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INTRACELLULAR STUDIES ON PACEMAKER AND FOLLOWER NEURONES IN THE CARDIAC GANGLION OF *LIMULUS*

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Intracellular studies on the cardiac ganglion cells of invertebrates have been confined mainly to the hearts of decapod and stomatopod crustaceans. The cardiac ganglia of these animals contain relatively few neurones, thought to be divisible into large follower cells and small pacemaker cells (for reviews see Hagiwara, 1961; Bullock & Horridge, 1965).

Studies on follower cells of crustacean cardiac ganglia are numerous but intracellular studies on pacemaker cells, with one exception (Bullock & Terzuolo, 1957) seem to be limited to the cardiac ganglion of the stomatopod, *Squilla* (Watanabe *et al.* 1967*a*; Watanabe, Obara & Akiyama, 1967*b*, 1968, 1969). In *Squilla* the most anterior cells exhibit pacemaker potentials and fire in bursts with each heart beat. However, all neurones in the ganglion are electrically coupled (Irisawa & Hama, 1965; Watanabe *et al.* 1967*a*), and pacemaker activity may also be initiated in the posterior part of the ganglion (Irisawa & Irisawa, 1957; Shibuya, 1961; Brown, 1964). The one study on pacemaker activity in a decapod heart was made on the two largest of the posterior cells of a crab heart (Bullock & Terzuolo, 1957). These workers found that the neurones fired a burst of overshooting spikes during each heart beat. No pacemaker potentials were present but Bullock & Terzuolo (1957) concluded that these two large cells were spontaneously active since there were no excitatory postsynaptic potentials (EPSP).

The *Limulus* cardiac ganglion is known to be composed of many hundreds of cells (Patten & Redenbaugh, 1899; Bursey & Pax, 1970). Electrical activity of ganglion cells was studied by Heinbecker (1933, 1936) and Prosser (1943). These workers recorded extracellularly from small pieces of the ganglion in an attempt to isolate single units. Prosser (1943) successfully recorded units firing once per burst and other units firing a train of spikes superimposed on a slow wave during each burst. Intracellular studies have been limited to one investigation of the activity of the largest cells in the ganglion, the large pigmented unipolars. These cells fire a train of attenuated spike potentials† superimposed on a slow depolarization, similar to that found in follower cells of crustacean cardiac ganglia (Palese, Becker & Pax, 1970).

In their exhaustive morphological study of the ganglion Bursey & Pax (1970) reported that there were five types of neurones present: large unipolars, bipolars and

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† *Attenuated spike potential*: the potential which is recorded across the non-spiking soma membrane of a neurone when the axon(s) of that cell are firing spikes.

Electrotonically recorded potential: a potential which originates outside of the cell and which is transmitted ephaptically.

multipolars and small bipolars and multipolars. Only the large unipolars have been studied electrophysiologically and these have been shown to be follower cells (Palese *et al.* 1970). A study of the remaining morphological types of neurones has therefore been undertaken in an attempt to assess their role in ganglionic activity.

METHODS

Specimens of *Limulus polyphemus* were kept at 10 °C in circulating sea water until used. Both males and females, averaging 6–8 in across the carapace, were used. Hearts were isolated as previously described (Abbott, Lang & Parnas, 1969) and immersed in a methylene blue solution (0·005–0·01 %) in *Limulus* saline (444 mM-NaCl, 37 mM-$CaCl_2$, 9 mM-KCl) for 10 min. A portion of the ganglion was isolated at the anterior end for suction-electrode recording. The entire heart was mounted on a glass rod which was large enough to stretch the heart sufficiently to eliminate mechanical activity in the circular direction. The heart was then stretched in a longitudinal direction and each end was tied tightly to the glass rod to eliminate longitudinal movement. This preparation was securely clamped in a Lucite chamber and immersed in *Limulus* saline. Impalement of somata was performed visually using a dissecting microscope at a magnification of 140×.

Intracellular recording from ganglion cells was accomplished using standard physiological techniques similar to those described for work on the heart muscle (Abbott *et al.* 1969; Parnas, Abbott & Lang, 1969). Electrode impedance was 20–40 MΩ.

Cells were stimulated intracellularly using a standard bridge circuit which permitted simultaneous stimulation and recording from the same electrode. A second microelectrode was inserted into other cells to see the effects of stimulation on these neurones. Current pulses ranged between 10^{-8} and 10^{-9} A except where otherwise described.

