Functional Organization of the Cardiac Ganglion of the Lobster, *Homarus americanus*

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ABSTRACT External recording and stimulation techniques were used to determine which neurons and interactions are essential for production of the periodic burst discharge in the lobster cardiac ganglion. Burst activity can be modulated by brief single shocks applied to the four small cells, but not by similar stimulation of the five large cells, suggesting that normally one or more small cells primarily determine burst rate and duration. Repetitive electrical stimulation of large cells initiates spike activity in small cells, probably via excitatory synaptic and/or electrotonic connections which may normally act to prolong bursts and decrease burst rate. Transection of the ganglion can result in burst activity in small cells in the partial or complete absence of large cell spike activity, but large cells isolated from small cell excitatory synaptic input by transection or by application of dinitrophenol do not burst. Generally, transections which decrease excitatory feedback to small cells are accompanied by an increase in burst rate, but mean spike frequency over an entire burst cycle stabilizes at the original level within 10–30 min for various groups of cells whose spike-initiating sites are still intact. These and previous results suggest that the system is two layered: one or more small cells generate the burst pattern and impose it on the large cells which are the system's motorneurons.

INTRODUCTION

Since its introduction to neurophysiology by Welsh and Maynard (1951), the lobster cardiac ganglion has been useful for studying how a small neural population generates a motor output pattern. (Early work in both the Maine lobster, *Homarus*, and the spiny lobster, *Panulirus*, is reviewed in Maynard, 1960; Hagiwara, 1961. More recent works are Cooke, 1966; Hartline, 1967 *a*, *b*; Connor, 1969; Hartline and Cooke, 1969; Mayeri, 1969 *a*, *b*; Rao et al., 1969; Van der Kloot, 1970; Anderson and Cooke, 1971; Tazaki, 1971 *a*, *b*; Livengood and Kusano, 1972; Mayeri, 1973.) Though the ganglion contains only nine neurons, its integrative mechanisms are complex. Five large cells are

motorneurons which drive the heart into contraction with each burst. Four small cells excite the large cells; no motor function has been found for them. Typically, bursts occur every 2.5 s. Each burst is composed of 80–150 spikes and lasts 500 ms (Fig. 4 A). A burst is initiated by a spike in a single small cell. During the burst each small cell discharges at slowly increasing intervals while each large cell discharges at more rapidly increasing intervals (Maynard, 1955; Hartline, 1967 *a*; Hartline and Cooke, 1969; Mayeri, 1973). The output pattern of the ganglion has many similarities to the output patterns of motor systems controlling other rhythmic behaviors in arthropods, such as insect flight and lobster swimmeret and stomach movements (for reviews, see Wilson, 1970; Evoy and Cohen, 1972). The functional organization of the cardiac ganglion for burst production is therefore of general interest.

Two mechanisms for burst generation have been proposed. Maynard (1955) (see also Hagiwara and Bullock, 1957; Watanabe, 1958) suggested that each of the nine cells is a simple oscillator capable of spontaneously discharging at uniform low frequency if isolated from the others; the generation of the periodic high frequency bursts of spikes is due to regenerative excitatory feedback via excitatory connections among all nine cells (Fig. 1 A) (see Mayeri, 1973). This mechanism assumes that the excitatory synaptic interactions among the interneurons and motorneurons of the system are essential for burst formation. On the other hand, Otani and Bullock (1959 *a*, *b*) suggested that bursts are generated in one or more small cells which are endogenous burst oscillators, and that the excitatory connections from burster cells to other cells serve simply to impose the burst discharge on the other cells (Fig. 1 B). According to this suggestion the system is two layered; one or more interneurons generate the burst discharge and impose it on the motorneurons, but the connections between cells do not play an essential role in the generation of the burst discharge per se.

Elucidation of the burst-generating mechanism and the functional organization of the cardiac ganglion has been hindered because many interactions and autogenic mechanisms operate simultaneously among the ganglion cells, making it difficult to establish the contribution of any one of them (Lewis, 1970). An additional complicating factor is that the somata of the large cells, where intracellular recordings are made, are located at some distance from the sites of cellular integration (Bullock and Terzuolo, 1957; Hagiwara and Bullock, 1957). Since the somata are electrically inexcitable (Hagiwara et al., 1959), the potential changes recorded from them may be somewhat attenuated.

The most prominent interaction to be demonstrated thus far is that each large cell receives excitatory synaptic input from every small cell (Fig. 1) (in *Homarus*, Hartline, 1967 *a*; Hartline and Cooke, 1969; in *Panulirus*, O. Friesen, personal communication). The excitatory inputs to each large cell, together

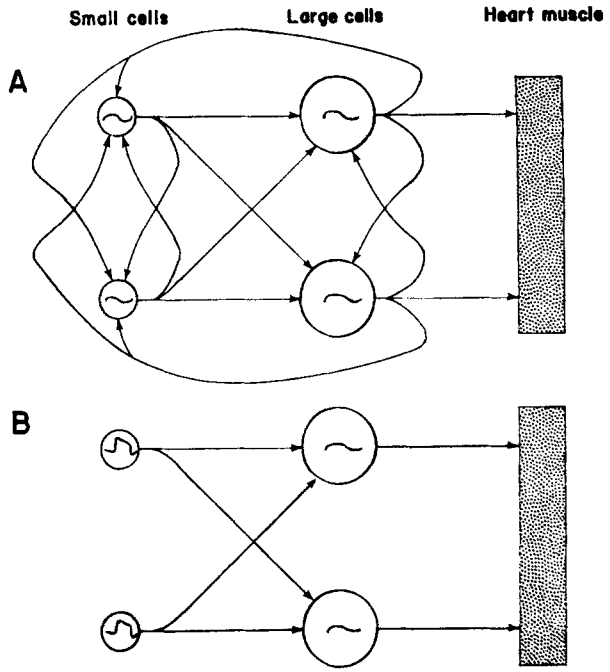

FIGURE 1. Two proposed burst-generating mechanisms for the lobster cardiac ganglion. Two representative small and large cells are shown for each case, and only the excitatory synaptic connections (arrows) which are essential for burst production are included. (A) All cells are simple oscillators and bursts are due to regenerative excitatory feedback among them. (B) One or more small cells are burst oscillators which impose the burst discharge on the remaining cells.

with attenuated spike potentials from the cell's axon, can account for almost all of the depolarizations during the burst period that are recorded intracellularly from large cell somata (Hartline and Cooke, 1969). These results alone suggest that the system might be two layered; burst generation occurs in the small cells. But since interactions also occur among large cells and from large to small cells, there is the possibility that these additional interactions also are essential for generating the burst discharge. Large cells are electrotonically coupled to one another (Watanabe, 1958; Hagiwara et al., 1959) such that a slow potential change induced in one cell body produces an attenuated potential change in others. There are also excitatory synaptic connections between some large cells (Otani and Bullock, 1959*b*; Hartline, 1967*a*). Finally, large cells, synaptically excite (Hartline, 1967 *a*) and/or are electrotonically coupled (Watanabe and Bullock, 1960; Tazaki, 1971 *b*) to the small cells. The investigators who have studied these interactions have suggested that they are relatively weak compared to the small-to-large cell interaction. One might therefore expect that they do not in themselves provide excitatory interactions which are strong enough to be an essential component of the burst-generating mech-

anism. However, more experiments are needed to convincingly demonstrate their functional roles.

The small cells have not been penetrated with microelectrodes (Hagiwara, 1961). However, the data from external recordings of small cell spike activity (Hagiwara and Bullock, 1957; Hartline, 1967 *a*; Mayeri, 1969 *b*, 1973) provide some support for the view that one or more of them are burst oscillators with many properties similar to pacemaker cells of the cardiac ganglion of the stomatopod, *Squilla* (Watanabe et al., 1967), or cardiac ganglion cells of the crab, *Eriocheir japonicus* (Tazaki, 1971 *a*), where intracellular recordings have been made. There is also evidence for relatively strong excitatory synaptic connections among some of the small cells (Maynard, 1955; Hartline, 1967 *a*).

