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THE BURST ACTIVITY OF DIFFERENT CELL REGIONS AND INTERCELLULAR CO-ORDINATION IN THE CARDIAC GANGLION OF THE CRAB, *ERIOCHEIR JAPONICUS*

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INTRODUCTION

The crustacean cardiac ganglion, which is an autonomous nervous system producing regular periodic burst discharges, is a useful preparation for experiments on spontaneity and integrative interaction among neurones. The neurophysiology of the ganglion has been studied by many investigators (for references see Hagiwara, 1961; Bullock & Horridge, 1965). In the lobster intracellular studies have been made on burst initiation, synchronous activity and intercellular co-ordination. Synaptic and electrotonic interactions are important for burst formation among ganglion cells. The burst is initiated in the pacemaker small cell, which fires repeatedly (Hagiwara & Bullock, 1957; Hartline, 1967). Recently, it was found that two small cells among four are pacemakers which control activities of large cells (or followers) through synaptic interactions; one of the two, which is the primary pacemaker, induces repetitive small synaptic potentials, increasing the excitability of large cells (Tazaki, 1971*b*). In a stomatopod, *Squilla*, the slow potential has been demonstrated to be a pacemaker potential for periodic burst discharge (Watanabe, Obara & Akiyama, 1967). In the crab intracellular studies on pacemaker cells have been made (Bullock & Terzuolo, 1957), and some characteristics of the pacemaker slow potential for repetitive spike generation have been reported (Tazaki, 1970, 1971*a*). Synaptic interaction among neurones in the crab has not, however, been studied in detail.

The present experiment is concerned with burst activity and cellular interaction in the crab cardiac ganglion. Electrical activity was recorded intracellularly from the soma and axon of a ganglion cell. In this material the neuronal activities of different cell regions could be simultaneously observed because soma and axon were impaled with little difficulty. Synaptic interactions mediated by impulses were examined among ganglion cells located in the anterior and posterior parts. The attempt was made to compare the present results with those obtained from cardiac ganglia in lobster and in *Squilla*.

METHODS

The crab, *Eriocheir japonicus*, was used. The heart was isolated from the body, and was pinned in a Lucite chamber containing a saline. The cardiac ganglion situated within the dorsal wall of the heart was exposed by cutting the ventral wall. Since the

ganglionic trunk lies under the muscles, these were carefully removed. The shape of the trunk and the situation of nerve cells were similar to those observed in *Cancer* (Alexandrowicz, 1932). Four large cell somata were located in the anterior part of the trunk and one in the posterior part which also contained two small cell somata. Distances between the anterior and posterior parts ranged from 3 to 4 mm. Mid part of the trunk consisted of many axons. Axons of anterior large cells ran backward along the posterior trunk.

Glass capillary microelectrodes filled with 3 M-KCl were used both for internal recording and for current injection. Their electric resistance ranged from 20 to 30 MΩ. The electrode was led to a high input-impedance pre-amplifier with a negative capacitance circuit and connected with a dual-beam cathode-ray oscilloscope through a main d.c. amplifier. Intracellular potential changes were recorded both from an anterior large cell soma and from its axonal region. Intrasomal recording was made from the posterior large cell. In some cases two microelectrodes were introduced into the same cell soma: one for recording potentials and the other for stimulating. Current pulses of relatively long duration ranging between 10^{-9} and 10^{-8} A were applied by a pulse generator through a resistance of 50 MΩ.

The bathing solution used in this study had the following composition: NaCl, 350 mM; KCl, 8 mM; $CaCl_2$, 15 mM; $MgCl_2$, 10 mM. The pH of the solution was adjusted at 7·3 with $NaHCO_3$. In this solution the ganglion was spontaneously active for several hours though various changes in each cell's activity took place with time. All experiments were carried out at room temperatures ranging from 20 to 23 °C.

RESULTS

Activity of the anterior large cell

Activity of different cell regions

Spontaneous activity was recorded from an anterior large cell. Various patterns of activities are shown in Fig. 1. Simultaneous recordings were made from the soma and from its axonal region in the mid part of the trunk (Fig. 1 A, B and C). The distance between these two cell regions was about 3 mm. That the impaled axon originated from the impaled cell soma was checked by means of the antidromic spike. The axon gave short high-frequency trains of spikes, while the soma gave a variety of forms of activity. The soma produced a slow potential of prolonged depolarization with superimposed spikes which were much smaller in amplitude than axon spikes. As described in a previous paper (Tazaki, 1968), the soma membrane is electrically inexcitable, as has been observed in the lobster (Hagiwara & Bullock, 1957; Hagiwara, Watanabe & Saito, 1959; Tazaki, 1972). The soma spikes are electrotonic potentials induced by the axon spikes, as witnessed by their exact correspondence. In Fig. 1 A repetitive small synaptic potentials, which did not appear in the axon, initiated the slow potential and spikes in the soma. When simultaneous external and internal recordings were made, these synaptic potentials were found to be preceded by impulses propagating from the posterior cell region to the anterior large cells. In some cases the large cell did not show the slow potential and spikes even though it responded with small synaptic potentials (Fig. 1 B). The important change took place in a later stage of this experiment. Small synaptic potentials disappeared, and the soma membrane became

