Electrical Properties of the Pacemaker Neurons in the Heart Ganglion of a Stomatopod, *Squilla oratoria*

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ABSTRACT In the *Squilla* heart ganglion, the pacemaker is located in the rostral group of cells. After spontaneous firing ceased, the electrophysiological properties of these cells were examined with intracellular electrodes. Cells respond to electrical stimuli with all-or-none action potentials. Direct stimulation by strong currents decreases the size of action potentials. Comparison with action potentials caused by axonal stimulation and analysis of time relations indicate that with stronger currents the soma membrane is directly stimulated whereas with weaker currents the impulse first arises in the axon and then invades the soma. Spikes evoked in a neuron spread into all other neurons. Adjacent cells are interconnected by electrotonic connections. Histologically axons are tied with the side-junction. B spikes of adjacent cells are blocked simultaneously by hyperpolarization or by repetitive stimulation. Experiments show that under such circumstances the B spike is not directly elicited from the A spike but is evoked by invasion of an impulse or electrotonic potential from adjacent cells. On rostral stimulation a small prepotential precedes the main spike. It is interpreted as an action potential from dendrites.

INTRODUCTION

In the *Squilla* heart ganglion the pacemaker for the periodic burst discharge is usually located in its rostral neurons (Shibuya, 1961; Watanabe and Takeda, 1963). With intracellular electrodes the pacemaker potential can be recorded from the somata in the rostral neurons as will be described in the following paper (Watanabe, Obara, and Akiyama, 1967). However, even after spontaneous firing has stopped and there are no slow potential components in intracellular records, the neurons can still generate large action potentials in response to applied stimuli. This paper is concerned with evoked

activity and interactions among pacemaker cells after spontaneous firing has ceased. It will serve as an introduction to subsequent papers, since the situation is simpler when there is no slow potential production in the ganglion, and comparison with other nerve cells is easier. It was found that, together with many properties which were common to other nerve cells, the *Squilla* ganglion cells also showed several unique properties. A major characteristic is that the activities of adjacent cells are closely correlated due to electrotonic coupling. A peculiarity is that by injecting a moderately strong current pulse the soma membrane can be stimulated initially before the axons fire. In the following description the main emphasis will be placed on these properties. Some relevant histological observations will also be described.

HISTOLOGY

The *Squilla* heart is a long tube 5–8 cm long and 1–2 mm wide in animals 12–16 cm long. 13 pairs of ostia open dorsally. Following Alexandrowicz (1934), notations Os 1, Os 2, etc., will be used for them, numbering from the rostral end of the heart. The ganglionic trunk runs longitudinally along the medial line of the dorsal surface of the heart on the outside of the wall (Fig. 1, upper). About 14 ganglion cells appear on the trunk (they will be called Gc 1, Gc 2, etc., from the rostral end of the trunk).

The heart ganglion is composed of two systems of neurons. *The local system* is made up of neurons whose somata lie in the ganglionic trunk. *The extrinsic nerves* (or *the nervi cardiaci dorsales*) are made up of neurons whose somata are in the CNS and whose axons innervate the local system and probably the heart muscle. There are three pairs of extrinsic nerves, which are called α, β, and γ nerves from the rostral side. They are also called regulator nerves since on stimulation of them acceleratory or inhibitory effects are observed in the heartbeat.

Irisawa and Hama (1965) found, with the aid of the electron microscope, that the nerve fibers in this ganglion were often tied close together, separated only by a very thin double plasma membrane (apparently a "tight junction"). This observation was recently confirmed by Dr. Kono (personal communication). What were probably these side to side appositions could also be observed with the light microscope (Fig. 2). The axons approach each other, become enclosed within a common sheath, and lose their round shape. Two or three (sometimes four) axons could be involved. The intervening sheaths become gradually thinner and finally are almost impossible to detect with the light microscope. These structures are called *side-junctions*.

In one preparation, we followed each axon in a ganglionic trunk between Gc 5 and Gc 6. About 30 junctions were observed in this region. Their location is represented schematically in the lower part of Fig. 1. It must be borne in mind that this representation is quite imperfect. It does not tell the length

of the junctions, which in fact ranged between 20 and 200 μ. Furthermore, the way in which the junctions are formed is sometimes too complicated to be represented by a single line. For example, a junction 1320 μ caudal from