RESULTS

In contrast to the heart of decapod crustaceans and like that of the stomatopods, the *Limulus* cardiac ganglion lies on the outside of the dorsal surface of the heart. Bursey & Pax (1970) reported that most of the somata were present in a ventral cellular portion of the ganglion while the dorsal portion of the ganglion consisted mainly of fibres. However, hearts immersed in methylene blue solutions almost invariably have from 20 to 80 stained somata clearly visible on the dorsal surface. In addition it was often possible to distinguish unstained somata by the small spots of bright pigment present on them. The large unipolar (120 μm diam.) and large bipolar (90 × 120 μm) neurones were easily distinguishable by size and shape. The bipolars often stained well, their fusiform shape being very evident. The monopolars had round somata which stained poorly but which often protruded outside the edge of the ganglion. The slightly smaller multipolar cells (60–80 μm) were distinguishable mainly by size as their somata appeared to be embedded in the fibre tract and, in addition, their processes were not visible. Of the two smallest types of neurones, the bipolars (30–40 μm) were the most easily recognizable. Other small somata appeared round rather than fusiform, and were embedded in the fibre tract which obscured the processes. These may represent the fifth type of neurone, the small multipolars.

Methylene blue appeared to have an excitatory effect on the ganglion, increasing burst frequency and slightly decreasing burst length. However, use of methylene blue was necessitated by the fact that it was impossible to see small cells and difficult to distinguish between large cell types without its use.

Ganglionic activity

Mounting the heart on a glass rod invariably resulted in an increase in burst frequency as well as in occasional firing of units between bursts. However, this method of preparation was necessary to eliminate movement entirely, permitting impalement of the small cells. Complete isolation of the ganglion was also attempted but this usually resulted in an increase in burst frequency, shortened bursts, and extensive firing of units between bursts. In both procedures the burst frequency usually decreased after 30–60 min but the burst duration did not usually increase proportionally in the isolated ganglion as might have been expected from previous work on crustacean cardiac ganglia (Maynard, 1955; Brown, 1964).

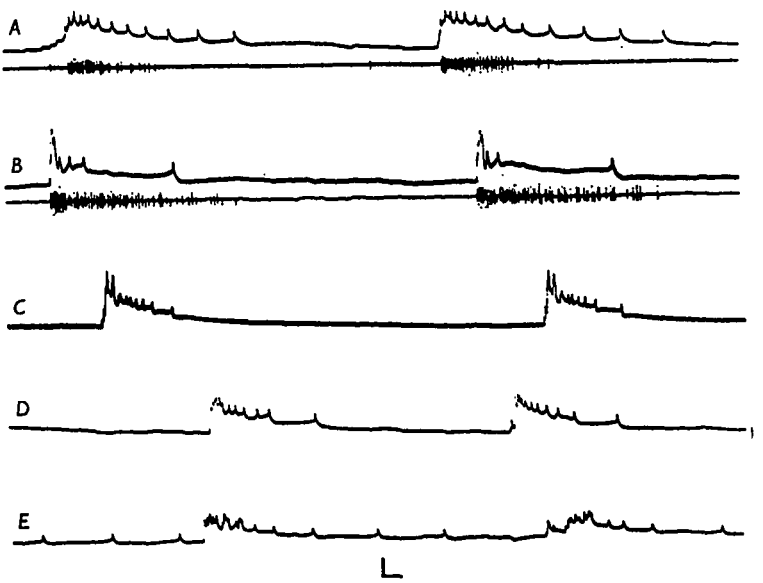

Fig. 1. Intracellular activity of large cells from the cardiac ganglion of five different *Limulus* hearts. *A* and *B* have simultaneous extracellular ganglionic activity (lower trace). Calibration: horizontal, 250 ms; vertical, *A*, *D*, 10 mV; *B*, *C*, *E*, 4 mV.

Intracellular activity – large cells

Regardless of morphological type, impalement of large cells always revealed a burst of electrical activity during each heart beat. Resting potentials were 30–50 mV. The height of the intracellular activity was partially dependent upon the resting potential, being higher for larger resting potentials, but the peak of activity was a maximum of 25–30 mV and was never overshooting (Fig. 1). The intracellular activity always occurred at about the same time as the extracellular ganglionic burst and was usually of the same duration. However, intracellular activity could be longer (Fig. 1 *A*) or shorter (Fig. 1 *B*) than the ganglionic burst recorded at the anterior end of the ganglion.