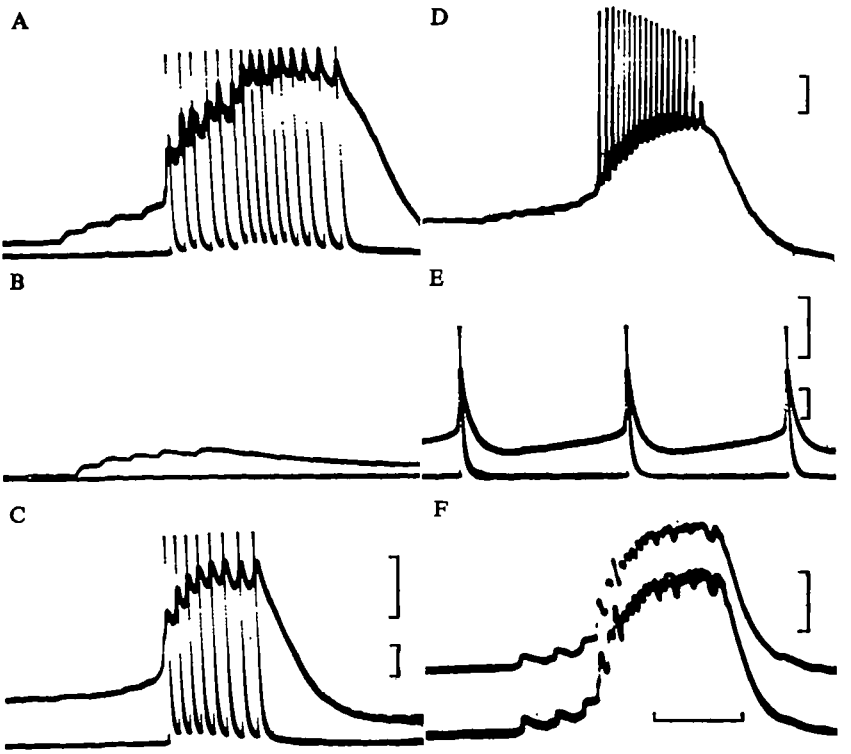

Fig. 1. Various patterns of burst activity of anterior large cells. A, B and C, Simultaneous recordings from the soma and the axon in the same preparation. Upper trace, the soma; lower trace, the axon. Distance between the two recording sites: 3 mm. D, Recording from the axonal region near the soma. E, Pacemaker potential arising in the soma. Upper trace, the soma; lower trace, the axon. F, Synchronous activity of two cell somata. Calibration: horizontal, 100 msec; vertical, 10 mV.

depolarized gradually during the interburst period (Fig. 1 C). The slow potential and spikes were initiated by a slow depolarization, though the number of spikes in a burst decreased compared to that in Fig. 1 A. This suggests that the large cell produces by itself the slow potential with multiple spikes without development of small synaptic potentials.

The axonal region distant from the soma did not show any slow potential or any small synaptic potentials (Fig. 1 A, C). Impalement of the axonal region at a short distance from the soma (within 0·1 mm) was successfully made (Fig. 1 D). The slow potential with superimposed large-sized spikes was initiated by a train of small synaptic potentials. The initial spike fired when the slow potential had developed slightly, by summation of successive small synaptic potentials. The slow potential developed progressively with repetitive firing and reached a plateau, being followed by an after-hyperpolarization. The result shows plainly that spikes are generated by the slow potential.

In some cases the spike was repeated at regular intervals, preceded by the pacemaker slow depolarization (Fig. 1 E). Simultaneous recordings were made from the soma and the axonal region. The distance between these two recording sites was within

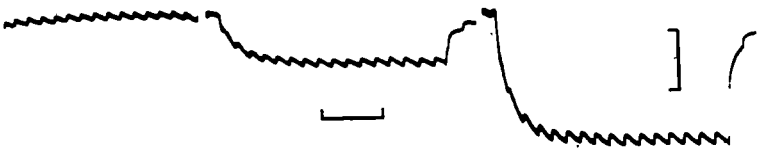

Fig. 2. Relation between the amplitude of small synaptic potentials and membrane potential. Inward current pulses were applied during development of small synaptic potentials. Left, at the resting membrane potential of about −55 mV. Calibration: horizontal, 100 msec; vertical, 10 mV.

1·5 mm. In this preparation the small cell failed to show spontaneous activity and the large cell did not show the slow potential with multiple spikes. The soma spike was not followed by an after-depolarization such as described later. The pacemaker potential appeared in the soma but not in the axon. This fact means that the large cell is capable of spike initiation without any synaptic inputs and that the pacemaker activity may be restricted to the soma-dendritic regions of the large cell.