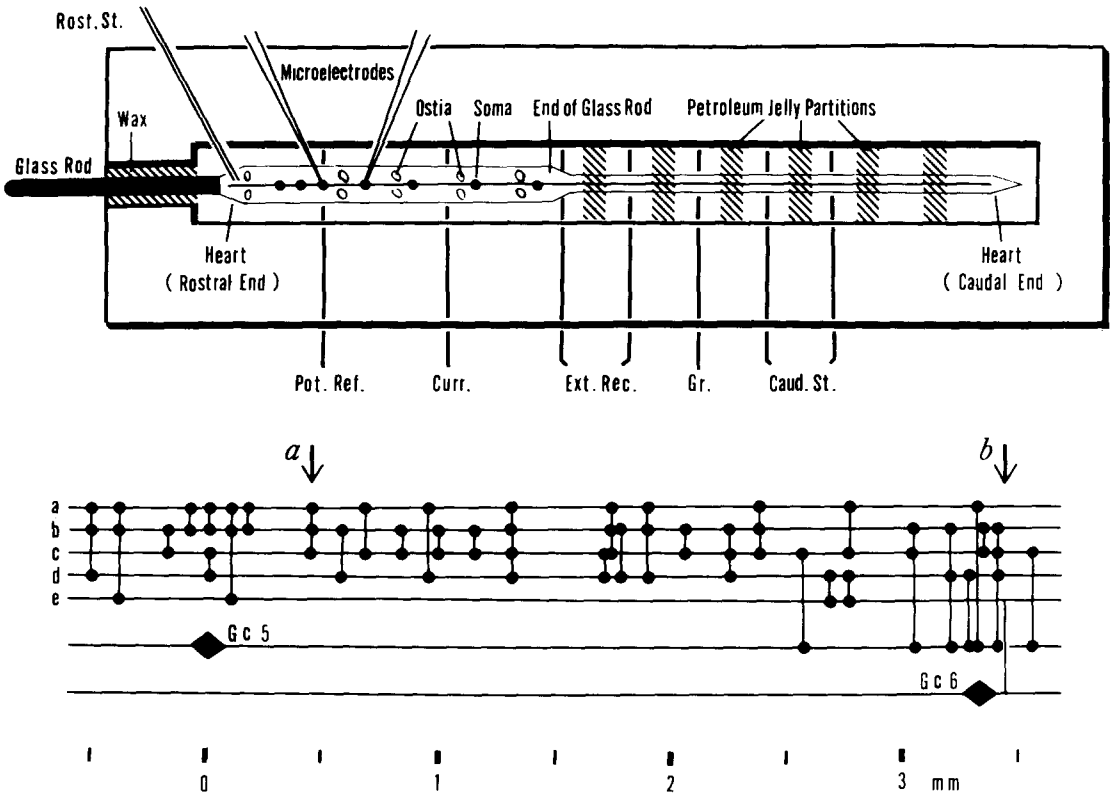

FIGURE 1. Upper, arrangement of the experimental setup. *Pot. Ref.*, reference electrode for the intracellular potential electrode; *Curr.*, a current electrode for receiving stimulating current through the intracellular current electrode; *Ext. Rec.*, a pair of electrodes for external recording; *Gr.*, a ground electrode; *Caud. St.*, a pair of electrodes for caudal stimulation; *Rost. St.*, a pair of electrodes for rostral stimulation. Lower, a schematic representation of the side-junctions (vertical lines) between Gc 5 and Gc 6. Axons *a*, *b*, *c*, *d*, and *e* correspond to those in Fig. 2 and arrow *a* shows the position the sections are shown in Fig. 2. Axon *e* is a branch of the caudal axon of Gc 6, and the point of branching is shown by a vertical line and marked with arrow *b*.

the center of Gc 5 is represented in the lower part of Fig. 1 as the junction of four axons. In fact the number of joined axons is, at the rostral end, only two, and increases to three, then four. In every case the axons which contribute to a side-junction separate again and their original number is restored. Thus the axons do not fuse, nor bifurcate on the formation of a junction. A very similar structure has been reported in the nervous system of *Ascaris* by