The intracellular potentials in the large cells usually showed an initial fast rise which appeared to be due to the summation of small potentials rather than to a single large potential (Fig. 1). In some cases there was no fast rise, and smaller potentials, much like excitatory postsynaptic potentials (EPSP), appeared to precede the first spike potentials (Fig. 1*A*) or were present between bursts (Figs. 1*A*, 2*A*, *B*). These EPSP differed from the attenuated spike potentials in both amplitude and decay time. They were smaller than the spikes and they showed a very slow decay, causing a sustained depolarization as they summated.

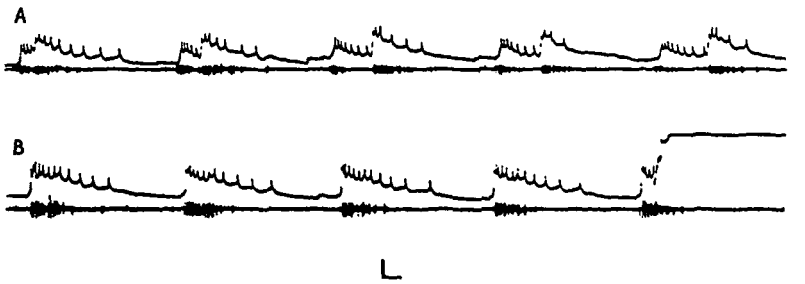

Fig. 2. Intracellular activity of large cell (upper trace) and extracellular ganglionic activity (lower trace). *A*, two independent trains of activity were present. After 10 min, normal activity began and lasted for over 30 min, at which time the electrode was withdrawn from the cell at the end of *B*. Calibration: horizontal, 500 ms; vertical, intracellular, 10 mV; extracellular, 20 μV.

The time course of repolarization of the large cells appeared to be partially dependent on the rate of firing of the spike potentials, whose after-hyperpolarizations brought the membrane potentials closer to resting level. This is particularly evident in Fig. 1*B* where there are spike potentials in the intracellular record for the first 200–400 ms. During the following second, there are no spike potentials and repolarization proceeds very slowly. Finally a single intracellular spike, near the end of the ganglionic burst, results in repolarization to nearly resting level. Slow repolarization without spike potentials is also evident in Fig. 1*C–D*.

During the course of one experiment the activity of a large cell and of the ganglion suggested that two pacemakers were simultaneously active in the ganglion (Fig. 2*A*). Two different trains of activity were present in the large cell and each train was associated with a burst of activity recorded from the whole ganglion. One of the trains had a larger sustained depolarization than the other and was associated with an extracellular ganglionic burst which contained more active units than the burst associated with the smaller train. The amplitude of the intracellular trains was not dependent on the number of attenuated spike potentials in it as the smaller train contained more attenuated spikes than the larger train. Likewise, frequency of spikes, at least for these two bursts, was not dependent on height of depolarization. The smaller train fired at a higher frequency than the larger train, suggesting that the sites of initiation may not be identical for both intracellular trains. After about 10 min of this uncoordinated activity the ganglion began to fire normally. Following 30 min of normal activity the electrode was removed from the cell to show a normal resting potential of 35 mV (Fig. 2*B*).

Intracellular activity – small cells

The two most easily distinguishable cell types in a methylene blue preparation were the large and small bipolar cells. The small bipolar somata stained darkly and were on the surface of the ganglion, above the fibre tract. In addition, some small somata were embedded within the fibre tract and it was difficult to distinguish whether they were the small bipolar or small multipolar cells. However, penetration of small cells, regardless of shape or location, always revealed pacemaker activity. Resting potentials were 40–60 mV. Between ganglionic bursts slowly depolarizing pacemaker potentials of 10–20 mV were observed. At the beginning of each ganglionic burst, monitored simultaneously with a suction electrode, the small cells fired overshooting spike potentials of 60–75 mV (Fig. 3). Spike rise times averaged 2–3 ms not including the