Four large cells exhibited synchronous burst activity as shown in Fig. 1F, where two cell somata of the four were impaled. Small synaptic potentials initiated slow potentials and spikes. Noticeable synchronization among individual small synaptic potentials, slow potentials and spikes was seen in these traces. Such synchrony was found in any pairs chosen from among the four large cells. Synchronization of pacemaker slow depolarization and slow potential has been described in a previous paper (Tazaki, 1971*a*).

The result illustrated in Fig. 1 indicates that integrative interactions are performed in the soma-dendritic regions and that impulses are generated in the axon.

Small synaptic potential

The small synaptic potential reached its peak (approx. 1 mV) at about 7 msec and decayed with an exponential time course. The time-constant of decay was about 40 msec. All large cells exhibited synchronous small synaptic potentials (Fig. 1F), showing that they were innervated by common pre-synaptic nerve fibres from the small cell. The trains of synaptic potentials were repeated at regular intervals, giving rise to the slow potential and spikes (Fig. 4D). However, individual synaptic potentials never fired spikes. These facts suggest that the small synaptic potentials have an excitatory effect on the large cells. The number in a train varied greatly from preparation to preparation. The change in the amplitude of small synaptic potentials during anodal polarization is given in Fig. 2. The amplitude was 1 mV at a resting membrane potential of about −55 mV, and it increased with an increase of membrane potential. The change during cathodal polarization was not examined because the slow potential and spikes arose superimposed on depolarization. The result of Fig. 2 suggests that the reversal potential is approximately at 0 mV.

Burst initiation

The development of small synaptic potentials in large cells gave rise to repetitive spike firing. An example is shown in Fig. 3, where recordings were made from the axonal region near the soma. In Fig. 3A spikes of low frequency fired on repetitive

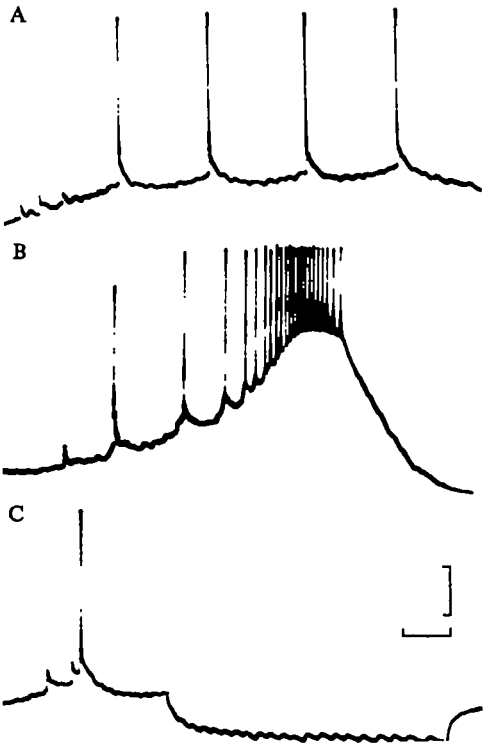

Fig. 3. Effect of a hyperpolarizing current pulse on repetitive spike initiation. A, Spikes were repeated on small synaptic potentials; the spike was followed by after-depolarization. B, The slow potential developed with high-frequency train of spikes. C, Applied hyperpolarization abolished repetitive spike firing; the small synaptic potentials remained. Calibration: horizontal, 50 msec; vertical, 15 mV.

small synaptic potentials which built up a slow, maintained depolarization. The spike was followed by a depolarizing after-potential. A transition from a maintained depolarization to the slow potential took place (Fig. 3 B). The frequency of spikes became progressively higher as the slow potential developed, which disappeared rapidly after completion of the high-frequency train of spikes. The repetitive firing was suppressed by a hyperpolarizing current pulse which was applied to the soma through another electrode (Fig. 3 C). On the other hand, the train of small synaptic potentials remained during the applied hyperpolarization. This finding supports the view that the large cells are activated by the small cell, their excitability being increased by the small synaptic potentials. Consequently, the small cell, which induces small synaptic potential, plays a role of a pacemaker for burst initiation, and the large cells are followers. Comparable observations have been made in the lobster (Hagiwara & Bullock, 1957; Tazaki, 1971*b*).

The anterior large cell produced the burst at regular intervals without being activated by small synaptic potentials. Effects of constant polarizing current pulses on the burst initiation were then examined. An example is shown in Fig. 4 A, B and C, where both intrasomal recording and current injection were performed. In the spontaneous activity the burst occurred with a certain rhythm, preceded by the slow depolarization