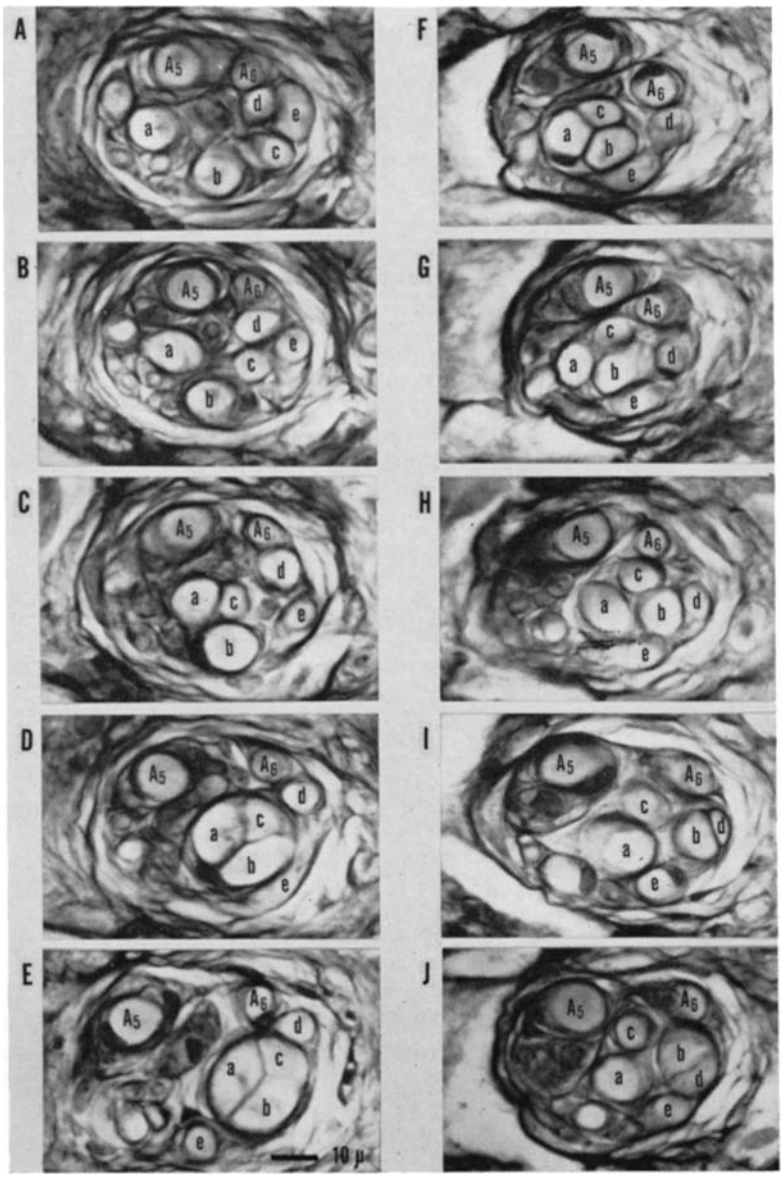

FIGURE 2. A part of the serial section of a ganglion trunk, showing two side-junctions. Fixation, 1% OsO_4. Paraffin embedding. Section thickness, 8 μ. Notations, A_5, a caudal axon of the Gc 5; A_6, a rostral axon of Gc 6; *a*, *b*, *c*, and *d*, axons of unidentified cell number; *e*, a branch of A_6. Distances of sections, *A–B* and *B–C*, 8 μ. *C–D*, *D–E*, *F–G*, and *G–H*, 16 μ. *H–I* and *I–J*, 24 μ. *E–F*, 88 μ. In *E*, *a*, *b*, and *c* form a side-junction. In *J*, *b* and *d* form a side-junction. The result of examination of this preparation is summarized in the lower part of Fig. 1.

Goldschmidt (1909) and has been reviewed by Bullock and Horridge (1965, p. 614).

In an earlier paper (Watanabe and Takeda, 1963), it was reported that the spikes in this ganglion spread throughout the local system. The routes for this spread were called side-connections, and from the physiological data it was presumed that they were so numerous that the fibers acted as a single fiber. Undoubtedly, the side-junctions, described above, supply the structural basis for the spread of spikes. In this paper we prefer to call them side-junctions, as this makes the nature of the connections clearer. It is supposed that the apposed parts form an ephaptic junction between neurons and allow the conduction of spikes in either direction (Kao and Grundfest, 1957; Watanabe and Grundfest, 1961; Bennett, Aljure, Nakajima, and Pappas, 1963).

Side-junctions are absent for some distance along the axon near a soma, as seen in the lower part of Fig. 1. This finding has been observed in several other examples. In the same preparation, the caudal axon of Gc 3 did not form any side-junctions for 1.5 mm. In a different preparation, the caudal axon of Gc 4 did not form any side-junctions for 1.8 mm. However, in one example, we observed some septum-like structures in the proximal part of the caudal axon of Gc 5. In this case, which was unlike other examples, one of the paired axons for the side-junction disappeared after a short distance in either direction. Further work is needed to describe the network completely, especially because the lack of junctions near the soma makes it difficult to interpret the existence of the electrotonic connections among adjacent somata (see Discussion).

Alexandrowicz (1934) also describes numerous dendrites and collaterals. These arise from the cell body and the proximal part of the axon, bifurcate extensively, and end in a fine network of fibrils inside or outside the trunk. Their sizes and shapes are variable, but probably some of them make contact with dendrites and collaterals from other neurons in the local system and in the regulator nerves.