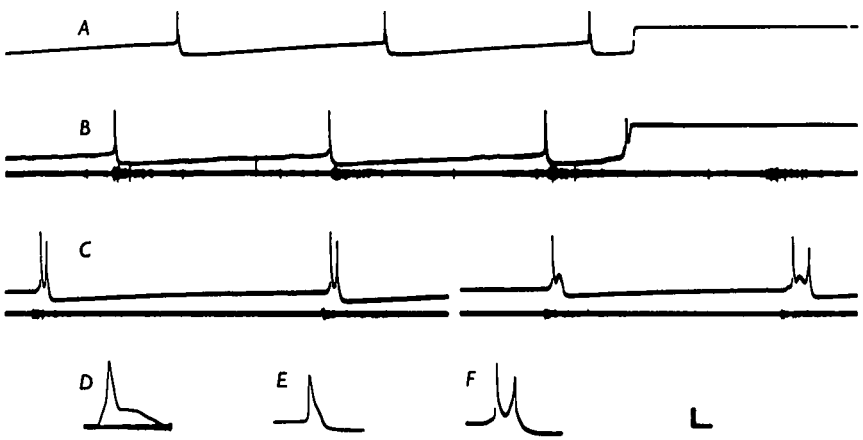

Fig. 3. Intracellular activity of small cells from three different hearts. *B*, *C* have simultaneous extracellular ganglionic activity (lower trace). Electrode was withdrawn at the end of *A* and *B*. *D–F* are expanded scales of *A–C*, respectively. Calibration: horizontal, *A–C*, 500 ms; *D*, 22 ms; *E*, 110 ms; *F*, 220 ms; vertical, *A*, 40 mV; *B–F*, 20 mV; extracellular, 20 μV.

prepotential. Total spike duration was long, due to the slow repolarization which occurred in two phases. An initial fast phase repolarized the cell 65–75% within 15–20 ms of spike initiation. A slower repolarization phase resulted in a plateau which lasted an additional 40–80 ms or longer (Fig. 3*D–E*). In a few preparations small cells were impaled that fired twice during each burst (Fig. 3*C*, *F*). The second spike arrived at variable times after the first spike but usually not until 100 ms after initiation of the first spike.

Intracellular stimulation of small cells

Intracellular stimulation of small cells was accomplished using a bridge circuit so that on-going activity could be continuously monitored. Depolarizing pulses introduced into a a pacemaker neurone resulted in an attenuated spike in the stimulated cell and a burst in the extracellular ganglionic recordings (Fig. 4*A–B*). In Fig. 4*A* the 100 ms pulse resulted in a burst before it would have occurred spontaneously. The following spontaneous burst was delayed, as shown in Fig. 4*B* which is a continuation of Fig. 4*A*. Following another spontaneous burst a second stimulus evoked an attenuated spike in the pacemaker cell and a large burst in the ganglion. The

following spontaneous burst was again delayed beyond the time when it would have normally occurred. Hyperpolarizing pulses delivered to pacemaker neurones were equally effective in producing a burst in the ganglion, usually at the end of the pulse or after a short latency. However, stimulation of the pacemaker cell never produced a large spike, as recorded from the soma, even when very short stimulating pulses were employed.

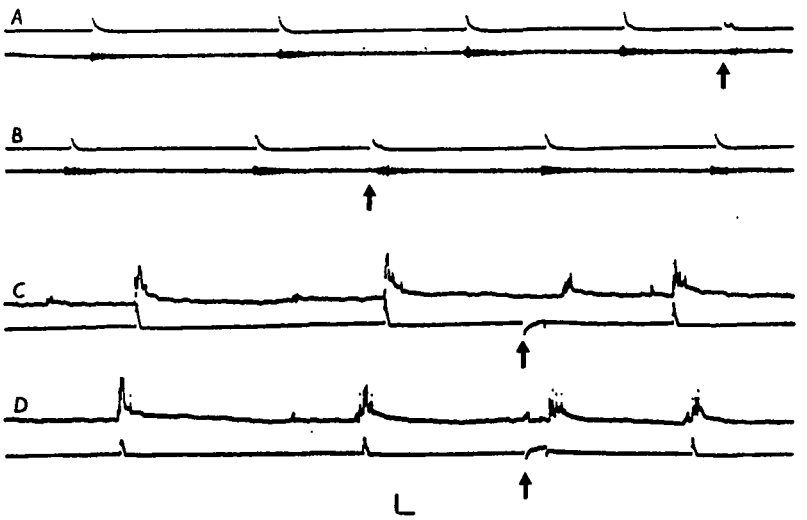

Fig. 4. Response of the ganglion and of a large cell to stimulation of pacemaker cells. *A–B*, stimulation of pacemaker cell (upper trace), at arrows, elicited burst in extracellular ganglionic record (lower trace). *B* is a continuation of *A*. *C*, *D*, stimulation of pacemaker cell (lower trace), at arrows, evokes bursts of spikes in large cell (upper trace). See text for details. Calibration: horizontal 500 ms; vertical, *A*, *B*, upper trace, 40 mV; lower trace 20 μV; *C*, *D*, upper trace, 10 mV; lower trace, 40 mV. Stimuli, *A*, *B*, 100 ms pulse; *C*, *D*, 500 ms pulse.