The present study of the functional organization of the ganglion combines stimulation and isolation techniques with the methods developed by Hartline (1967 *a*) for identification of individual ganglion cells using extracellular recordings. Evidence is presented to suggest that the small cells (or a subgroup of them) generate the burst pattern and impose the pattern upon the large cells. Thus the anatomical separation of the nine cells into two populations is functionally preserved. The small cells serve to generate the pattern of burst activity that is characteristic of this ganglion, while the large cells serve to impose the pattern on the heart muscle (Fig. 1 B).

MATERIALS AND METHODS

600-g (1 1/4-lb) Maine lobsters (*Homarus americanus*), supplied by a local wholesaler, were kept in tanks of running sea water for 2 wk before they were used. Recordings were made from 67 lobsters.

The dorsal half of the cephalothorax was removed and pinned ventral side up in a small chamber continuously perfused with physiological saline (Cole, 1941). The ventral heart wall was cut lengthwise and pinned down, exposing the cardiac ganglion lying on the inner surface of the dorsal heart wall. Heart muscle partially covering the ganglion was removed, exposing the main trunk and portions of the principal nerves to the heart muscle. Small side branches emanating from the main trunk and containing dendritic processes which insert to heart muscle (Alexandrowicz, 1932) were cut except for a few preparations in which the effects of ganglionic transection were being studied. In these preparations muscle contraction was greatly diminished by the time the ganglionic transections were made and the results were the same as for preparations in which the small branches had been cut. Eight Ag-$AgCl_2$ suction electrodes were placed on the main trunk and principal nerves. Activity from any four electrodes could be amplified, displayed on a four-trace oscilloscope, and recorded by a four-channel Ampex SP-300 tape recorder (Ampex Corp., Redwood City, Calif.). Differential amplification was used, but one side of each amplifier input was grounded to the perfusion fluid via a resistor whose value was similar to that of suction electrodes. Any two of the eight electrodes could be selected for stimulation and stimulus isolation was used. Since burst rate and burst duration were affected by small changes in perfusion rate and temperature, the perfusion rate was kept within

20% of 15 ml/min, and the temperature was held constant within 0.2°C. To control perfusion fluid temperature the perfusion fluid was passed from a continuously aerated reservoir through a Graham condenser to the chamber, and cooled by a refrigerated bath connected to the jacket of the condenser. The experiments were performed at temperatures within 2°C of the temperature at which the animals had been stored, 11–16°C. Generally, burst activity was still stable at the end of the experiments, after 16 h in one instance. For a typical preparation, mean spike frequency (MSF) (spikes per burst times burst rate) decreased from its original value at a fairly constant rate of 3% per hour.

Spike Identification

Hartline (1967 *b*) has mapped the axons of the ganglion and identified their respective cell bodies. Spikes were identified in the present experiments in a similar manner and the results are in agreement with his description. A maximum of eight electrodes (but not less than four) were placed at appropriate locations on the main trunk and efferent nerves. Electrical signals were tape-recorded from combinations of four electrodes at a time until samples of spontaneous spike activity had been taken from each electrode. Once recordings had been made from the different combinations of electrodes, one combination was usually sufficient for identification during the remainder of the experiment. Spikes were identified from examination of four-channel tape recordings which were filmed at 0.5 or 1 m/s or displayed on a storage oscilloscope. A spike from a particular cell appeared with a fixed temporal relationship in the four simultaneous recordings. Spikes were identified by (*a*) their presence (or absence) at a particular electrode location, (*b*) their size and shape, and (*c*) direction of conduction (for details, see Hartline, 1967 *b*). Spikes from different small cells could be distinguished from one another (and from the large cells) by using the above criteria, but their respective cell bodies were usually not identified because that required a larger number of electrodes on the posterior main trunk of the ganglion than was used. In some cases, when the identification of a particular large cell was important, the large cells were stimulated antidromically and the elicited spikes were compared to the spontaneous ones.

Measurement of Burst Duration and Burst Rate

To reduce the effects of random fluctuations from one burst to the next, all burst duration, silent period, and MSF measurements mentioned in the results were averaged over at least 25 successive bursts. Two successive measurements of natural burst activity averaged in this manner were generally within 1% of each other. Burst duration, taken as the time from the first to the last small cell spike in the burst, was measured from the display of a storage oscilloscope.

Transection of the Ganglion

During transection experiments, the recording electrodes remained at the same locations before and after the transection. Occasionally additional electrodes were placed on one of the isolated parts to ensure precise spike identification. It was important to know whether MSF after transection of the ganglion stabilized at the same level as

before transection. MSF after transection was considered to be stabilized at the same level as measured a few minutes before transection provided the one and usually two measurements made afterwards (one at 30–60 min and the other at 90–120 min) were within 10 % of the original level. In cases where more than one transection was made on the same preparation, changes in MSF and burst rate were compared to the values of these parameters before the first transection. MSF for a group of cells was measured as the total number of spikes in 25 consecutive bursts divided by the interval from the onset of the first burst to the onset of the 26th. In order to count spikes, each spike in the burst triggered a pulse generator by means of a Schmitt trigger and the pulses were counted by a decade scaler. The accuracy of the method was checked routinely by simultaneously displaying a sample of spike activity and the output of the pulse generator on a storage oscilloscope and verifying that each spike produced a single pulse. 5–10 % of the spikes in the burst were not counted by the scaler because two spikes sometimes occurred at the same time. But this error was of the same sign and about the same proportion before and after transection of the ganglion, so that the error of the percentile differences in MSF was lower than 5 %. MSF was measured at the same recording site before and after transection. To measure small cell MSF in the posterior section of the ganglion, counts were made of spike activity of the small cells plus large cells 1, 2, and 3 *c*, as recorded on the main trunk, at a site slightly anterior to the point of origin of the two posterolateral nerves (Fig. 8). Counts made of the same spike activity in large cells 1, 2, and 3 *c*, as recorded on one of the posterolateral nerves, were subtracted from the other counts to obtain the MSF of the small cells alone. Counts for various groups of large cells could be made from sites on the anterolateral nerves or posterolateral nerves, where there are only large cell fibers (Hartline, 1967 *b*; Mayeri, personal observation).

RESULTS

Modulation of Burst Activity by Electrical Stimulation of the Small Cells

Fig. 2 shows the effect of delivering a brief single shock (3.5 V, 0.8 ms) to the ganglion at different times during the burst cycle (arrow, B, C, and D). The shock elicited a burst if presented during the latter portion of the silent period (Fig. 2 B), terminated a burst if presented in the latter two-thirds of the burst period (Fig. 2 C), and was followed by a "reset" burst if presented in the first third of the burst period (Fig. 2 D). The placement of the two stimulating electrodes was in the posterior portion of the main trunk (Fig. 2, below). In this region run the fibers of all the small cells, all the large cells except cell 4, and regulatory fibers from the ventral cord (Alexandrowicz, 1932; Hartline, 1967 *b*; Mayeri, personal observation). The modulations of burst activity can be attributed, however, to the direct effect of the stimulus on one or more of the small cells. The firing thresholds of the large cells and regulatory fibers are lower than those of the small cells. If the stimulus strength was adjusted so that spikes were elicited from all of the large cells and regulatory fibers but from none of the small cells, the shock had no effect on duration of bursts or silent period no matter what phase of the burst cycle the shock was presented.

