganglion is complex, and

neither the number of impulses per burst generated by the pacemaker cell nor its $\bar{f}$ provide a measure (though they are parallel) of the process controlling burst rate. However, our observations indicate that consistent rate *and* length increases are observed only when the acting pacemaker is affected by application of our active substances.

Efficacy of synaptic interactions. The degree to which effects of the active substances are localized has already been discussed and implies the inability of impulse-mediated synaptic interaction alone to substantially increase the $\bar{f}$ of post-synaptic units. Despite the known synaptic input of small cells onto other cells (Hartline & Cooke, 1969; in *Panulirus*, Hagiwara & Bullock, 1957; Bullock & Terzuolo, 1957; Friesen, 1973, 1974), in the present experiments, we fail to find a good correlation between the amount of excitation of small cells affected by an application, and that of the unaffected cells.

Enhancement of an endogenous burst mechanism by XPO. When, more than 24 h after isolation, a cardiac ganglion is firing irregular, poorly co-ordinated bursts, application of XPO to the small cells, even at concentrations normally subthreshold, will restore the ganglion to co-ordinated, rhythmic bursting. This 'organizing' effect of XPO is irreversible, in the sense that it persists for an hour or more after removing the application and rinsing.

5HT application to small cells of deteriorated ganglia revealed some ability of the substance to restore co-ordinated bursting. However, upon removing the 5HT, the ganglion in most cases reverted to a less co-ordinated pattern than before the application.

In the case of a surgically isolated Cell 9, irregular trains of impulses were converted to rhythmic trains (bursts) by application of XPO. The ability of isolated small cells to fire bursts in the absence of impulse activity in other cells (unpublished observations; Mayeri, 1973*a*) is consistent with the existence of an endogenous bursting mechanism. XPO appears capable of enhancing this mechanism.

Generalizations and exceptions

The generalizations presented above can be summarized as follows. Cells are most sensitive to the test substances in the region of their trigger zone and proximal axon. They are sensitive, but less so, in the inactive segment, and are not affected by application to the soma or axon distal to the trigger zone. In terms of Table 1, units 'exposed' to test substances (i.e. in shaded boxes) are expected to exhibit increases in $\% \Delta \bar{f}$ of $> 25\%$, those not in shaded boxes $< 25\%$. Rate *and* length increases are observed only when the pacemaker (during the response) is affected. XPO has no effect on these parameters unless small cells are affected. 5HT affects burst rate at any point of application.

There are a considerable number of entries in the table which do not conform in all details. The majority of these can be interpreted as resulting from one or a combination of the following. (A) Anatomical variability between ganglia is consistently observed, and no individual ganglion conforms exactly in its gross or functional anatomy to the typical pattern. It is reasonable to suppose that other variation, not observable by our methods, such as the position of axon collaterals and sites of synaptic interaction, also occurs and with it a variation of the region sensitive to the test substances. Our strictly topographical classification makes no allowance for this variability.

(B) The site of application and the size of the areas exposed to applied substances is to some extent uncertain, in some cases, to as much as ± 1 mm. Further, the extent of diffusion may vary as a result of the presence of exiting processes or adhering tissue. (C) The ganglion or one or more cells may not have completely recovered from a previous application. Since perturbation of individual units did not necessarily change burst rate and length (the parameters observable during the experiment), such inadequately recovered units were not detected until full analysis of the data was carried out. (D) The extracts of pericardial organs were made from single animals and may therefore have varied in concentration and proportion of active substances (Belamarich & Terwilliger, 1966; Maynard & Welsh, 1959; Cooke & Goldstone, 1970).

DISCUSSION

The observations lead to the following general conclusions. (1) XPO and 5HT have excitatory effects on each of the lobster cardiac ganglion cells: both increase the average firing frequency, $\bar{f}$, though in a different pattern. (2) The neurones are responsive to these substances only in the functionally and topographically definable region lying between the soma (but excluding it) and the proximal site of impulse initiation ('trigger zone'). (3) The mean firing frequency of exposed units may be increased as much as threefold while unexposed units usually increase their $\bar{f}$ only slightly ($< 15\%$). Our manipulations never caused failure of synchrony and rhythmicity of the total ganglionic impulse traffic, and if anything, strengthened it.