Stimulation of pacemaker cells and simultaneous monitoring of the intracellular activity of a large cell indicated that the large cells would produce a burst of spikes, or occasionally a single spike, in response to the stimulus. Fig. 4*C* shows the effect of a hyperpolarizing pulse which did not appear to produce a spike in the pacemaker cell (lower trace) but did produce a burst in the follower cell (upper trace). A second hyperpolarizing pulse evoked a larger burst in the follower cell (Fig. 4*D*, upper trace) again with no apparent effect on the pacemaker neurone. Depolarizing pulses were equally effective stimuli for large cells.

Small neurones often showed intracellular potentials that were greatly reduced, often less than 10 mV in height. In addition, intracellular stimulation of small cells often reduced the spike height in the stimulated cell whether it had previously given large spikes or small ones. However, neurones with spikes of reduced size, whatever the cause, were not appreciably effected in their ability to cause firing of a large cell or of the whole ganglion.

Electrical coupling between ganglion cells

Most attempts to find electrotonic connexions between two large neurones were unsuccessful. However, in one experiment, simultaneous recording from two large

cells, particularly when they fired repetitively between bursts, suggested that there may be coupling between cells. In Fig. 5*A*, attenuated spikes appeared simultaneously in the somata of two large cells which were separated by 1–2 mm. In a few cases where a spike in one cell was not accompanied by a spike in the second cell, a small potential was present instead. These small potentials did not possess the slow decay characteristic of the EPSP described above (Fig. 1*A*) but, rather, they decayed

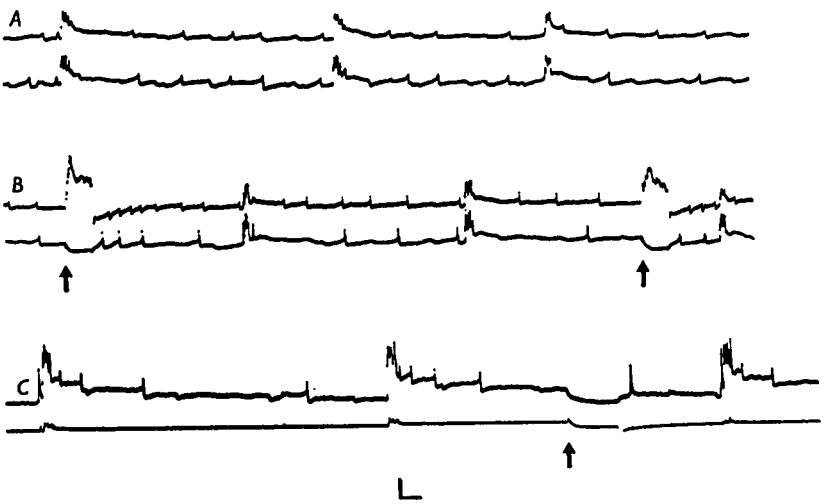

Fig. 5. Simultaneous intracellular record of two large cells. The ganglion was firing repetitively between bursts. *A*, activity in the cells suggested that they were coupled. *B*, strong hyperpolarizing pulses introduced into upper electrode (at arrows) caused small hyperpolarizing potentials in lower record. *C*, similar experiment in another heart; hyperpolarizing pulse introduced into electrode from lower trace. Calibration: horizontal, 500 ms; vertical, *A*, *B* upper trace, 10 mV; lower trace, 4 mV; *C* upper trace; 4 mV; lower trace, 20 mV.

rapidly. When strong hyperpolarizing pulses of long duration (500 ms) were introduced into a cell, hyperpolarizing responses were observed in a second large cell (Fig. 5*B–C*). At the end of the pulse the stimulated cell usually fired one or more spikes. These stimulating pulses were much larger than those necessary to stimulate either the large cell by itself or to stimulate a small cell to trigger bursts. Currents ranged from 5×10^{-8} to 10^{-7} A.