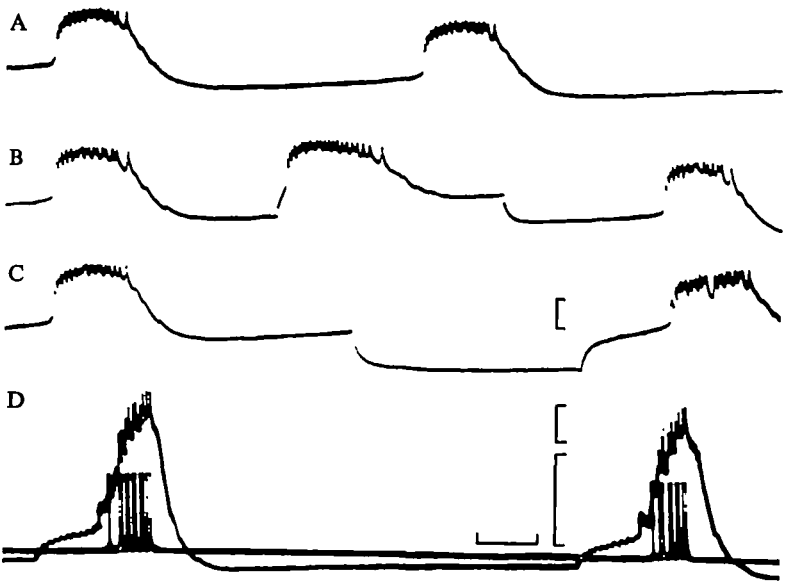

Fig. 4. Effects of polarizing current pulses on burst initiation. A, Control. The burst was initiated at regular intervals. B, The burst arose on the depolarization applied during the interburst period. C, The burst was abolished during the hyperpolarization applied in the period when it would occur normally; it was initiated as a rebound. D, Typical burst activity preceded by small synaptic potentials. Upper trace, the axon; lower trace, the soma. Calibration: horizontal, 100 msec; vertical, 15 mV.

(Fig. 4A). A depolarizing current pulse was applied during an interburst period. The burst similar to the natural one was evoked superimposed on the applied depolarization, and the rhythm of the following burst was determined by the elicited burst (Fig. 4B). On the other hand, the burst was inhibited by a hyperpolarizing current pulse given during the period when it would rise normally (Fig. 4C). The burst was caused by the rebound from the applied hyperpolarization. The pacemaker slow depolarization arising continuously during the interburst period was completely abolished during the hyperpolarization. This experiment shows that the anterior large cell becomes a pacemaker for burst initiation. Small synaptic potentials appeared superimposed on the burst. This will be described in a later section. The typical pattern of burst in a follower large cell is illustrated in Fig. 4D, which is put together in order to make easier a comparison between follower and pacemaker activities of anterior large cells. A part of this record has been shown in a previous paper (Tazaki, 1970). In the soma, the slow potentials were periodically initiated by repetitive small synaptic potentials and followed by the after-hyperpolarizations. In the axon, short high-frequency trains of spikes were propagated. In view of the observation of follower and pacemaker activities in large cells it seems reasonable to think that the slow potential is the most important for repetitive firing in the axon.

Slow potential

As shown in Fig. 3, the spike was followed by the after-depolarization of approximately 6 mV amplitude and 15 msec duration. It was occasionally found during multiple spike discharges when recordings were made from the soma and the axona

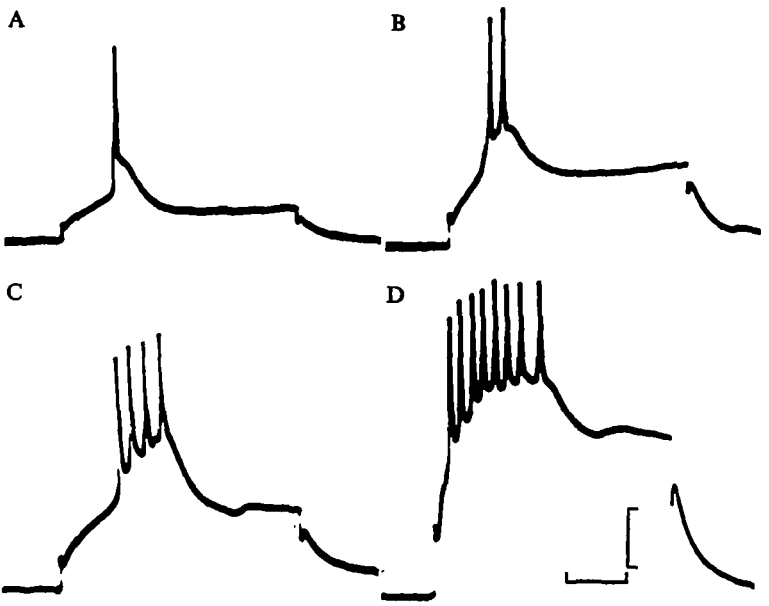

Fig. 5. Development of the slow potential during depolarization. Depolarizing current pulses with varying intensities were applied. The slow potential developed with increasing current intensity. Calibration: horizontal, 40 msec; vertical, 10 mV.

region near it. It was abolished by the hyperpolarization (Fig. 3); it occurred with directly elicited spikes (Fig. 5); and it was in some cases preceded by the pacemaker slow depolarization (Fig. 6). From these facts it is considered that the after-depolarization is a non-synaptic event in the large-cell membrane. The record of Fig. 3 suggests that the slow potential results from repetitive spikes. When the membrane potential attains a critical depolarization, the initial spike is generated and followed by the after-depolarization. The second spike is evoked on this after-depolarization, and its after-depolarization adds to the residual one of the first spike. When the spikes fire in succession with progressively shortening intervals, the resultant potential has sufficient magnitude to cause repetitive firing of the axon.