Patterns of excitation. We have used the average firing frequency, $\bar{f}$, of individual units as the most easily quantifiable measure of their activity levels under the variety of pattern changes observed. The degree to which $\bar{f}$ is maintained approximately constant by units whose sensitive region is not exposed to the test substances is one of the striking observations to emerge in this study. This observation lends support to a suggestion that $\bar{f}$ is controlled endogenously. In attempting to compare the pattern of excitation produced by the two test substances, we must first consider to what extent the excitation they produced was equivalent. The mean values for all $\% \Delta\bar{f}$'s of all entries in Table 1 are within 7 % for XPO and 5HT (27·4 % and 29·2 %, $n = 94$ and 95, respectively). The response pattern to these substances probably does not change qualitatively as a function of concentration, since log concentration *vs.* response plots (for such parameters as burst rate and length) for isolated ganglia perfused with these test substances were continuous and often linear over several orders of magnitude (Cooke, 1962*a*, 1966). In spite of the uncertainties about the strength and composition of XPO arising from the use of crude extract of organs from single animals, the consistency of responses permits some generalizations to be made.

For comparable changes of $\% \Delta\bar{f}$, cells responding to XPO show much greater increase in intraburst frequency (i.e. pattern contraction) than in response to 5HT. This is interpretable as a direct consequence of the lack of effect on burst rate produced by XPO, unless the exposed cell is or becomes the pacemaker. 5HT, in contrast to XPO, causes a burst rate increase wherever applied, though most dramatically if small cells are exposed. With the exception of applications involving the pacemaker, we can generalize the difference in response pattern to the test substances as follows: XPO increases $\bar{f}$ by increasing the frequency and number of impulses per burst; 5HT,

increases $\bar{f}$ by increasing the frequency of bursts, with minor increase in number of impulses per burst. When the pacemaker is affected, 5HT produces larger rate increases than does XPO (% $\Delta\bar{f}$ for the pacemaker being comparable).

Localization of sensitivity. Evidence for lack of sensitivity of the large somata is quite strong; the difference in responsiveness of soma *vs.* proximal axon is more marked than our technique would have led us to predict. Since we did not locate the small cell bodies visually, we cannot state our relative point of application as precisely. The more distal limit of sensitivity is less clearly delimited. We chose the trigger zone as a functionally identifiable anatomical indicator. However, the precise point of impulse initiation varies, and therefore location of the 'zone' is imprecise. In addition, our electrodes have finite width, and drug droplets occupied at least 200 μm, with further diffusion to an unknown distance. The trigger zone was consistently highly sensitive, as was the region between it and the soma. A few units were highly sensitive just distal to the trigger zone. The sensitive region corresponds to the anatomical region at which numerous collaterals are given off to neuropile. Electron microscopy (Ohsawa, 1972) and intracellular recordings which show synaptic potentials of larger amplitude than impulses (Cooke, 1966; Hartline & Cooke, 1969), indicate the proximal axon and its collaterals to be sites of synaptic input (see Hartline, 1967*a*).

Sensitivity of the trigger zone does not indicate a direct effect of the substances on the spike initiation mechanism. These substances were not effective at sites of distal impulse initiation. The degree of individual variability precludes a firm conclusion about whether the sensitivity to 5HT extends more distally than that to XPO. The impression of more localized sensitivity to XPO than to 5HT would be consistent with the presence of specific receptors for the neurohormone. There is no evidence thus far for any physiological role of 5HT. Experiments in which block (by LSD) or tachyphylaxis to 5HT has been established without interference with response to XPO (Cooke, 1962*a*, *b*; 1966) indicate different sites of action for the two substances.

Integrative mechanisms. Our ability to excite one or a few units while monitoring all units of the relatively intact ganglion provides a means of examining the role of individual units in the functioning of the ganglion. Excitation of small cells produces much larger effects on overall burst characteristics (rate and length) than does perturbation of large cells. Thus the synaptic interactions which have been shown to exist between large cells and from large to small cells (Hartline, 1967*a*; Mayeri, 1973*a*) are of minor influence on the overall output of the ganglion. A similar conclusion, based on selective electrical stimulation, is reached by Mayeri (1973*a*). In clarification of an earlier statement (Cooke & Hartline, 1973, p. 433), strong synaptic driving of large cells by small cells is evidenced by the maintained synchronization of large cell impulse trains with that of the small cells during significant changes in burst rate. However, excitation of small cells did not produce marked changes in $\bar{f}$ of unexposed cells. This is unexpected in view of the known strong excitatory synaptic input to large cells from small cells (Hartline, 1967*a*; Hartline & Cooke, 1969; Mayeri, 1973*a*), and postulated input to more anterior small cells (Hartline, 1967*a*). An explanation of the observations may lie in extending the properties of defacilitation seen in synaptic transmission between small cells and large (Hartline & Cooke, 1969; in *Panulirus*, Hagiwara & Bullock, 1957; Bullock & Terzuolo, 1957; Friesen, 1973, 1974) to all impulse-mediated synaptic interactions of the ganglion. The practical effect of the

defacilitation is that, after the first two or three impulses, the mean post-synaptic depolarization produced by firing of a pre-synaptic unit reaches a constant value which is approximately independent of its firing frequency.