Stimulation of pacemaker cells with very strong hyperpolarizing pulses never resulted in hyperpolarizing pulses in large cell somata when recorded simultaneously. The same was true when two pacemaker cells were impaled, with one exception. In one experiment, a strong hyperpolarizing pulse in one pacemaker cell resulted in a small (less than 0·5 mV) hyperpolarization in another pacemaker cell.

DISCUSSION

In their detailed study of *Limulus* cardiac ganglion Bursey & Pax (1970) classified the nerve cells into five distinct classes, large unipolars, bipolars and multipolars and small bipolars and multipolars. In the methylene blue preparations of the present study the large unipolar and large bipolar cells were easily distinguished both by their size and because they stained well. The large multipolar cells were smaller than either

of the other two large types, and since their somas did not stain darkly enough to distinguish processes they were difficult to classify except by process of elimination they were too small to confuse with the unipolars and were quite unlike the bipolar cells. However, without histological verification, which has not yet been attempted, it was uncertain whether large multipolar cells were impaled. Likewise, the small bipolar cells were very distinct while the small multipolars could only be described as those small somata which did not appear to be bipolar.

An added complication in identifying cell types in the *Limulus* cardiac ganglion was suggested by Bursey & Pax (1970). In general, the larger the animal the larger the ganglion. This was both in terms of number of cells and size for a given cell type. Thus it appears that the ganglion is continuously adding new cells, and any given type of cell can be of any size, up to the maximum for the variety. In fact, bipolar cells of intermediate size (40–80 μm) were occasionally observed; these always showed follower cell activity.

Intracellular recording – large cells

Previous work on the large ganglion cells of neurogenic hearts has been confined mainly to the large cells of decapod crustaceans, particularly of the spiny lobster, *Panulirus*. Spike potentials in these preparations were attenuated, occasionally being as large as 30–35 mV (Watanabe, 1958; Connor, 1969). However, they were usually 15 mV or less (Hagiwara, 1961) suggesting, as Hagiwara & Bullock (1957) showed, that the soma were electrically inexcitable.

The source of the activity of the large neurones of cardiac ganglia has been the subject of some debate. Hagiwara & Bullock (1957) concluded that the potentials in *Panulirus* follower cells were a combination of EPSP and attenuated spike potentials. In addition, there was probably a third source, electrotonically conducted potentials, which were ephaptically transmitted from other cardiac ganglion neurones (Hagiwara, Watanabe & Saito, 1959). Connor (1969), on the other hand, concluded that soma potentials were solely the result of attenuated spike potentials in the large cells of *Homarus* heart ganglion. He reported that EPSP were never found (Connor, 1969).

The activity in large cell somata in *Limulus* appeared to arise from at least two different sources. Small, slowly decaying potentials (Fig. 1*A*, 2*A*, *B*) appeared to be EPSP. Larger attenuated spike potentials were also present (Figs. 1, 2). In addition, a third source was probably a small depolarization conducted electrotonically from other large cells. In this regard it should be noted that Palese *et al.* (1970) failed to find electrotonic coupling between pairs of large unipolar neurones in this preparation. Since definite morphological classification was not always possible in the present study, it is not known whether the electrotonic coupling is found only between cells of a specific morphological type or whether it is only occasionally present.

The large cell soma potential appeared to be the summation of activity from more than one site in the neurone. In one experiment both the ganglion and a monitored soma were firing with two bursts of different frequencies and different properties. The smaller train contained more attenuated spikes in the intracellular record in spite of the lower depolarization. The other train had a larger slow potential. This suggested that the activity was present in different axons or in branches of an axon and that each could fire independently of the other.

Intracellular recording – small cells

The small cells in the posterior portion of the decapod cardiac ganglion are believed to be the pacemaker neurones (Maynard, 1955; Hagiwara, 1961; Hartline, 1967). Intracellular studies have not been reported for the four posterior cells in the lobster heart. In the crab Bullock & Terzuolo (1957) found that the activity of the two large posterior cells was quite unlike that found in the anterior large cells of this cardiac ganglion or in the lobster cardiac ganglion. Spike potentials were very large, over 50 mV, and often overshooting zero potential. Normally spike potentials occurred in bursts. There appeared to be no synaptic potentials; pacemaker potentials were absent, or if present, very small. Bullock & Terzuolo (1957) concluded from the foregoing that these cells must be spontaneously active since they received no apparent input. In addition, they suggested that pacemaker potentials were present in distant portions of the axons and were not conducted into the somata.