The above interpretation was supported by the experiment which is shown in Fig. 5. Depolarizing current pulses with varying intensities were applied. The elicited spike was followed by the after-depolarization (Fig. 5A). The number of spikes increased with increasing current intensities, and summation of after-depolarizations took place (Fig. 5B, C and D). The amplitude of the resultant potential increased with repetitive spike firing. It decayed with an approximately exponential time course. In the directly initiated spikes the after-depolarizations showed summation, similarly to the spontaneous activity. When the spike was not followed by the after-depolarization, only a train of spikes occurred on the applied depolarization, not producing the slow potential (Tazaki, 1970). The present result demonstrates that the after-depolarizations are cumulative and can develop into the slow potential.

Simultaneous recordings from the soma and axonal region are shown in Fig. 6. The distance between the two recording sites was about 2·5 mm. The train of small synaptic potentials did not appear, indicating that the large cell was a pacemaker and developed

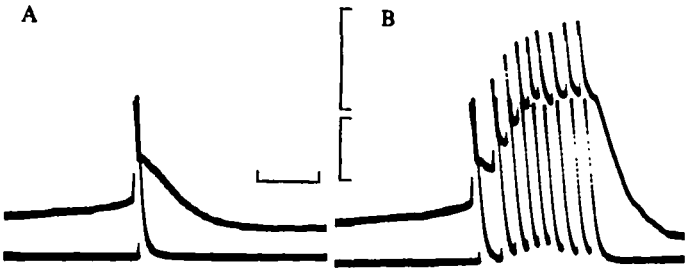

Fig. 6. Simultaneous recordings from the soma (upper trace) and the axon (lower trace). Distance between two recording sites: 2·5 mm. A, After-depolarization in the soma. B, After-depolarizations summating and developing the slow potential. Calibration: horizontal, 50 msec; vertical, 10 mV.

the slow potential by itself. In this case single and multiple spike firing were occasionally intermixed. In the soma the pacemaker slow depolarization preceded the spike which was followed by the after-depolarization, while neither depolarization appeared in the axon (Fig. 6A). From this finding it is concluded that the after-depolarization does not result from the spike activity in the axon, and that it is a separate component such as a local potential originating in the soma-dendritic regions. The slow potential developed with multiple spike firing in the same way as in Fig. 5 (Fig. 6B). A comparison with Figs. 1E and 6 confirms the conclusion that the after-depolarization is associated with repetitive firing. As shown in Fig. 1A and C, the slow potential disappeared in the axonal region at a distance of about 3 mm from the soma. It originates in the soma and spreads electrotonically along the axon, generating a brief period of repetitive spikes (Tazaki, 1970). The slow potential remains when all impulses are blocked by tetrodotoxin (Tazaki, 1971*a*). It is concluded from the foregoing that the slow potential is an electrogenic component different in nature from the synaptic potential, and that it is an endogenous event in the large-cell membrane.

Under normal conditions the excitatory state leading to a brief high-frequency train of spikes is maintained by two processes, one being synaptic activation from the small cell (small synaptic potential) and the other endogenous electrogenesis in the large cell (slow potential).

Activity of posterior large cell

Synaptic potential

The posterior large cell is also innervated by the small cell, indicating that it is a follower. An example is shown in Fig. 7. Small synaptic potentials were repeated similarly as in the anterior large cell (Fig. 7A). Constant polarizing current pulses were applied to the same cell soma. Hyperpolarization abolished the development of small synaptic potentials (Fig. 7B). This was very different from what happened in the anterior large cell such as shown in Fig. 3C. In contrast, depolarization evoked small synaptic potentials whenever it was applied during spontaneous activity (Fig. 7C). This experiment shows that polarization in the posterior large cell (follower) affected the activity in the small cell (pacemaker). The explanation of this fact is that electrical interaction such as seen among large cells (see later section) is present between large and small cells in the posterior part. Probably, two cells are electrically inter-

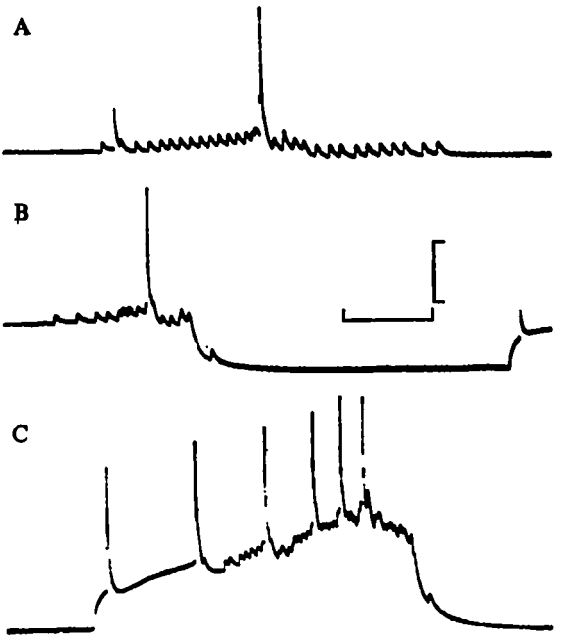

Fig. 7. Effects of polarizing current pulses on small synaptic potentials in the posterior large cell. A, Control. Repetitive small synaptic potentials in the posterior large cell. B, Hyperpolarization abolished development of the small synaptic potentials. C, Depolarization initiated small synaptic potentials and repetitive spikes. Calibration: horizontal, 200 msec; vertical, 10 mV.