The response patterns of all units not exposed to the test substances are interpretable by the generalization that they adjust their firing pattern to reflect the smallest change of mean firing frequency consistent with co-ordination of the burst. The characteristic $\bar{f}$ of individual units and its stability have been previously noted (Maynard, 1955; Hartline, 1967*a*). The results of these experiments suggest that mean impulse firing frequency is an endogenously determined property of each of the active units of the cardiac ganglion. A similar conclusion, based on observations that $\bar{f}$ stabilizes at original levels following transections of the ganglion, is reached by Mayeri (1973*a*). It may also be that the organization of impulse firing into rhymically recurrent trains is an inherent property of each neurone, as suggested by our observation of this in an isolated Cell 9 as well as observations by others (see below). The major function of electrotonic and excitatory synaptic interaction within the ganglion would then be seen as serving to ensure synchronization of the spontaneous trains of impulses of all the cells to form the burst (see also e.g., Hagiwara, Watanabe & Saito, 1959, for *Panulirus*).

Driver potentials and the test substances. An hypothesis to account for the excitatory responses to XPO and 5HT and their difference in pattern invokes an effect of the test substances on an endogenous 'driver potential' having the characteristics described by Watanabe, Obara & Akiyama (1967) in cardiac ganglion pacemaker cells of a stomatopod. There is reciprocity between recurrence rate of driver potentials and the duration of their plateau; a driver potential can be triggered by a depolarization. Mayeri (1973*b*) has demonstrated response patterns to stimulation of *Homarus* small cells consistent with the presence of such potentials in the small cells. Their existence in small cells is also suggested by observations of bursting in isolated small cells. Potentials suggestive of driver potentials have been described in cardiac ganglion large cells of other species (Watanabe, 1958; Tazaki, 1971, 1972, 1973). However, Connor's (1969, *Homarus*) and Tazaki's (1973, *Panulirus*) conclusion that large cells have endogenous burst formation capabilities must be considered as unproven. It has been shown (Mayeri, 1973*a*; also our unpublished observations) that isolated small cell axons are capable of producing bursts and activating large cells in *Homarus*. Neither Connor nor Tazaki apparently monitored for remaining small cell activity.

Our hypothesis is that XPO enhances driver potentials: it increases their recurrence rate by lowering threshold depolarization for their occurrence or increasing the rate of depolarization in the interburst interval. During the burst, it either augments the amplitude and duration of the depolarized plateau or decreases the threshold for spike initiation. Livengood & Kusano (1972) have provided observations of a role of decreasing potassium conductance following the burst, set against hyperpolarizing current from electrogenic sodium pumping, in determining the rate of depolarization and hence burst rate. However, it seems premature to speculate on the mechanisms by which XPO might produce its effects. Enhancement by XPO of the driver potential would account both for the change of pattern of individual cells and the effects of the localized applications on overall ganglion pattern. Since burst rate is presumably determined by the cell which has the fastest recurrence of the driver potential, XPO produces a rate increase only when it acts on the pacemaker cell, or one which can

become the pacemaker. Since driver potentials of large cells are normally driven by the depolarizing synaptic activity of small cells at rates far higher than their spontaneous rate, there is no effect on burst rate from XPO applications to large cells only. Since the last third of total burst length normally represents small cell impulse activity, significant burst length increase is observed only following applications including small cells.

If driver potentials arise in membrane not capable of impulse activity, such as that of the soma, proximal axon and its collaterals, they can serve as a source of current for impulse generation at the trigger zone. The increased intraburst frequency (pattern contraction) and increased number of impulses per burst, characteristic of response to XPO, would be accounted for in our hypothesis by an increase in the depolarized level and duration of the plateau phase of the driver potential or by decreased threshold for initiation of impulses at the trigger zone. Intracellular recordings from large cells during perfusion with XPO do not show a clear change in the depolarized level during bursts, but synaptic and impulse activity obscures any plateau, and makes a conclusion impossible (Cooke, 1966). XPO does not act strongly at distal trigger zones and seems to be effective well proximal to the proximal trigger zone, observations which make an effect on impulse threshold seem the less likely suggestion.