METHODS

Mantis shrimps (*Squilla oratoria* de Haan) with body length between 12 and 16 cm were used. The method of dissection and mounting of the heart was similar to that described in the earlier paper (Watanabe and Takeda, 1963), but several alterations were necessary for studying the rostral cell group. After isolation of the heart, its rostral end was opened, and a glass rod about 10 cm long and 2 mm thick (made from a glass tube filled with dye and sealed at both ends) was inserted from the rostral end down to a position near Gc 8. This provided mechanical support for the ganglion. Then the preparation was laid inside a trough made from two pieces of Lucite plate (Fig. 1, upper). Across the caudal part of the heart, several partitions were made with petroleum jelly for applying stimuli and for recording the trunk action potential. The heart muscle around the trunk was not removed at the recording sites, and therefore the external records were a mixture of the action potentials from the ganglion

trunk and the heart muscle (Irisawa and Irisawa, 1957). The rapid time course of the nerve impulse can be easily distinguished from the slow time course of the muscle action potential which follows it.

Rostral stimulation was performed with an insulated silver wire, the cut end of which was pressed against the nerve trunk. A bare silver wire, set near the stimulating electrode, served as the indifferent electrode. The intracellular electrodes were conventional glass capillary microelectrodes filled with 3 M KCl. Most results were obtained from Gc 4 and Gc 5. The extracellular medium was natural sea water. The experiments were performed at a room temperature of 18–22°C.

RESULTS

Spontaneous Activity

When the preparation had been mounted in the experimental trough, discharges of the ganglion were checked with the external recording electrode. Spontaneous rhythmical burst activity was observed from most preparations. The burst activity could be kept for more than 5 hr in some preparations. In many others, however, the interval between bursts gradually increased and spontaneous activity eventually stopped.

With intracellular electrodes it was found that the potential changes in spontaneously firing ganglion cells were composed of two factors: spikes and slow potentials, of which the latter were the source of the spontaneous activity (Watanabe, Obara, and Akiyama, 1967). Corresponding to the time course of the extracellular records, the slow potentials were more labile than the spikes and were apt to disappear during the experiment. However, even after the slow potential had stopped, electrical stimuli produced spikes. The resting potentials of cells ranged between 50 and 60 mv. On trunk stimulation, action potentials of 40–60 mv were observed.

The Electrotonic Coupling among Neurons

The effective resistance of the cell membrane ranged between 2 and 8 MΩ. The variation can probably be explained by the variation of cell size (from 30 to 80 μ in length). A small rectification was always observed in the I-V relation of a cell (Fig. 3).

Electrotonic coupling was not observed between cells in the caudal region. In cells in the rostral region, however, couplings were easily demonstrated. Two intracellular electrodes were inserted into one cell, one for recording and the other for passing current, while a third electrode was used for recording the potential in an adjacent cell (Fig. 3, insert). The electrotonic potentials were recorded (Fig. 3 *A*, *B*, and *C*) and their I/V relations were drawn (Fig. 3, right). The latter, expressed in ohms, are listed in Table I. Their ratios represent the degree of attenuation.

The Response to Trunk Stimulation

In Fig. 4, A_1 and B show examples of the responses to stimuli delivered at caudal and rostral sites of the nerve trunk. The spike amplitude was 40–60 mv. Overshoots were infrequent. A notch on the rising phase was invariably