Although there have been no intracellular studies on the four small posterior cells in lobster cardiac ganglion, the axon-mapping experiments of Hartline (1967) do quantify their activity. By extracellular recording he was able to identify the axons of all nine neurones in the cardiac ganglion, and in addition was able to show their pathways. He concluded that small cells did not send axons outside of the ganglion and that the burst was usually initiated in cell 6 or 7, the two most anterior of the four posterior small cells. In addition, it is clear from his records that all of the small cells fire repetitively during each burst.

The most complete study on cardiac ganglion pacemaker neurones has been made on the *Squilla* heart. This ganglion consists of 14–16 cells, the anterior three (GC 1–3) and the most posterior few being smaller than the others (Alexandrowicz, 1934). Location of the pacemaker neurones has been variously reported as being at the posterior end (Irisawa & Irisawa, 1957) and the anterior end (Watanabe *et al.* 1967*b*). Shibuya (1961) and Brown (1964) reported that ganglionic bursts could be initiated at either end of the ganglion. Electrical potentials of pacemaker cells (GC 4–6 were used) consisted of bursts of large spikes which nearly reached zero potential. Pacemaker potentials usually preceded each burst (Watanabe *et al.* 1967*a*, *b*). However, all neurones in the ganglion are coupled via low resistance pathways, resulting in a functional syncytium (Irisawa & Hama, 1965; Watanabe *et al.* 1967*a*). In particular, the pacemaker cells communicate via ephaptic connexions which permit passage of both slow potentials and spike potentials (Watanabe *et al.* 1967*a*).

For the *Limulus* cardiac ganglion Bursey & Pax (1970) reported the presence of both small bipolar and small multipolar neurones in the anterior three segments of the ganglion. Since Garrey (1930) and Heinbecker (1936) had failed to find initiation of spontaneous activity anterior to the third pair of ostia, Bursey & Pax suggested that neither type of small neurone was likely to be the pacemaker.

However, Bullock, Burr & Nims (1943) and Palese *et al.* (1970) were able to evoke bursting activity in the ganglion in response to extracellular stimulation of the anterior portion of the ganglionic cord. In the former case continuous stimulation with a bipolar electrode increased the burst frequency up to twice its control rate (Bullock *et al.* 1943). Palese *et al.* (1970) applied stimuli, using pulses from a monopolar suction electrode at either the anterior end of the ganglion or at a side branch

nerve while recording intracellularly from one or two large monopolar neurones. In general, they recorded two types of response in the large cells: (1) small bursts, with one or a few attenuated spikes, which had no effect on the normal ganglionic rhythm or (2) normal bursts which apparently re-set the ganglionic rhythm. When recording from a pair of large cells stimultaneously they found that one of the pair could have the first type of response while the other was unaffected. However, if one cell gave a normal burst in response to a stimulus, the second neurone also gave a normal burst, regardless of how stimulus parameters were varied (Palese *et al.* 1970). This suggests that, when the ganglionic rhythm was re-set, they were stimulating pacemaker cells, either directly or indirectly.