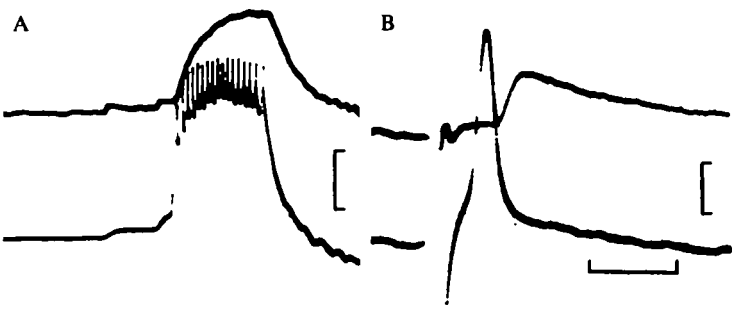

Fig. 8. Synaptic interaction between anterior (lower trace) and posterior (upper trace) large cells. A, Spontaneous activities recorded from two cell somata. B, Synaptic potential induced by the axon spike. The anterior cell soma was stimulated with a brief current pulse. Calibration: horizontal, A, 100 msec; B, 20 msec; vertical, A, 10 mV; B, upper trace, 2 mV; lower trace, 10 mV.

connected whereby potential changes in the large cell have some effects on the small cell. Such pacemaker–follower interaction through electrical connexion was found in the lobster (Watanabe & Bullock, 1960; Tazaki, 1971*b*).

Fig. 8 illustrates interaction between anterior and posterior large cells. Simultaneous recordings from the anterior and posterior large-cell somata are shown in Fig. 8A. Small synaptic potentials were evoked synchronously in two cells. Depolarized potential changes of about 18 mV amplitude developed in the posterior large cell during burst discharge of the anterior large cell. In this preparation the anterior cell was then impaled in the axonal region in the mid part of the trunk. When a brief current pulse

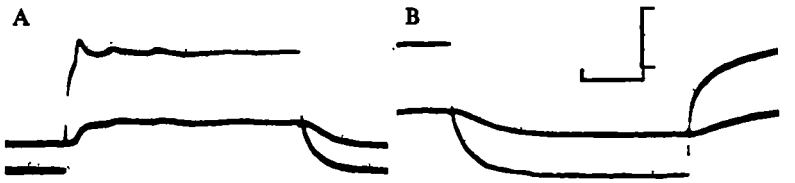

Fig. 9. Electrotonic coupling between anterior and posterior large cell somata. Polarizing current pulses were applied to the anterior large cell soma. A, Depolarization. B, Hyperpolarization. The attenuated polarized potential occurred in the posterior large cell soma. Calibration: horizontal, 100 msec; vertical, polarized cell, 10 mV; second cell, 5 mV.

of 0·5 msec duration was applied to the anterior cell soma, it elicited the spike in the axon. The posterior large cell responded with a small depolarizing deflection of 2 mV in amplitude to the axon spike (Fig. 8B). It rose to its peak at about 6 msec, and the time constant of decay was about 30 msec. It was tentatively regarded as a synaptic potential though the effect of displacement of membrane potential was not examined. This synaptic potential seemed not to result from electrical transmission such as described in a later section because synaptic delay was much larger. This finding makes it clear that the depolarizing potential change of the posterior large cell in Fig. 8A represents summation of these synaptic potentials evoked by repetitive spikes from the anterior large cell. Their amplitude can be explained by a simple summation of synaptic potentials. It is, therefore, unlikely that the posterior large cell produces the slow potential in its own soma.

Electrotonic coupling

As shown in Fig. 4, small synaptic potentials appeared superimposed on the burst. When polarizing current pulses were applied, they occurred consistently accompanying the burst. Since they appeared only during the burst discharge on the applied depolarization and they disappeared during the applied hyperpolarization, the effect of depolarization is to increase the burst discharge and the effect of hyperpolarization is to decrease it. This is interpreted as follows: the burst of the anterior large cell induces repetitive synaptic potentials in the posterior large cell which build up some amount of depolarization and this depolarization activates the small cell through electrical connexion in the same way as shown in Fig. 7C. This interpretation may be supported by the fact that the depolarization due to summation of synaptic potentials has sufficient magnitude to affect the small cell, as seen in Fig. 7.