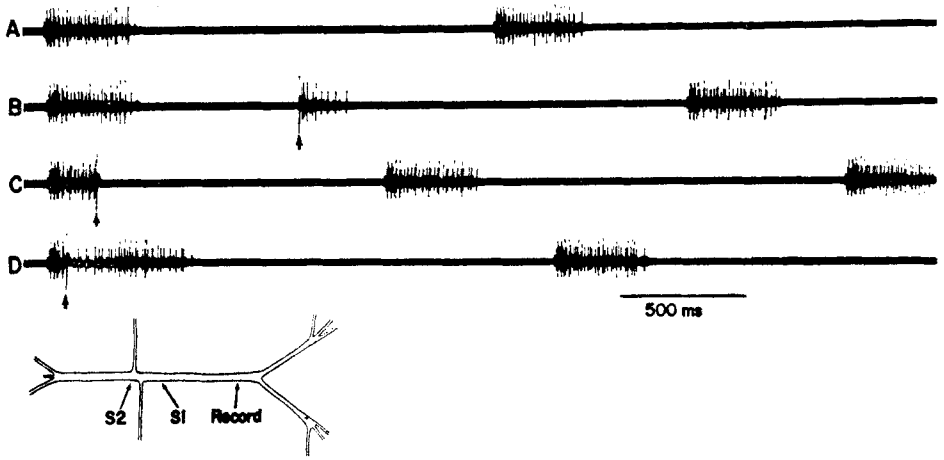

FIGURE 2. Modulation of burst activity by brief single shock stimulation (arrow, B, C, and D) of the small cells. The largest spikes in each trace are from large cells; the intermediate and small spikes are from small cells. (A) Natural bursts. (B) A burst (after arrow) elicited by a shock delivered during the silent period. (C) A burst (before arrow) terminated by a shock delivered late in the burst period. (D) A burst (after arrow) reset by a shock delivered early in the burst period. Below: Locations of stimulating electrodes (S1 negative, S2 positive) and recording electrode on the main trunk of the ganglion.

Furthermore, the shock did not produce repetitive activity in the activated cells. Only when the stimulus strength was strong enough to elicit a small cell spike during the silent period (as in Fig. 2 B) were strong effects on burst and silent period durations observed. In this case a small cell spike occurred within 10–30 ms, depending on time in the silent period, and was invariably followed immediately by a burst discharge. General features of the elicited bursts were similar to normal bursts, in that (*a*) large cell spike density was highest near the start of the burst and usually declined rapidly, whereas small cell spike density declined more slowly (*b*) the ratio of total number of spikes per burst to burst duration was similar (typically to within 15% of each other), and (*c*) variations in burst duration about the mean value were of similar magnitude.

The small cell bodies lie posterior to the stimulating electrodes and send their axons anteriad, each of them branching at the bifurcation of the main trunk and ending at the level of the cell bodies of the large cells 1 and 2 (Fig. 8). For Fig. 2 the stimulating electrodes were located in the region of the posterior main trunk that contains the small cell trigger zones (Hartline, 1967 *b*; Mayeri, personal observation). With single shocks delivered at various other locations, bursts could be elicited in any region of the ganglion containing small cell axons, large cell axons, and regulatory fibers (i.e., the main trunk), but not in the nerves containing only large cell axons or regulatory fibers (or three such nerves stimulated simultaneously). However, shocks were

effective in terminating or resetting bursts only with the stimulus electrodes located in the posterior portion of the main trunk, suggesting that this region is especially important for burst generation under normal conditions. Additionally, increases in stimulus strength above the threshold for exciting the first small cell *at a particular time* in the silent period did not affect the duration of the elicited burst. The stimulus therefore appeared to elicit a spike in a single small cell which in turn initiated the burst discharge in a manner similar to the normal burst.

The threshold for exciting the small cells and thereby modulating burst activity changed systematically through the burst cycle. Only one stimulus strength was used to produce the modulations of burst activity shown in Fig. 2. It was chosen so that bursts would be elicited early in the silent period and yet still be effective in terminating or resetting bursts when presented during the burst period.

In the succeeding paper (Mayeri, 1973) I illustrate that the firing threshold for small cells and the accompanying elicited burst discharge is highest for a brief shock delivered at the start of the silent period and decreases exponentially to a minimum threshold during the silent period. I also found that the small cell threshold changes during the burst cycle are qualitatively consistent with a model describing modulations of burst activity produced by small cell stimulation such as shown in Fig. 2. On the other hand, modulations of burst activity do not occur with single shock stimulation of the large cells, and large cell firing threshold variations during the burst cycle are small compared to small cell variations (Mayeri, 1973). By themselves, these results demonstrate that there are important functional and physiological differences between some or all of the small cells, on the one hand, and the large cells, on the other. They suggest that burst duration and burst rate are determined primarily by small cells. More evidence for these differences is presented below.

Excitatory Feedback from Large Cells to Small Cells

Watanabe and Bullock (1960) and Hartline (1967 *a*) have reported that large cells excite small cells. Since small cell spike activity at the start of each burst causes spiking in the large cells (Hagiwara and Bullock, 1957; Bullock and Terzuolo, 1957; Cooke, 1966), there is the possibility that excitatory feedback to the small cells during the burst regeneratively excites the small cells, and that the excitatory feedback is an essential component of the burst generating mechanism. I therefore investigated the large-to-small cell interaction in greater detail.

In Fig. 3 B–E large cells were antidromically stimulated with trains of four brief (0.25 ms, 2.0 V) shocks spaced 10 ms apart at various delays after the first small cell spike potential at the left of each trace. The stimulating electrodes were located on one of the posterolateral nerves which only contain

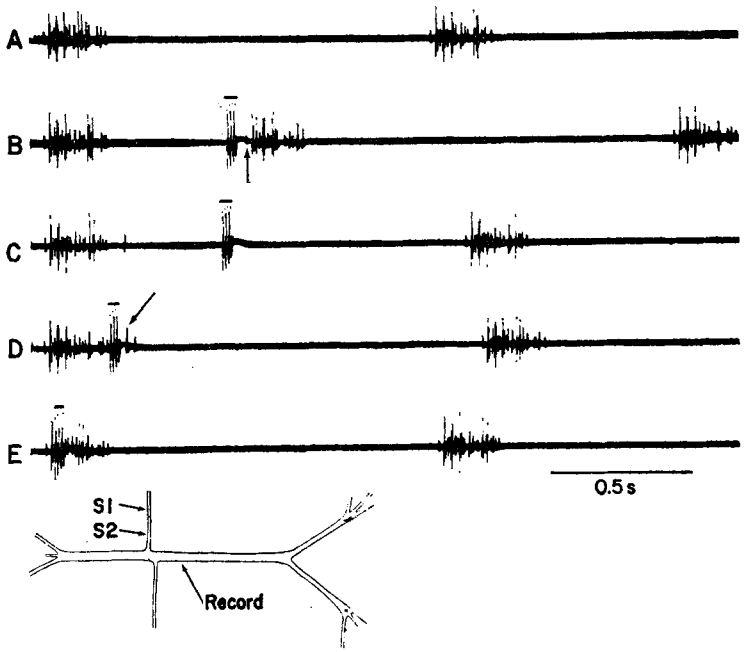

FIGURE 3. Repetitive antidromic stimulation of large cells. Generally, the larger spikes are from large cells and the smaller ones from small cells. The largest small cell spike is denoted by the arrow in D. (A) Two successive natural bursts. (B–E). Examples of repetitive antidromic stimulation (denoted by bars over the stimulus artifacts) applied at various phases of the burst cycle. Below: Location of the stimulating electrodes (S1 and S2 with S1 positive) and the recording electrode.

fibers from large cells 1, 2, and 3 (Hartline, 1967 *b*; Mayeri, personal observation). (Stimulation of the anterolateral nerves, containing four large cell fibers produced similar results.) All three large cells were excited by the first three shocks in each train and cell 3 *c* by the last shock. A burst was initiated when the pulse train was delivered at a delay of 640 ms (Fig. 3 B) or more. But at a slightly shorter delay, 620 ms (Fig. 3 C), and for other trains delivered earlier in the silent period, a burst was not initiated unless there were more pulses in the train. Similarly, for delays greater than shown in Fig. 3 B, three-pulse trains were sufficient to initiate a burst. If the stimulus intensity was raised so that large cell spike excitation was prevented by anodal block (not shown), the shocks had no effect on on-going burst activity, thus ruling out the possibility that the small cells were being directly excited by the stimulating electrodes.