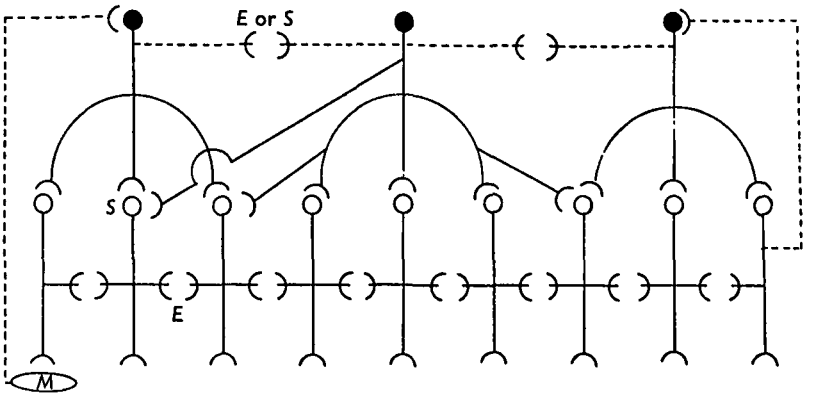

Fig. 6. Model of ganglionic organization. Darkened circles, pacemaker cells; open circles, follower cells; *E*, electrotonic connexions; *S*, synaptic connexions; dashed lines, possible, but uninvestigated influences. Synapses at the same horizontal level are presumed equivalent with the exception of the feed-back from muscle and follower cells to the pacemaker neurones. The former may be transmitted through dendritic arborization of the pacemaker neurones and thus would be neither synaptic nor ephaptic. Neuromuscular junctions have been shown to be synaptic (Parnas *et al.* 1969). Pacemaker neurones are coupled in some fashion, probably ephaptically, and their processes synapse on or near the somata of several follower cells. Follower cells are innervated by one or several pacemaker neurones and are also electrotonically coupled to other follower cells. Pacemaker cells may be influenced directly by follower cells or may be influenced directly or indirectly by muscular activity. Extrinsic cardioregulatory nerves and possible neurosecretory influences are not included.

In the present study it was shown that the small neurones fired an overshooting spike at the beginning of each ganglionic burst. Between bursts there was a slowly depolarizing pacemaker potential in the small neurones. Intracellular stimulation of small cells caused bursting in the ganglion and in single large neurones, re-setting the ganglionic rhythm. These characteristics suggest that the small neurones are the pacemaker cells of the ganglion. The possibility that another cell type is driving the small cells is not supported by any available evidence. In fact, no spikes are found in the extracellular ganglionic record between bursts and no EPSP were ever seen on the pacemaker potentials or preceding the spike in the small cells.

If the small cells are indeed the pacemakers, an as yet unresolved question concerns the coupling of these neurones in the ganglion. It is known that the whole ganglion does not fire synchronously. The burst begins in one of the middle segments and is conducted within less than 100 ms to both ends (Edwards, 1920). Carlson (1904)

noted that this was particularly evident in the contractions of preparations that had been isolated for a few hours, the conduction time increasing significantly. Thus it appears that not all pacemaker cells fire simultaneously, and for a given burst there may be one cell that is firing before all other cells. The fact that the bursts are so well co-ordinated does suggest that all pacemaker cells are coupled in some fashion. The present study does not show conclusively whether this coupling is through synaptic or ephaptic transmission, or both. The presence of electrotonic coupling has been suggested by one experiment but whether there is synaptic transmission, in addition, is unknown.

Models of ganglionic organization have been proposed previously for both *Limulus* cardiac ganglion (Krijgsman, 1952; Pax & Sanborn, 1967) and for crustacean (Maynard, 1955) cardiac ganglia. New modifications are suggested in Fig. 6. The pacemaker neurones (top row, dark circles) are coupled in some fashion and each innervates several follower cells (open circles). Follower cells are innervated by one or several pacemaker cells, and in addition are electrotonically coupled to other follower cells. Pacemaker neurones may be affected by follower cell activity as in lobster cardiac ganglion (Maynard, 1955). Contraction of the muscle may likewise feed-back to ganglion cells, perhaps via stretch receptors or perhaps by direct action on neurones themselves. Extrinsic cardioregulatory nerves and possible neurosecretory influences are not included in this model for sake of simplicity.

The physiological characteristics of *Limulus* cardiac ganglion cells belie the morphological diversity. Large cells never failed to exhibit follower cell activity and small cells never failed to exhibit pacemaker activity. Due to lack of eutely and to the large number of cells it is not possible to map the *Limulus* cardiac ganglion completely, as in crustacean ganglia, but additional work may uncover differences in function of the different large cell types or the different small cell types.

SUMMARY

1. The three types of large neurones in *Limulus* cardiac ganglion exhibit activity of the follower cell type. Large neurones are connected by ephaptic junctions.

2. Small bipolar cells and perhaps small multipolar cells behave like pacemaker neurones. They have slowly depolarizing pacemaker potentials between heart beats and single overshooting spike potentials which initiate each ganglionic burst.

3. Intracellular stimulation of pacemaker neurones elicits a burst of spikes in the ganglion, as recorded extracellularly, between spontaneous bursts. Stimulation of pacemaker cells causes a train of spikes in large ganglion cells, as recorded intracellularly.

4. Pacemaker neurones may be electrotonically coupled.

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