Although electrical interaction was present between anterior and posterior large cells, it was very weak. Fig. 9 shows simultaneous recordings from anterior and posterior large cell somata, the former being polarized by constant-current pulses. Two large cell somata were electrotonically coupled. The attenuation ratio (Hagiwara, 1961) of the potential amplitude in the second cell to that in the polarized cell was calculated from the records. The ratio was about 9·5. It is considered that the slow potential changes arising in the anterior large cell have little effect on the posterior cells through electrical connexions. Such electrical coupling was observed among four anterior large cells. The attenuation ratios ranged from 2·0 to 3·5. Four large cell somata are situated close together in the anterior part, distances between them being 0·8 mm. Probably they are closely interconnected electrically. The electrotonic interaction between them

is more effective for synchrony of slow potentials (Tazaki, 1971*a*). This interaction also applies to the posterior cell somata. On the other hand, anterior and posterior cell somata were 3 mm or more apart. The synaptic interaction mediated by impulses is important for co-ordinated burst discharges between them.

DISCUSSION

It was found that the small cell of the pacemaker induces periodic trains of small synaptic potentials in the large cells, giving rise to slow potentials in the anterior ones. The slow potential maintains multiple spike firing. The anterior large cells have pacemaker activity for burst initiation, generating slow potentials by themselves in their own somata.

In the lobster cardiac ganglion pacemakers for burst initiation are located among four small cells (Maynard, 1955), and they induce synchronous synaptic potentials in large cells (Hagiwara & Bullock, 1957; Hagiwara *et al.* 1959). It has been reported that the primary pacemaker is the small cell which evokes repetitive small synaptic potentials in large cells, and that these small synaptic potentials increase the excitability of the post-synaptic membrane of the large cell (Tazaki, 1971*b*). Similar small synaptic potentials were observed in the present experiment. Their shape and size were almost the same as those examined in the lobster (Tazaki, 1971*b*). They caused the anterior large cell somata to generate multiple spike discharges (Fig. 3). As in the lobster, repetitive activation due to these synaptic potentials increases excitability of the large cell membrane. Synaptic interactions of the lobster have been studied in *Panulirus*. In the large cells of *Homarus*, however, Connor (1969) reported that the soma action potentials were to be ascribed to endogenous activity of the respective cells and not to synaptic potentials. The present study has shown that potential changes during the burst are mainly composed of both synaptic and endogenous potentials.

In contrast to a great deal of information on the electrical activity of large follower cells, almost nothing is known about intracellular activity of the small pacemaker cells. In the crab *Cancer* Bullock & Terzuolo (1957) found that the two posterior large cells which apparently receive no synaptic input behave like pacemakers, initiating a train of large spike potentials. In the present material, however, the posterior large cell was not a pacemaker but a follower controlled by two pre-synaptic nerve cells (Figs. 7, 8). Intracellular studies on the pacemaker cells have been made on the *Limulus* cardiac ganglion (Lang, 1971). They exhibit slowly depolarizing pacemaker potentials which generate single overshooting spike potentials. Intracellular records were taken from one of the two small pacemaker cells of the lobster (Tazaki, in preparation). It discharged repetitive spikes, each of them being preceded by the pacemaker potential. They evoked large synaptic potentials in large cells. In the present experiment, although successful impalement of the small pacemaker cell in the posterior part has not been made, it produces probably a train of pre-synaptic impulses, initiated by the pacemaker potential.

The anterior large cells have pacemaker activity, being capable of developing slow potentials in their own somata without activation by small synaptic potentials. The pacemaker potential appeared continuously in the soma, while it faded out in the axon (Figs. 1E, 6). Furthermore, the slow potential is restricted to the soma and its

vicinity (Tazaki, 1970). Bullock & Horridge (1965) suggest that the pacemaker locus is located in the soma-dendritic regions of the neurone. This agrees with the present observation. The slow depolarization and slow potential of the pacemaker take place in the soma-dendritic regions, and they spread over the axon, generating repetitive spikes.

In the crab *Neptunus* it has been reported that the slow potential is necessary to produce a burst of spikes (Minamisawa, 1961). The slow potential is a local potential; it is generated synchronously in four anterior large cell somata; and it has characteristics similar to those of the generator potential in the mechanoreceptor (Tazaki, 1970, 1971*a*). In the lobster the slow potential is found in large cells when the activities of pacemaker cells are stopped or delayed (Watanabe, 1958; Tazaki, 1971*b*). However, the large cells in the lobster are controlled by two small cells of primary and secondary pacemakers which evoke small and large synaptic potentials respectively; this large synaptic potential fires spikes under co-ordinated synaptic interaction (Tazaki, 1971*b*, 1972). In the crab the small pacemaker cell innervates the large cells, resultant small synaptic potentials giving rise to slow potentials in their own somata; this slow potential is responsible for repetitive spike discharges. In the *Squilla* cardiac ganglion synaptic interaction, except for electrical transmission, is not found among ganglion cells, and the slow potential is a pacemaker potential for burst initiation (Watanabe *et al.* 1967). It is of interest that the cardiac ganglion of the crab has some characteristics similar to those of ganglia in lobster and in *Squilla*.