The appearance of a burst after the train was presented was contingent on the appearance of a small cell spike (arrow, Fig. 3 B), suggesting that the effect was due to large cell spikes exciting at least one small cell which in turn initiated the burst discharge. This view is also supported by the finding that each shock in the train elicited only one spike in those large cells whose axons

are located in the stimulated nerve; the activated large cells did not discharge repetitively in the absence of small cell spikes even when other patterns of stimulus trains were presented. Also, many features of the bursts elicited during the silent period were similar to bursts elicited by single shock stimulation of small cells. In both cases, a greater amount of excitatory drive to the small cells (i.e., number of large cell spikes in the former case and stimulus strength in the latter), is needed to bring one of the small cells to spike threshold as the stimulus is presented earlier in the silent period. And in both cases the duration of the elicited burst is a function of the time in the silent period at which the burst is initiated, rather than the magnitude of the excitatory drive above the threshold value. These findings confirm the existence of feedback from large to small cells mediated by synaptic excitation, electrotonic interaction, or a combination of the two mechanisms.

In Fig. 3 D, a train of four shocks delivered to the large cells at the end of a spontaneously occurring burst produced one or a few small cell spikes but never a discharge approaching the duration of normal bursts. In Fig. 3 B–D, the train affected the next silent period duration by prolonging it 100 ms or so (about 10% above normal silent period duration), but a train presented 10 ms after the start of a burst had a comparatively small effect on the burst and silent period durations (Fig. 3). Each large cell showed approximately the same ability to activate the small cells; a train of five shocks which excited a single large cell was as effective as two shocks which excited three large cells.

Burst Activity in Isolated Populations of Small Cells

The results of the antidromic stimulation of the large cells raised the possibility that normal burst activity is due to regenerative excitatory feedback from large to small cells. The following results show that whatever function such feedback serves, it is not essential for generation of the burst discharge in small cells.

Fig. 4 shows one experiment where transection of the ganglion 0.5 mm posterior to the bifurcation (dashed line across the main trunk of the ganglion) resulted in regularly occurring burst discharges in the small cells in the posterior half of the ganglion in the complete absence of large cell spike activity. A and B each show a burst recorded simultaneously from three electrodes. A was recorded before transection, B, 11 min after transection. In A all large cell spikes were identified from records filmed at 1 m/s with the help of three additional electrodes in the anterior portion of the ganglion (not shown). Activity in large cells 1 and 2 are distinguished as the only spike types present in the top trace. They are present in the lower two traces as well. The spikes of large cells 3, 4, and 5 are recorded in the bottom trace only. The small cell spikes are distinguished from the large because each of the small cells is recorded in the lower two traces in both A and B and their direction of propaga-

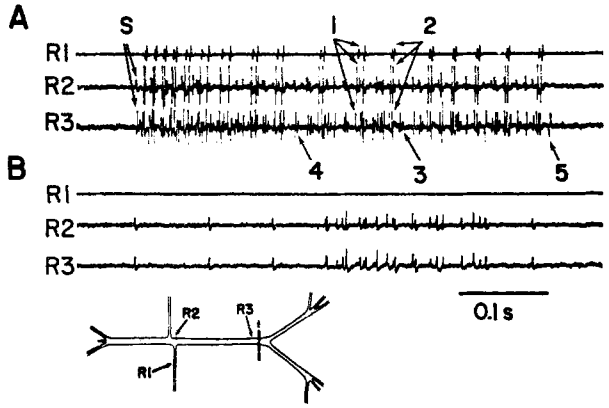

FIGURE 4. Small cell burst discharge in the absence of large cell spike activity after transection of the ganglion. Simultaneous recordings from three locations are shown. (A) A burst recorded before transection with representative large (1–5) and small (S) cell spikes denoted. (B) A burst recorded 11 min after transection with no large cell spikes present. Below: Location of recording electrodes (R1, R2, and R3).

tion is anteriad. The small cells are therefore the only cells spiking after transection (Fig. 4 B).

As with other transections described below, the pattern of burst activity after the transection remained quite stable, comparing favorably to stability in preparations where no transections were made. 11 min after transection the MSF of the small cells (spikes per burst times burst rate) was decreased 13% compared to before transection, even though burst rate had increased 91% from 1/2.83 to 1/1.48 s. 34 min after transection MSF was only 8% less than before transection.

These results show that burst production can occur in small cells isolated from the spike activity of large cells. Since large-to-small cell feedback is not necessary for burst production in small cells, it is unlikely that the feedback is an essential component of the burst-generating mechanism under normal conditions.

Behavior of Isolated Populations of Large Cells

Other transections were made to see whether large cells are capable of burst activity when isolated from small cell synaptic input. Complete isolation of the large cells was only successful with a transection located immediately posterior to the bifurcation, as shown in Fig. 5. There was no burst activity in the anterior section of the ganglion just after transection (Fig. 5 A, taken 2 min after the transection). Instead, the large cells fired spontaneously at regular intervals without any apparent relation to one another. The somata of the four small cells are located posteriorly on the main trunk of the ganglion and their axons travel anteriad, branching at the bifurcation and ending at the level of

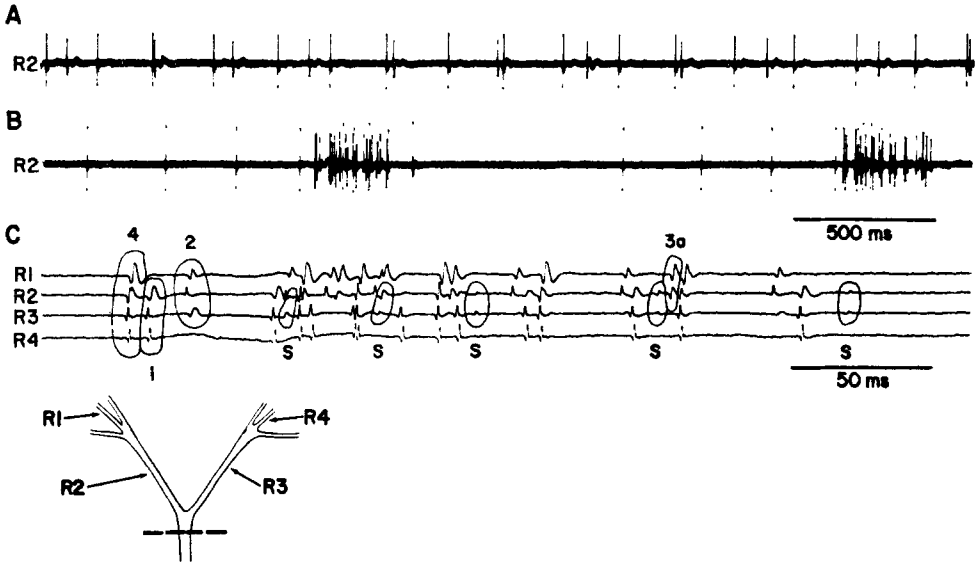

FIGURE 5. Spike activity in the anterior section of the ganglion after transection. (A) 2 min after transection two large cells discharge at uniform low frequency in the absence of small cell spike activity. (B) 20 min after transection the large cells are bursting again. (Bursting resumed 5 min after transection.) (C) Four simultaneous records of a burst occurring within a few seconds of those in B, showing the presence of small cell spikes (S). Expanded time scale is used. Below: Location of recording electrodes (R1, R2, R3, R4).

the large cell soma in each anterior branch of the main trunk (Fig. 8; Hartline, 1967 *b*; Mayeri, personal observation). Therefore, there were axons from all four small cells in the anterior portion of the transected ganglion. Spike activity was recorded from them in the anterior portion before the transection was made, but after the transection, whenever the large cells were observed firing at regular intervals (Fig. 5), no small cell spike activity was observed.