Our hypothesis would provide an explanation also for the 'organizing' effect of XPO: its ability to restore co-ordination in an aged preparation would be attributed to simultaneous enhancement of the driver potential in each of the cells, following which the positive feedback mechanisms normally operative (excitatory synaptic potentials, electrotonic inter-connections) would sustain the effect. Similarly the induction of trains from steady firing in isolated units would be attributed to induction of a driver potential by XPO.

This hypothesis is consistent with the great majority of the observations, including those with intracellular electrodes (Cooke, 1966). It does not require that there be a change of membrane potential during interburst periods nor changes of apparent cell resistance in response to XPO.

Our hypothesis for the action of 5HT on the cardiac ganglion is that it produces a depolarization localized in the non-impulse sustaining membranes of the proximal axon or its collaterals. Such a depolarization would act in two ways: it would sum with driver and synaptic potentials at the trigger zone to increase the number of impulses fired; it would sum with other depolarizing influences to bring on driver potentials in pacemaker cells earlier than normal. The most striking difference between the effects of XPO and 5HT is that unlike XPO, 5HT creates an increase in burst rate even when applied only to large cells. In view of evidence that large cells have only weak impulse-mediated excitatory effects on other cells, excitation must be transmitted via electrotonic connexions. We postulate that depolarization of the affected cell spreads via the known electrotonic pathways to promote the earlier onset of the driver potential in the pacemaker cell. Rate increases are much more dramatic when small cells are affected; this is attributable to the much shorter electrotonic path length over which depolarization is attenuated in affecting the pacemaker. Re-analysis of earlier experiments (Cooke, 1966) in which intracellular potentials were recorded from large cells during perfusion with 5HT show a small depolarization (3 mV on average) in 8 of 10 experiments. That ganglionic burst rate can be influenced by relatively small brief currents

applied intracellularly to large cells has been demonstrated (*Panulirus*, Watanabe & Bullock, 1960; authors' unpublished observations on *Homarus*). Preliminary experiments show that 5HT application to a large cell can be imitated by passage of steady depolarizing current into a large cell soma. However, the current levels required produce substantial depolarization of the cell. This can be reconciled by postulating that 5HT depolarization is confined to collaterals.

This appears to be the nearest approach yet achieved to a complete analysis of an integrative nervous system at the level of all single unit activity, and the first detailed study of how a neurohormone acts in such a system. The methods of analysis used are prohibitively time consuming, and further exploitation of the techniques awaits development of more automated data reduction methods.

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References

Alexandrowicz, J. S. (1932). The innervation of the heart of the Crustacea. I. Decapoda. *Q. Jl microsc. Sci.* **75**, 181–249.

Alexandrowicz, J. S. (1953). Nervous organs in the pericardial cavity of the decapod Crustacea. *J. mar. biol. Ass. U. K.* **31**, 563–80.

Belamarich, F. A. & Terwilliger, R. (1966). Isolation and identification of cardioexcitor hormone from the pericardial organs of *Cancer borealis*. *Am. Zool.* **6**, 101–6.

Berlind, A. & Cooke, I. M. (1970). Release of a neurosecretory hormone as peptide by electrical stimulation of crab pericardial organs. *J. exp. Biol.* **53**, 679–86.

Bullock, T. H. & Terzuolo, C. A. (1957). Diverse forms of activity in the somata of spontaneous and integrating ganglion cells. *J. Physiol., Lond.* **138**, 341–64.

Cole, W. H. (1941). A perfusing solution for the lobster (*Homarus*) heart and the effects of its constituent ions on the heart. *J. gen. Physiol.* **25**, 1–6.

Connor, J. A. (1969). Burst activity and cellular interaction in the pacemaker ganglion of the lobster heart. *J. exp. Biol.* **50**, 275–95.

Cooke, I. M. (1962*a*). The neurohumoral regulation of the crustacean heart. Ph.D. Thesis, Department of Biology, Harvard University.

Cooke, I. M. (1962*b*). Effects of the pericardial organ neurosecretory substance on the crustacean heart. *Gen. comp. Endocr.* **2**, 29.

Cooke, I. M. (1964). Electrical activity and release of neurosecretory material in crab pericardial organs. *Comp. Biochem. Physiol.* **13**, 353–66.

Cooke, I. M. (1966). The sites of action of pericardial organ extract and 5-hydroxytryptamine in the decapod crustacean heart. *Am. Zool.* **6**, 107–21.