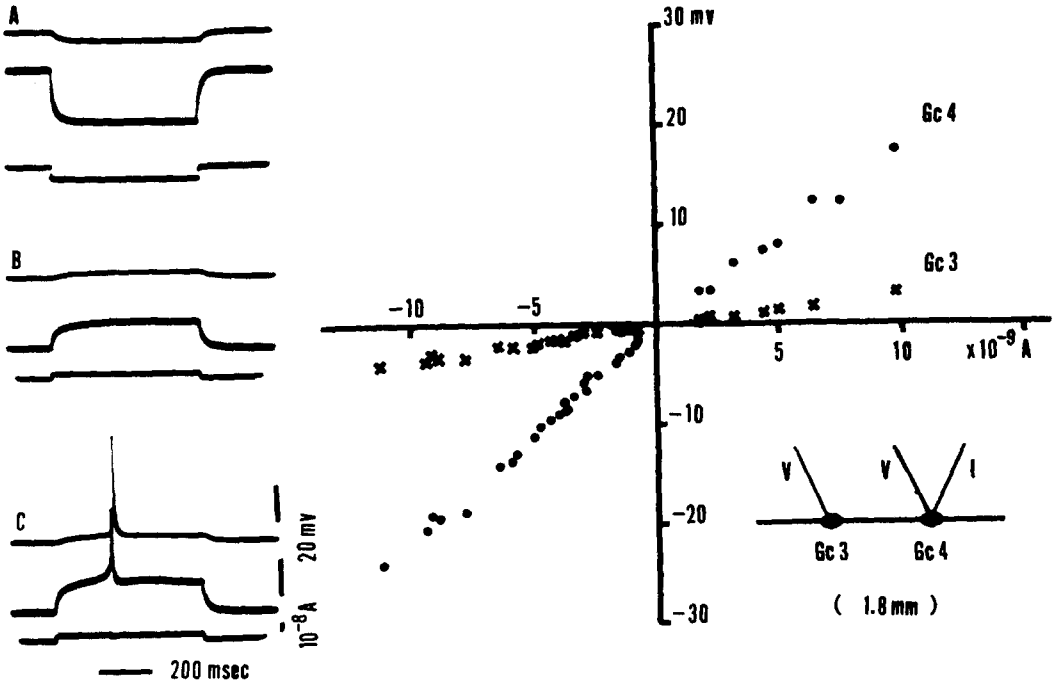

FIGURE 3. I–V curve and electrotonic connection. Left, electrotonic potentials in two cells. Upper beam, Gc 3, cell 59. Middle beam, Gc 4, cell 58. Lower beam, current injected into the Gc 4. *A*, inward current. *B*, outward current. *C*, stronger outward current with a spike. Right, the I–V curve.

found at a level about 20–25 mv above the resting potential. In the present paper, the initial part of the action potential will be called A spike, and the later part B spike, in accordance with the notation adopted by Fuortes, Frank, and Becker (1957). The origin of these components will be discussed in the next section.

Simultaneous Block of Spikes on Repetitive Stimulation

A specific feature of the response of cells in the pacemaker region is that on repetitive stimulation the late component of the action potential is rather easily blocked at the notch, in contrast with the action potential in caudal

cells. The critical stimulus frequency is different in different preparations, but in some of them even with stimuli at one per sec, block is produced. Simultaneous recordings from two adjacent cells revealed that block occurred simultaneously among the cells. In Fig. 4, one example is presented. With repetitive stimulation the step became gradually more conspicuous, and the B spike appeared after some delay which progressively increased in the course of repetitive stimulation. The third (Fig. 4 A_2) and the seventh (Fig. 4 A_4) stimuli elicited repetitive firing, but otherwise the response was a single spike with a marked notch. The order of appearance of the B spikes in Gc 4 (upper trace) and Gc 5 (middle trace) was gradually reversed; at first the B spike in Gc 5 preceded the B spike in Gc 4 (A_1), but later the B spike in Gc 5 appeared at the falling phase of the A spike (A_4). With the eighth stimulus the B spikes in the two cells blocked simultaneously (A_5). Throughout this period of repetitive stimulation, the delay for the A spike remained almost constant. After the third stimulus (A_2), the extracellular recording (lower trace) picked up impulses travelling caudally. The number of impulses was usually one (A_3) except for the third (A_2) and seventh (A_4) responses, in which the intracellular potentials also showed the repetitive firing.

TABLE I

Cells (a) in which current (I_a) was injected	V_a/I_a in 10^6 Ω	Cells (b) in which tonic spread appeared	V_b/I_a in 10^6 Ω	$(V_b/V_a)I_a$	Distance between cells
					mm
Gc 4	2.0	Gc 3	0.35	1/5.7	1.8
Gc 4	4.7	Gc 5	0.58	1/8.1	4.8
Gc 4	7.0	Gc 5	2.4	1/2.9	3.5
Gc 5	8.9	Gc 4	1.2	1/7.4	4.0
Gc 5	4.6	Gc 4	1.4	1/3.3	4.8

An analysis of the time relations is presented in Fig. 5. It shows that the delay for the A spike was almost constant, that the delay for the B spike changed markedly, and that in spite of this the delay from the B spike to the rostrocaudally travelling impulse was almost constant (except for the third stimulus) and slightly longer than the conduction time of caudorostrally travelling impulses.

Such behavior of the A spike is consistent with the usual interpretation (Coombs, Curtis, and Eccles, 1957) that the initial part of the soma action potential is composed of the electrotonic spread from the action potential of the initial segments (IS spike).