Data from four transected preparations were analyzed. In three of them a form of bursting did reappear in the anterior section 5–30 min after transection (Fig. 5 B and C). The reappearance of burst activity was always accompanied by the reappearance of spikes in one or more small cell axons during the bursts (S, Fig. 5 C). This form of bursting was common in ganglia that were transected or injured in other ways. It was characterized by the large proportion of large cell spikes which preceded the first small cell spike in the burst and by the fact that the interval between the first and last small cell spike in the burst was shorter (usually less than half) than the same interval in bursts recorded before the transection or injury. In a similarly transected preparation which displayed the same type of burst activity, a burst was elicited during the silent period by a brief single shock (0.1 ms, 3.6 V) which produced a spike in a small cell axon (S, Fig. 6 A), but no burst was elicited when a slightly weaker

shock (0.1 ms. 3.5 V) which was just subthreshold for the small cell, was presented at the same phase of the silent period (Fig. 6 B). From these results I suggest that the elevated large cell spike frequency during this type of burst activity is due almost entirely to small cell synaptic input.

The large cells that are active before the first small cell spike in the bursts shown in Fig. 5 B and C include the two large cells that were spontaneously active in Fig. 5 A, before bursting resumed. Hagiwara and Bullock (1957)

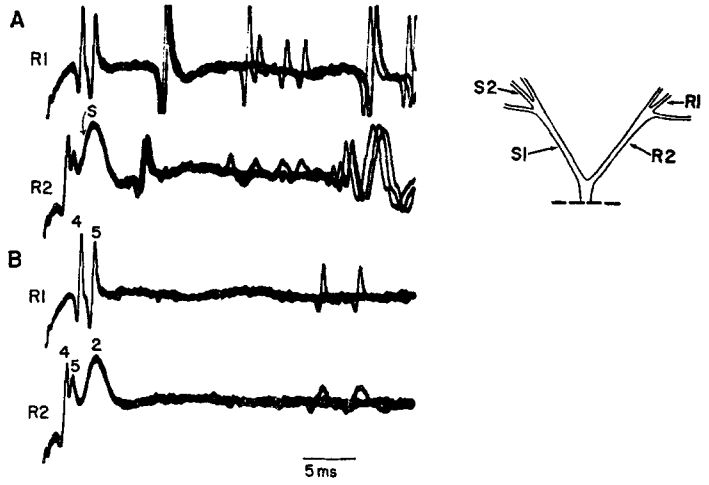

FIGURE 6. Initiation of a burst is contingent upon antidromic activation of a spike in a small cell axon. The ganglion was transected as in Fig. 5. (A) Three superimposed responses to a brief shock which excites large cells 4, 5, and 2, and a small cell axon (S) and is followed by a burst. The S and cell 2 spike potentials overlap, but S occurs slightly earlier. (B) Three superimposed responses to a slightly weaker shock which excites only large cells 4, 5, and 2, and is not followed by a burst. All shocks were presented at the same phase in the silent period. At right: Location of stimulating electrodes S1 and S2 and of recording electrodes R1 and R2.

recorded intracellularly from large cells under conditions where they displayed a similar pattern of discharge. They concluded that the series of large cell spikes just preceding each burst is not part of the burst-generating mechanism but is instead due to their ability to discharge spontaneously at regular intervals. They based this conclusion on the findings that in a single large cell each of the spikes preceding the burst was preceded by a gradual depolarization instead of a synaptic potential, that the rhythm of firing could be reset by antidromic activation of the cell, and as in the present study, that there was no other externally recorded spike potential that could be interpreted as being a presynaptic input to it. Furthermore, they suggested that the temporary depression of spontaneous discharge in these large cells after each burst occurred because the spike frequency of the large cells during the burst was elevated

above the level of spontaneous spiking frequency by the excitation that the large cells received from presynaptic inputs. Thus, temporary depression could also be induced when spike frequency was artificially elevated by direct depolarization of a large cell. In the tonic crayfish stretch receptor neuron (Sokolove and Cooke, 1971; Nakajima and Takahashi, 1966) temporary suppression also occurs when spike frequency is artificially elevated. Sokolove and Cooke (1971) suggested that it is due to an electrogenic sodium pump which causes a hyperpolarization of the cell after the end of the imposed high frequency spiking. However, Livengood and Kusano (1972) have found that in lobster cardiac ganglion large cells, the electrogenic pump does not contribute to the the hyperpolarization which follows each burst. Therefore, other hypothetical mechanisms, such as accumulated refractoriness (Wilson, 1966), or a prolonged hyperpolarizing after-potential resulting from an increased potassium conductance (Noble and Tsien, 1968) are necessary to account for temporary suppression.

From these considerations, it is likely that the large cell burst pattern of Fig. 5 C was caused by small cell synaptic input rather than by a capability for bursting inherent to the large cells themselves. Small cell synaptic input to the large cells during the burst causes a high frequency discharge in the large cells and results in the temporary suppression of some spontaneous large cell activity during the earlier portions of the next silent period. But the small cell synaptic drive is not sufficient to suppress all large cell spiking for the entire silent period, and large cells are again spontaneously active before the first small cell spike of the burst.

Another procedure which isolated the large cells from small spike activity produced results that are also consistent with this interpretation. When the metabolic inhibitor 2,4-dinitrophenol was added to the perfusion fluid (10^{-4} M), all spike activity in the ganglion ceased within an hour. It diminished small cell activity much faster than large cell activity, so that at the time when all of the small cells became silent, all of the large cells were still spiking. The two traces in Fig. 7 A are parts of a continuous record taken during one of the last instances when small cells were spiking. The burst in the upper trace was not followed by another one for 2 min. It was followed by a short silent period and then by large cell spikes occurring at uniform low frequency. The same burst and a sample of the uniform low frequency spike activity of the large cells are shown in expanded time scale in Fig. 7 B and C, respectively. Small cells (S, Fig. 7 B) were active only during the burst, as was true for other similar records from this preparation.

The results of metabolic poisoning are in agreement with the transection experiments; they suggest that interactions among the large cells are too weak to produce bursts and that burst production in the large cells during normal burst activity requires synaptic activation of them by small cells.