Cooke, I. M. & Goldstone, M. W. (1970). Fluorescence localization of monoamines in crab neurosecretory structures. *J. exp. Biol.* **53**, 651–68.

Cooke, I. M. & Hartline, D. K. (1973). Alteration of ganglionic integrative properties by a neurohormone. In *Neurobiology of Invertebrates* (ed. J. Salanki), pp. 431–37. Tihany: Hungarian Acad. Sci.

Friesen, W. O. (1973). Two components of facilitation in motor neurons of *Panulirus interruptus* cardiac ganglion. *Program Third Annual Meeting, Society for Neuroscience*, Nov. 1973, p. 291.

Friesen, W. O. (1974). Physiology of the spiny lobster cardiac ganglion. Ph.D. Thesis, Dept. of Neuroscience, University of California, San Diego.

Hagiwara, S. (1961). Nervous activities of the heart in Crustacea. *Ergebn. Biol.* **24**, 287–311.

Hagiwara, S. & Bullock, T. H. (1957). Intracellular potentials in pacemaker and integrative neurons of the lobster cardiac ganglion. *J. cell. comp. Physiol.* **50**, 25–47.

Hagiwara, S., Watanabe, A. & Saito, N. (1959). Potential changes in syncytial neurons of the lobster cardiac ganglion. *J. Neurophysiol.* **22**, 554–72.

Hartline, D. K. (1967*a*). Integrative physiology of the lobster cardiac ganglion. Ph.D. Thesis, Department of Biology, Harvard University.

HARTLINE, D. K. (1967*b*). Impulse identification and axon mapping of the nine neurons in the cardiac ganglion of the lobster *Homarus americanus*. *J. exp. Biol.* **47**, 327–40.

HARTLINE, D. K. & COOKE, I. M. (1969). Postsynaptic membrane response predicted from presynaptic input pattern in lobster cardiac ganglion. *Science, N.Y.* **164**, 1080–82.

LIVENGOOD, D. R. & KUSANO, K. (1972). Evidence for an electrogenic sodium pump in follower cells of the lobster cardiac ganglion. *J. Neurophysiol.* **35**, 170–86.

MAYERI, E. (1973*a*). Functional organization of the cardiac ganglion of the lobster, *Homarus americanus*. *J. gen. Physiol.* **62**, 448–72.

MAYERI, E. (1973*b*). A relaxation oscillator description of the burst generating mechanism in the cardiac ganglion of the lobster, *Homarus americanus*. *J. gen. Physiol.* **62**, 473–88.

MAYNARD, D. M. (1955). Activity in a crustacean ganglion. II. Pattern and interaction in burst formation. *Biol. Bull. Mar. biol. Lab, Woods Hole* **109**, 420–36.

MAYNARD, D. M. & WELSH, J. H. (1959). Neurohormones of the pericardial organs of brachyuran Crustacea. *J. Physiol. (Lond.)*. **149**, 215–27.

OHSAWA, K. (1972). Morphological organization and fine structures of the cardiac ganglion of the lobster *Panulirus japonicus*. *Sci. Rep. Tokyo Kyoiku Daigaku*, B **15**, 1–24.

TAUC, L. & GERSCHENFELD, H. M. (1962). A cholinergic mechanism of inhibitory synaptic transmission in a molluscan nervous system. *J. Neurophysiol.* **25**, 236–62.

TAZAKI, K. (1971). The effects of tetrodotoxin on the slow potential and spikes in the cardiac ganglion of a crab *Eriocheir japonicus*. *Jap. J. Physiol.* **21**, 529–36.

TAZAKI, K. (1972). Electrical interaction among large cells in the cardiac ganglion of the lobster, *Panulirus japonicus*. *J. exp. Zool.* **180**, 85–94.

TAZAKI, K. (1973). Impulse activity and pattern of large and small neurones in the cardiac ganglion of the lobster, *Panulirus japonicus*. *J. exp. Biol.* **58**, 473–86.

WATANABE, A. (1958). The interaction of electrical activity among neurons of lobster cardiac ganglion. *Jap. J. Physiol.* **8**, 305–18.

WATANABE, A. & BULLOCK, T. H. (1960). Modulation of activity of one neuron by subthreshold slow potentials in another in lobster cardiac ganglion. *J. gen. Physiol.* **43**, 1031–45.

WATANABE, A., OBARA, S. & AKIYAMA, T. (1967). Pacemaker potentials for the periodic burst discharge in the heart ganglion of a stomatopod, *Squilla oratoria*. *J. gen. Physiol.* **50**, 839–62.