The time relations in Fig. 5 further show that, when the safety factor is reduced by repetitive stimulation, the B spike in Gc 5 results from receiving an impulse from Gc 4. The delay between responses was more or less con-

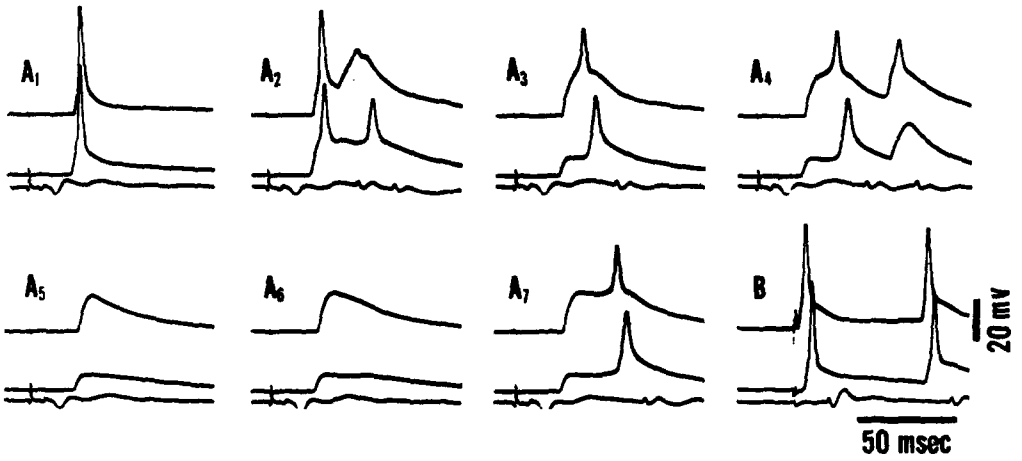

FIGURE 4. Responses to trunk stimulation in two adjacent cells. Upper beam, Gc 4, cell 178. Middle beam, Gc 5, cell 179. Lower beam, extracellular records across a petroleum jelly partition set at 28 mm caudally from Gc 5. *A*, caudal stimulation with a frequency of 5/sec. Stimulus number, A_1, No. 1; A_2, No. 3; A_3, No. 5; A_4, No. 7; A_5, No. 8; A_6, No. 10; A_7, No. 11. *B*, rostral stimulation.

stant after the fourth stimulus, and when Gc 4 failed to fire, the B spike in Gc 5 was also blocked. This means that the B spike in Gc 5 is not composed of only the action potential of the soma-dendritic membrane of Gc 5. There must be some basic activities which represent the excitatory influence from Gc 4. In the caudal cells, the main spike can be subdivided by hyperpolari-

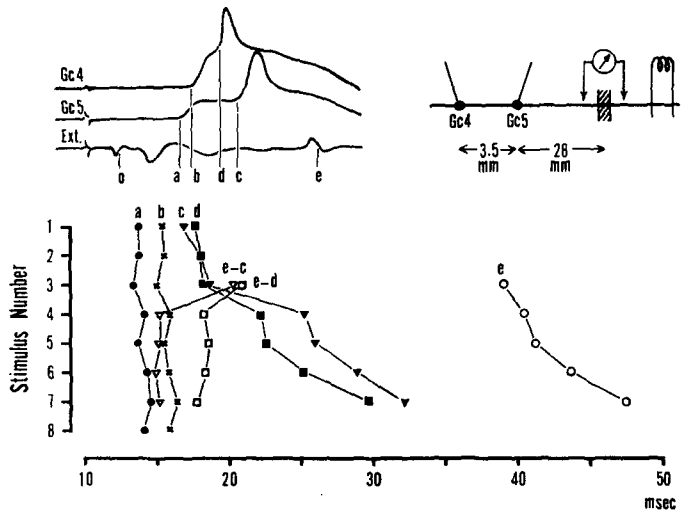

FIGURE 5. Analysis of the experiment shown partly in Fig. 4. Notations and setup are shown in the two inserts. Descriptions in text.

zation into two parts, A_2 and B; the A_2 spike is generated by the axon which is not stimulated, whereas the B spike is generated from the soma-dendritic membrane (Watanabe and Takeda, 1963). In the rostral cells, block usually takes place simultaneously in adjacent cells and therefore the main spike is not separable (see Fig. 6 for an exception). For that reason, we call this spike simply a B spike, which in the present paper contains A_2 and B spikes in the earlier paper.

Extra impulse generation (A_2–A_4) and repetitive firing (A_2 and A_4) are frequently observed in this ganglion, especially in a state of refractoriness (Watanabe and Takeda, 1963).