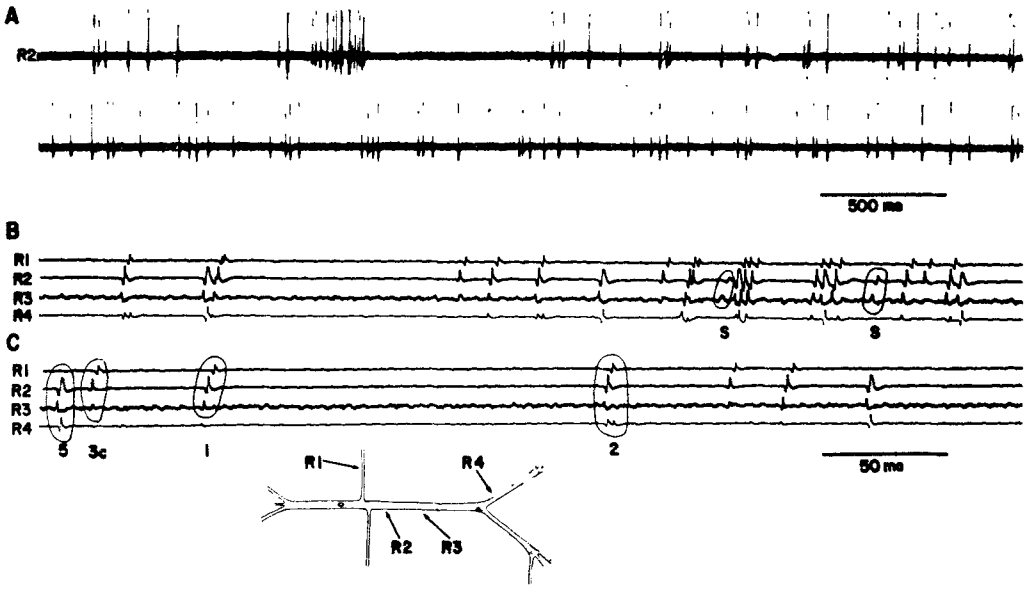

FIGURE 7. Cessation of small cell spike activity after the addition of 10^{-4} M dinitrophenol to the perfusion fluid. (A) One of the last bursts to occur (upper trace), followed by a silent period and then large cell spikes at uniform frequency. (B) Four simultaneous records of the burst in A., showing the presence of two small cell spikes (S) in the burst. Expanded time scale is used. (C) A sample of four simultaneous records illustrating that when large cells were not bursting, no small cell spikes were observed.

Increase in Burst Rate and Recovery of MSF after Transection of the Ganglion

Despite the physical injury associated with transection at various places on the ganglion, the spike activity of those cells which remained active after transection often stabilized to a new burst pattern within 10–30 min. Thereafter, the burst rate, burst duration, and number of spikes per burst remained stable for hours. Depending on the location of the transection, the MSF of various groups of cells after stabilization of the burst pattern was the same as for a few minutes before the transection, but burst rate was increased.

MSF for the groups of ganglion cells indicated in Table I was measured from the same locations a few minutes before transection and 30–60 min afterwards. MSF was often measured again after another 30 min or more to confirm that the level was still within 10% of the value measured immediately before the transection. In 19 of 27 transections, the MSF of at least one of the groups returned to the original level. Of the 19, burst rate increased an average of 34.5%, including four cases in which burst rate remained essentially the same. In all 27 transections, MSF stabilized at a level higher than 10% only once, and burst rate decreased once.

In general, a group of cells that was isolated by transection regained original

TABLE I

INCREASE IN BURST RATE AND STABILIZATION OF MSF AT THE ORIGINAL LEVEL AFTER TRANSECTION OF THE GANGLION

	Small cells				Large cells		
Location of transection, section of ganglion studied	Neurons counted	Number of transections	MSF* remains constant (number)	Burst rate‡ increase (%)	Large neurons counted	Number of transections	MSF* remains constant (number)
A or B, posterior	Small or small + large cell 5	3	2	7, 12	1, 2, 3*c*	5	3
C or D, posterior	Small or small + large cell 5	7	4	3, 15 22, 89	1, 2, 3*c*	6	1
E, posterior	Small or small + large cell 5	5	4	2, 39 75, 102	1, 2, 3*c*	5	0
E, anterior	Small + large cells 2, 3, 4, 5	3	1	211	2, 3, 4, 5	4	2
F, anterior	Small or small + large cells 2, 3, 4, 5	4	2	13, 20	1, 3, 4, 5 or 1, 2, 3	3	2
G, anterior	Small or small + large cells 2, 3, 4, 5	2	2	17, 28	1, 3, 4, 5 or 1, 2, 3	2	2

* Number of transections in which MSF stabilized after transection to within 10% of the MSF level before transection.

‡ For instances when MSF stabilized at the original level.

MSF provided none of the cells of the group were isolated from their normal spike-initiating sites. The normal spike-initiating sites of the cells were determined by external recordings; they agree well with previous studies (Hartline, 1967 *a*, *b*). For a given cell, it is usually 1 or 2 mm distal to the cell body (Fig. 8 B), but the exact locations of cell bodies and spike-initiating sites can vary from one ganglion to another, and the locations shown are most typical. A transection in region A or B (Fig. 8 A) isolated the axon and spike-initiating site of large cells 1 and 2 respectively, from its cell body, but the trigger zones of all ganglion cells, including the one in question, were in the isolated posterior section of the ganglion and the MSF of all cells usually returned to the original level (Table I, transections A and B, posterior). However, more posterior transections in regions C and D isolated the axon from both the cell body and spike-initiating site of cells 1 and 2, respectively, and at E the cell body and sometimes the spike-initiating site of the cell 3 *c* axon was isolated in addition to cells 1 and 2 (see Fig. 8). Even after the burst pattern had stabilized after these transections, the MSF of large cells 1, 2, and 3 *c*, recorded from the posterolateral nerve, was usually reduced (Table I, transections C, D, and E, posterior). However, the small cells and large cell 5 were left intact, having been cut distal to their trigger zones, and their MSF, recorded on the posterior main trunk, usually returned to the original level (Table I, same transections).

Figure 9 B shows a single burst (left of arrow) recorded 35 min after the

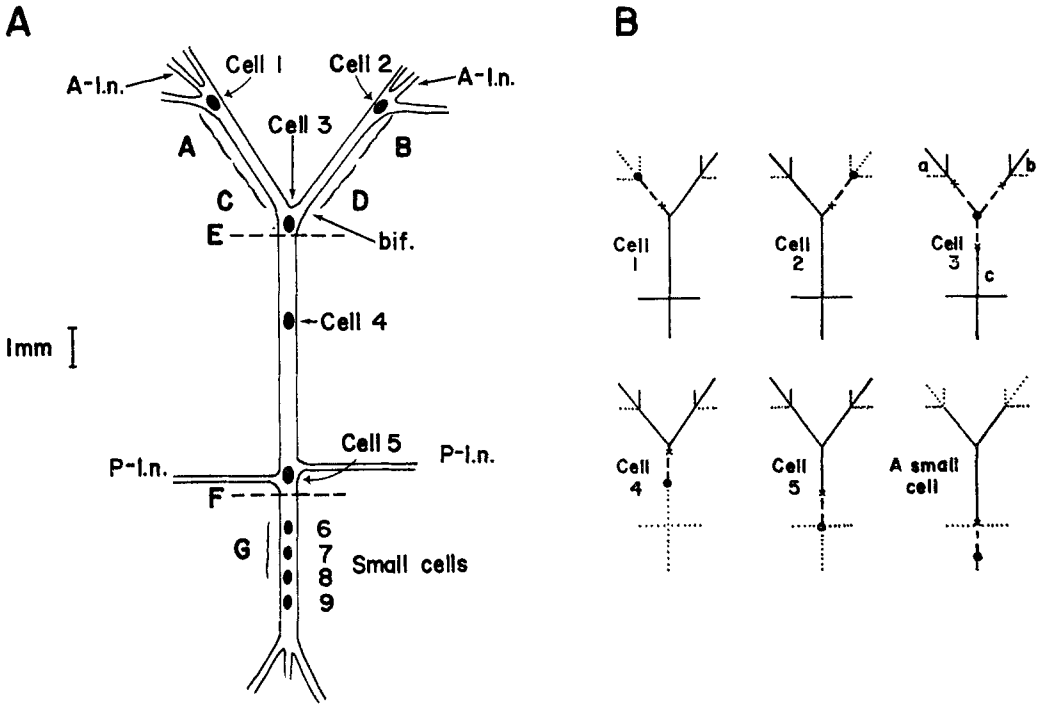

FIGURE 8. (A) Location of transections of the lobster cardiac ganglion listed in Table I. Large cell somata are numbered 1–5; small cell somata are numbered 6–9. A-l.n., Antero-lateral nerve; bif., bifurcation; P-l.n., Postero-lateral nerve. (B) Maps of axons of the neurons in the main trunk and principal nerves of the ganglion. Spikes arising at each spike initiation site (×) propagate decrementally towards the cell body (●) along the inactive segment (dashed line) and away from it nondecrementally along the axon solid line). Cell 3 has three independent spike initiation sites.