It is well known that prolonged synaptic excitation builds up a maintained depolarization, leading to repetitive spike generation (Eccles, 1964). The present experiment shows that non-synaptic maintenance of membrane depolarization is associated with multiple spike firing (Fig. 5). The after-depolarization following the spike is not a true after-potential which is a sequel of the spike potential (Fig. 6). It is an interposed potential like a synaptic potential and a local potential for spike initiation. In the lobster the soma potential is found to be a distinct component occurring in the soma and its processes, and it is interposed between the pacemaker potential and the spike (Bullock & Terzuolo, 1957). The after-depolarization seems to be similar in nature to the soma potential. It was additive, developing a large depolarization which sustained repetitive spike discharges. A similar relation between the after-potential and repetitive firing has been demonstrated by Kandel & Spencer (1961) in the hippocampal neurone. They concluded that an excitatory state underlying repetitive firing is partly maintained by an endogenous process that is intrinsic to the nerve cell membrane. Comparable observations have been made in the stretch-receptor neurones of the crayfish, in which the electrical property of membrane maintains the excitatory state (Eyzaguirre & Kuffler, 1955; Gramp, 1966). In the slow grower-type neurone of the *Squilla* cardiac ganglion the summated after-potentials form the plateau of large depolarization; the slow potential is the result of repetitive spikes (Watanabe *et al.* 1967). The present findings are in accordance with the above-mentioned conclusion. In the crab the excitatory state is not only maintained by the endogenous electrical event but also by the synaptic activation from the small cell. The large cells cause a tetanus in cardiac muscles (Maynard, 1955; Brown, 1964; van der Kloot, 1970). It is therefore important for a tetanic contraction in muscles that the excitatory state underlying a brief period of repetitive firing is sustained by intrinsic and extrinsic processes in the large cells.

Electrical interaction such as seen in the lobster (Watanabe, 1958; Hagiwara *et al.* 1959; Tazaki, 1972) was observed among ganglion cells of the crab. Electrotonic coupling among cardiac ganglion cells has been observed in *Callinectes* (Saito, 1965). Anterior large-cell somata in the present material are closely interconnected electrically and they produce slow potentials of same shape and size (Tazaki, 1971*a*). Electrotonic coupling plays an important role for synchronous production of slow potentials. When large cells become pacemakers their membranes gradually become depolarized to a certain level and generate slow potentials with spikes. In this case also synchronous activity of the pacemaker's slow depolarization is accomplished through electrical connexions. This has been discussed in a previous paper (Tazaki, 1971*a*).

The posterior large cell received synaptic inputs from two pre-synaptic nerve fibres, one being the small pacemaker cell and the other the anterior large cell. The activities of all large cells are well co-ordinated by common pre-synaptic nerve fibres of the small pacemaker cell. Depolarization applied to the posterior large cell facilitated the activity of the small pacemaker cell, suggesting that the electrotonic coupling was present between posterior cells and indicating that the effect of depolarization was positive feedback (Fig. 7). Such positive feedback of the slow potential change has been shown among lobster cardiac ganglion cells (Watanabe & Bullock, 1960; Tazaki, 1971*b*). The finding that the burst of the anterior large cell activates the small pacemaker cell (Fig. 4) may be further proof of the presence of positive feedback in the ganglion. Since electrotonic coupling between anterior and posterior large-cell somata is extremely weak, it is inferred that the attenuated electrotonic potential of the slow potential is very small in the posterior cells, having little effect on their activities. It is possible that the burst activity of the anterior large cell affects the small pacemaker cell indirectly through the posterior large cell. This effect may be in fact the positive feedback occurring in the spontaneous burst activity. It is another point of interest in this ganglion that co-ordination between the anterior large cell and posterior small cell may be mediated by the posterior large cell. However, this interpretation remains speculative because intracellular studies on small cells have not been made.

From the above-mentioned findings it is concluded that all ganglion cells are coupled by either synaptic or electrotonic connexions and that these couplings are important for the synchronous, co-ordinated burst discharges required of the cardiac ganglion.

SUMMARY

1. Various patterns of burst activity in the cardiac ganglion cells of the crab *Eriocheir japonicus* were observed by means of intracellular electrodes.

2. The pacemaker for burst initiation is located among small cells, and it induces small synaptic potentials in the large cells, increasing their excitability. The anterior large cells generate slow potentials by synaptic activation.

3. The slow potential is the spike generator. The anterior large cells are capable of initiating slow potentials in their own somata without synaptic activation from the small cell.

4. Non-synaptic maintained depolarization takes place in the anterior large cell membrane. The after-depolarizations are cumulative and can develop the slow potential, leading to repetitive firing.

5. The posterior large cell is innervated by two pre-synaptic nerve fibres, one being the small pacemaker cell and the other the anterior large cell, showing that it is a follower.

6. Electrical interaction is present among ganglion cells. Positive feedback through electrical connexions is observed between large and small cells.

7. The cardiac ganglion of the crab has some features common and similar to those found in the ganglia of both the lobster and *Squilla*.

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