When the repetitive stimulation was continued, the B spike sometimes re-

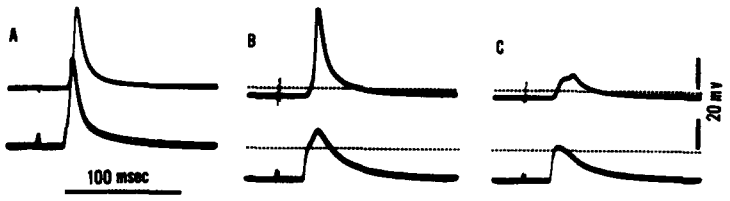

FIGURE 6. The effect of hyperpolarization on the action potentials from the adjacent cells. Caudal stimulation. Upper beam, Gc 3, cell 59. Lower beam, Gc 4, cell 58. *A*, control. *B* and *C*, hyperpolarizing current was applied to Gc 4. The resting level is indicated by dotted lines. The distance between the somata was 1.8 mm.

appeared on the A spike, as seen in Fig. 4 A_7. In Fig. 4 A_6 the A spikes in the two cells had a small addition of potential on the falling phase. It is probably a local response, and its periodic development may be due to an intrinsic membrane rhythm, which sometimes makes its appearance even after the spontaneity has ceased. The synchronization of such local responses among adjacent cells is probably performed through the electrotonic coupling between the pacemaker neurons. This would mean that the electrotonic coupling is a contributing factor to the synchronized appearance of B spikes in adjacent cells, although the decisive factor must be the communication through impulse transmission.

Simultaneous Block of Action Potentials by Hyperpolarizing the Membrane of One Cell Soma

A hyperpolarizing current can block an action potential elicited by trunk stimulation at the notch of the rising phase. In Fig. 6, action potentials were evoked by caudal stimulation and were recorded from Gc 3 and Gc 4 simultaneously. A third electrode was inserted into Gc 4 for the application of hyperpolarizing currents.

A 20 mv hyperpolarization in Gc 4 blocked part of the B spike, leaving

a partial response which had a rather sharp peak. With a very small increase of the hyperpolarizing current, the main part of the spike in Gc 3 was also blocked, and at the same time the peak of the response in Gc 4 disappeared, leaving a smooth A spike.

In two other examples we did not observe the intermediate stage shown in Fig. 6 *B*; the B spikes in two cells were blocked simultaneously. The situation is thus similar to the effect of repetitive stimulation, which causes a simultaneous block of the B spikes in two adjacent cells. An essentially similar explanation can be applied to the effect of hyperpolarization; the communication

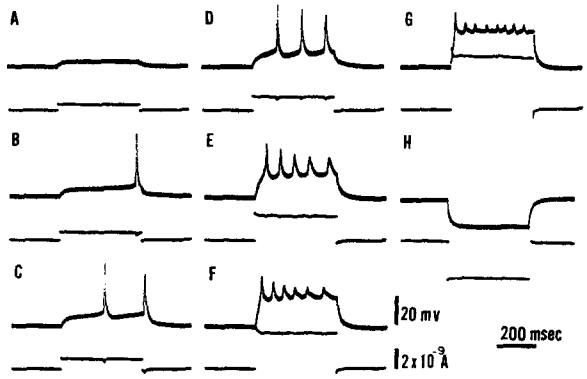

FIGURE 7. Direct stimulation. Upper beam, electrotonic potential with spikes. Lower beam, injected current. *A–G*, current is outward through the membrane. *H*, current is inward through the membrane. Gc 5, cell 69.

among cells results from electrotonic coupling with or without active impulse propagation. In the example of Fig. 6 the communication was probably electrotonic, since in Fig. 6 *B* the spike in Gc 3 occurred almost simultaneously with the peak in Gc 4. But in another example (not shown) a delay was recognized, and was interpreted to mean that the impulse was actually propagated. The third example belonged to the "electrotonic" type in which the B spike in two cells persisted until the point at which both of them disappeared at a critical hyperpolarization.

Repetitive Firings on Direct Stimulation

When depolarizing current of 10^{-8}-10^{-9} A intensity is injected into the soma, action potentials are elicited as shown in Fig. 7. When the duration of the current pulse is sufficiently long, repetitive firing takes place, and the number of spikes is increased with increased stimulus intensity. This is different from the effect observed in caudal cells of the ganglion, in which the spike number is not increased to more than two with a DC stimulus (Watanabe and Takeda, 1963). The process of accommodation is probably slower in pacemaker cells than in caudal cells.

Dependence of the Firing Level on Stimulus Strength

The firing level for the first spike was conventionally measured as the membrane potential level just before the sharp rising phase of the spike. It was found that the firing level increases with increased stimulus intensity. In the example of Fig. 7, the firing level is about 8 mv at the threshold current (Fig. 7 *B*). It rises with the stimulus intensity, and attains about 20 mv on stimulation with about seven times threshold current (Fig. 7 *G*).