ganglion had been transected in regions C and D through the spike-initiating sites of cells 1 and 2, respectively. The MSF of cell 3 and the isolated axons of cells 1 and 2, recorded in the R2 trace was decreased to 10.6 spikes/s from 14.0 spikes/s before transection (Fig. 9 A). A third transection, just posterior to the bifurcation, was made at the arrow in Fig. 9 B. It isolated cells 1 and 3 still further from their spike-initiating sites and also isolated the cell 3 *c* axon recorded in R2 from its cell body It was followed by a prolonged discharge in the small cells and cell 4 (smaller-sized spikes in upper trace with no corresponding spikes in lower trace) lasting 700 ms, and an injury discharge in cells 1 and 2 lasting approximately 2 min. Cells 1 and 2 were permanently silent after the injury discharge and the remaining cells were silent for a number of minutes after the prolonged discharge. 80 min after the third transection (Fig. 9 C), burst activity had already resumed and was stabilized with only large cell 3 active at a reduced level in the lower trace, indicating that its

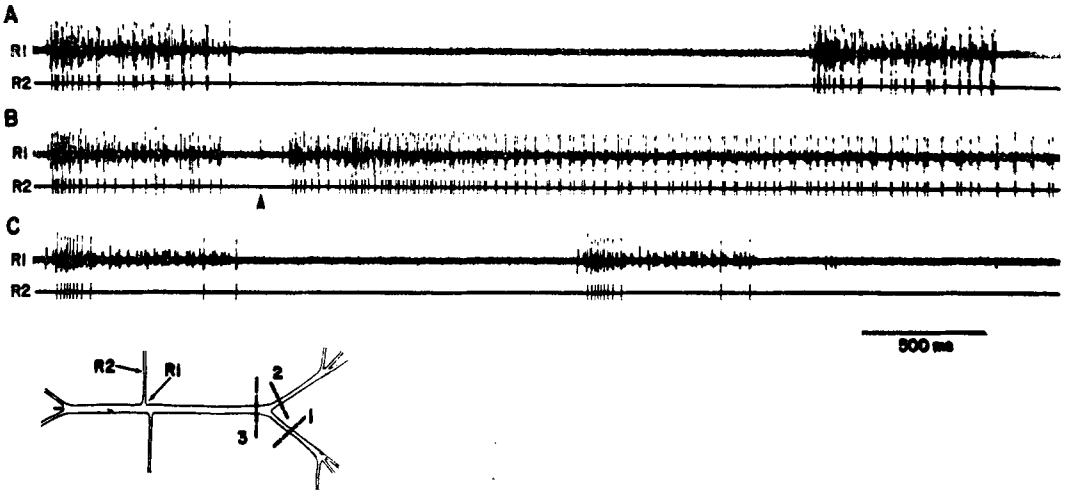

FIGURE 9. Changes in burst activity produced by successive transections. R1 records activity from both large and small cells with small cells generally having smaller spike amplitudes. R2 simultaneously records spike activity from large cells 1, 2, and 3 *c* only. (A) Two successive bursts recorded before the first transection. (B) Left of arrowhead: 35 min after transections 1 and 2. Third transection at arrowhead. (C) 80 min after the third transection.

spike-initiating site and small cell synaptic input were still at least partially intact.

On the other hand, the MSF of the group of small cells plus large cell 4 regained their original level after the second and third transections. Before the first transection it was 21.1 spikes/s, compared to 21.6 spikes/s after the second and 23.0 after the third. However, burst rate increased from 1/3.02 s to 1/2.65 and 1/2.10 s, respectively; consequently, spikes per burst decreased. Burst duration was relatively unchanged, but in many other cases it decreased.

As with the large cells the ability of the small cell fibers to recover MSF also occurred provided the spike-initiating sites of all of them remained in the isolated part of the transected ganglion from which the recordings were made. These sites are usually distributed along the main trunk of the ganglion from the level of the posterolateral nerves to just posterior to the bifurcation (Hartline, 1967 *b*; Mayeri, personal observation). Thus any transection which isolated this section of the ganglion intact usually resulted in recovery of MSF of the small cells (Table I, transections A to E, posterior; F, G, anterior).

The transection results show that the ability of ganglion cells to maintain their levels of spike activity is independent of major changes in the anatomy of the cell and pattern of spike activity. It is also independent of major changes in interactions among cells, since small cells regained MSF when some or all large cell spike activity was destroyed. One possible explanation of these re-

sults is that autoregulation of MSF is a feature of the active ion transport mechanisms of each cell. When MSF is altered by sustained changes in the autoactive mechanism, in frequency of excitatory input, or by cell injury, the concentration gradients across the cell membrane will change over a period of time but the active transport mechanisms will act to oppose the concentration gradient changes and alter "resting" membrane potential in a manner which restores MSF at or very near to its original level. It is conceivable that an electrogenic sodium pump might be an important component of autoregulation because in lobster cardiac ganglion cells a small increase in intracellular sodium concentration stimulates the pump and leads to a hyperpolarization of the cell (Livengood and Kusano, 1972). Thus when MSF is increased the additional spike activity may produce an increase in intracellular sodium concentration which stimulates electrogenic pump activity and causes it to hyperpolarize the cell, thereby reducing cell excitability and restoring MSF to its original level.

DISCUSSION

The results of the present study together with those reported in the following paper (Mayeri, 1973) suggest that the ganglion is organized into what is essentially a two layered system. The small cells, or a subgroup of them, generate the burst discharge and impose the burst pattern upon the large cells. Thus the anatomical separation of the nine cells into two populations is functionally preserved. The small cells serve to generate the pattern of burst activity that is characteristic of this ganglion, while the large cells impose the pattern on the heart muscle.

These conclusions were made from the following considerations. First, stimulation of the small cells with brief single shocks produced modulations of the burst discharge, and the firing threshold for the small cells decreased during the silent period (Mayeri, 1973). By contrast, single shock stimulation of the large cells did not produce modulation of the burst discharge, and large cell firing threshold was essentially constant during the silent period. These results showed that the integrative properties of small and large cells differ significantly from one another. Furthermore, the properties of the small cells are consistent with a model which describes many aspects of the burst-generating mechanism (Mayeri, 1973), suggesting that it is primarily the small cells which control burst duration and burst rate.

Second, functional isolation of the small cells from the large cells, by transection of the ganglion, resulted in burst activity in small cells. The small cells therefore have a burst-generating capability that does not depend on large cell spike activity. Third, populations of large cells fired spontaneously at regular intervals when small cell synaptic input was stopped by transection or by application of DNP. Whenever burst activity did reappear in the large cells of

these preparations, it was invariably accompanied by small cell spike activity. These results indicate that large cell burst activity depends on synaptic excitation from small cell axons. Together with the results of single shock stimulation of the small and large cells, the results suggest that large cells, either individually or as a group, do not have an independent capability for burst generation. Therefore, burst production in the small cells is sufficient and also necessary for burst production in the large cells. The results of several previous studies (Hagiwara and Bullock, 1957; Bullock and Terzuolo, 1957; Hagiwara et al., 1959; Hartline and Cooke, 1969) are consistent with the view that excitatory synaptic input to the large cells from small cells is strong enough to produce the burst pattern observed in the large cells. The present results together with previous ones (Watanabe, 1958; Hagiwara et al., 1959; Otani and Bullock, 1959 *a*, *b*; Hartline, 1967 *a*) also support the conclusior that electrotonic and synaptic coupling among the large cells is not strong enough to cause bursting.

The interpretation that the large cells do not have an independent capability for burst generation is based on three lines of evidence: isolation of the large cell population by transection, isolation by addition of DNP, and stimulation of the large cells. No one line of evidence can be regarded as conclusive for demonstrating the hypothesis. Furthermore, for the large cell isolation experiments, one cannot rule out the possibility that the isolation procedures themselves destroyed an inherent capability for burst activity in one or more large cells. Nevertheless, in each of the procedures tried, the small cells served as a basis for comparison to large cell behavior, and the small cells always displayed the properties to be expected of burst-generating neurons whereas the large cells did not. For instance, in the isolation experiments, whenever the small cells were spontaneously active they were bursting, even when all of the large cells were silent; but in the absence of small cell spike activity, the large cells always discharged at regular intervals. Contrary to this view, Conner (1969) concluded that sustained depolarizations recorded intracellularly from large cells during burst activity in transected ganglia of *Homarus* are mainly endogenous to the individual large cells and are not the result of excitatory postsynaptic potentials. But in these experiments the spike activity of other ganglion cell fibers located in the isolated part of the ganglion was apparently not monitored to make sure that small cell activity was silenced by the transections, nor was the large cell hyperpolarized to distinguish postsynaptic potentials from endogenous activity. Instead, it was assumed that removal of all small cell somata from the isolated part of the ganglion silenced activity in their axons. The results of Fig. 5 of the present study show that spike activity in small cell axons occurs even after the transections made by Connor. Similarly, in isolated large cells of *Panulirus*, Watanabe (1958) reported trains of three or four spikes superimposed on a slow depolarization of 10 mV, but pre-

potentials were also present, suggesting that synaptic input from other cells might be responsible for the spike train. So far, the large, smoothly graded, slow depolarizations characteristic of burst-generating neurons in mollusks (Frazier et al., 1967) and reported for cardiac ganglion neurons of the crab (Tazaki, 1971 *a*), have not been reported for lobster large cells, despite numerous studies.

It is known that the cell bodies of the large cells are electrically inexcitable (Hagiwara et al., 1959), and that the sites of integrative activity are located at some distance from them (Bullock and Terzuolo, 1957). It is therefore not surprising that large cell axons can function quite well when isolated from their cell bodies (Fig. 9). Similarly, spike activity from small cells cannot be recorded from external electrodes placed over their cell bodies, and the axons of the small cells can recover original MSF when some or all of them are isolated from their cell bodies (Table I, transections F and G, anterior). These results suggest that the small cell somata are also electrically inexcitable. In molluskan neurons, axons can be synaptically activated despite removal of the cell body (Tauc, 1962). But in contrast to lobster cardiac ganglion cells, burst activity can occur in the isolated cell body of molluskan pacemaker cells (Alving, 1968). Since Alving did not examine activity in the isolated pacemaker axon, there still is the possibility that endogenous activity may also reside in other parts of the cell.

Possible Significance of Interactions Which Are Not an Essential Component of the Burst-Generating Mechanism

Confirming the results of Hartline (1967 *a*) repetitive stimulation of the large cells during the silent period could excite a small cell and thereby initiate a burst. But the data of the present study indicate that this excitatory feedback is not essential for the generation of burst activity. What, then, is the function of this interaction? One possibility is that it serves to coordinate the relative activities of the two populations so that the burst pattern of motor output from the large cells to the heart muscle is optimal for efficient heart function under diverse conditions. The results of Fig. 3 B showed that when repetitive large cell spike activity was artificially imposed late in the silent period by electrical stimulation, the excitatory drive it provided to the small cells speeded the onset of the next burst. Similarly, in some transected preparations large cells were observed to fire repetitively late in the silent period before the first small cell spike initiated the burst (Fig. 5). The functional consequences of this kind of burst activity, observed in preparations where the heart muscle is still attached to the ganglion, is that heart contraction is less efficient; the large cell spikes preceding the burst occur at relatively long intervals and produce twitches in heart muscle. Furthermore, the heart contraction occurring with the burst is weaker than would be the case if

all large cell spikes were more densely clustered in time. Yet even under these conditions, the electrical stimulation results suggest that the large cells which are active before the first small cell spike in each burst cause an earlier onset of the burst than would be the case if no feedback were present. Therefore, the net effect of the feedback under conditions where large cell spikes precede the small cell-initiated burst may be to hasten the onset of each burst, thereby reducing the number of large cell spikes preceding it. Consequently, the large cell spikes are more densely clustered in time and heart contraction is improved.

Though trains of large cell spikes presented late in the silent period hasten the onset of the next burst, the same train presented earlier in the silent period or at the end or middle of a burst delays the onset of the next burst (Fig. 3 B–D). The delay is particularly pronounced for trains presented near the end of a burst (Fig. 3 D), probably because the small cell discharge is prolonged beyond its normal duration. Since large cell spikes normally occur only after the small cell burst has started, excitatory feedback to the small cells can be expected to increase the number of small cell spikes per burst and decrease burst rate during normal burst activity compared to the values of these parameters which are endogenous to the small cells by themselves. Inversely, any procedure which decreases the normal amount of excitatory feedback during the burst may have just the opposite effect; the number of spikes per burst will decrease and the burst rate will increase. The results of the transection experiments are consistent with this view, although there are other plausible explanations of these results. When all large cell feedback to the pacemakers was eliminated by complete isolation of the pacemakers (Fig. 4), MSF of the small cells returned to the same level as before transection but the number of spikes per burst decreased and burst rate increased. The burst rate also increased in other transections when MSF returned to the original level in the small cells but was reduced in the large cells (Table I, transections C, D, and E, posterior) suggesting that excitatory feedback was also reduced. The same results occurred even with transections in which both small and large cells returned to their original MSF's (Table I, transections F and G). In this case one can assume that some of the large-to-small cell synapses were removed by the transection, thus reducing the amount of feedback the small cells received.

Thus the feedback has two distinctive modes of action which act within a single burst cycle to coordinate the relative activities of the small and large cells. These corrective actions may be important when there are rapid temperature changes, or when ganglionic activity is modulated by cardioregulatory input from the central nervous system (Maynard, 1961, 1966), by hormonal influence via the neuroendocrine product released by the pericardial organ (Cooke, 1966), or by stretching of the heart (Alexandrowicz,

1932; Bullock et al., 1954; Hartline, 1967 *a*). In a more general sense the feedback mechanism may have evolved to correct damage to the motor system that occurs under conditions of severe stress, such as anoxia, when there is temporary or permanent damage to parts of the motor system. The feedback may also serve to correct errors in the assembly of the ganglion cells which might occur through developmental accidents. I have encountered two ganglia with abnormal anatomical arrangements where fewer than nine cells were active (Mayeri, personal observation).

In locust flight Wilson (1968) has similarly found that a major function of exteroceptive feedback to the centrally generated oscillator controlling flight is to correct inborn asymmetries in motor output. It can also correct for surgically induced asymmetries produced by removal of one of the animal's four wings. Thus in control of heartbeat, as in grasshopper flight, feedback to the neurons which generate the motor pattern may assure adaptive function even when the motor control mechanisms are damaged or inherently deficient.

One function the electrotonic interactions may serve is to couple autoregulatory mechanisms among the large cells and between large and small cells. By equalizing the membrane potentials among them the electrotonic interactions may ensure that all neurons are operating at similar MSF's. The present results are consistent with this interpretation, though they do not provide direct support for it. It is known that each large cell widely innervates the heart musculature and each muscle cell receives polyneuronal innervation from the large cells (Anderson and Cooke, 1971; Van der Kloot, 1970). Obviously, these anatomical features together with the electrotonic interactions ensure that excitatory drive to the myocardium is smooth and evenly distributed. They may also provide a certain amount of redundancy to the system which may be important for the animal's survival.

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