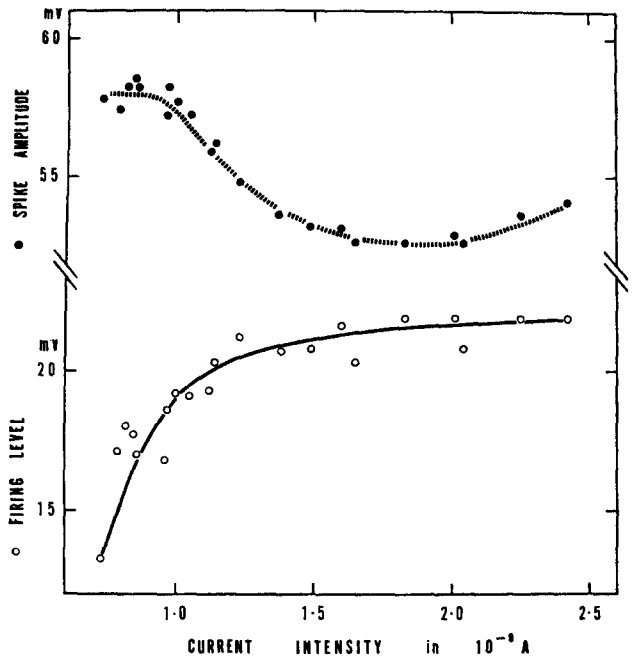

FIGURE 8. The amplitude and firing level of the directly evoked soma spike with change in injected current intensity. Gc 4, cell 178. Pulse duration, 277 msec. Stimulation was applied at 20 sec intervals.

In Fig. 8, the firing level of a Gc 4 is plotted against the stimulating current intensity. With the threshold current it is about 13 mv, but it rises with the stimulus intensity to about 22 mv.

In a homogeneous membrane, the firing level usually remains constant until the accommodation process starts (see Hagiwara and Oomura, 1958). In the present material, the stronger stimulus decreases the latency of the spike, and therefore the accommodation process does not explain the rise of the firing level.

A simple explanation can be supplied by assuming that the neuron has a heterogeneous membrane. In this view the membrane of the neuron is made

up of a high threshold membrane, which covers the soma and the proximal part of the axon, and a low threshold membrane, which covers the peripheral part of the axon. It will be shown in the following sections that this hypothesis explains many of the experimental facts.

According to the hypothesis, on weak current stimulation the spread of electrotonic potential from the soma is enough to initiate a spike from a proximal part of the axon, even though at the soma the depolarization does not reach the threshold level. The site of spike initiation in the axon will be called the *trigger zone*. The spike produced at the trigger zone conducts in two directions, one towards the soma and the other away from it. Thus the firing level measured at the soma does not represent the threshold potential of the soma membrane, since the action potential is a conducted one. As will be seen below, a strong stimulating current evokes an action potential directly from the soma membrane, and in that case the firing level does represent the threshold of the soma membrane. In the example of Fig. 7, the threshold depolarization of the soma membrane is about 20 mv.

The Lowest Firing Level in Different Cells

When the injected current pulse has a duration of more than 40 msec, and the intensity is at the threshold value, the firing level takes its lowest value. Several measurements of the lowest firing level revealed that the value is different between Gc 4 and Gc 5. Five examples from Gc 4 gave an average value and an SD of 17.0 $\pm$ 4.1 mv, with a range of 11 to 21 mv. Six examples from Gc 5 gave an average value and an SD of 7.4 $\pm$ 0.8 mv, with a range of 4.6 to 10.0 mv. The difference in the lowest firing level can be ascribed either to the difference in threshold of the membrane or to the difference in the structure of the neuron. It is possible that in Gc 5 the distance between the trigger zone and the soma is less than that in Gc 4.

Spike Amplitude on Long Pulse Stimulation

With increased stimulus intensity the amplitude of the elicited initial spike is often decreased. In Fig. 7 *A–D*, the amplitude of the initial spike is 43–45 mv, whereas in *E* and *F* it is 35–36 mv. Since this was an unexpected effect, several checks were made to be sure that the decrease was not due to an error or to some subsidiary effects. The effect of refractoriness has been excluded, since the decrease appeared even when the stimulus interval was as long as 20 sec, and the decrease depended only on the stimulus intensity and not on the order of application of strong and weak stimuli. The artefacts due to an external field or to a capacitative coupling between recording and current electrodes were checked by withdrawing the recording electrode from inside the cell. In most cases the artefacts were negligible.

The decrease of spike height using stronger stimuli has been clearly ob-

served in eight of ten cells examined, and in both Gc 4 and Gc 5. An example from Gc 4 is presented in Fig. 8, where the spike height and the firing level are plotted against intensity of the injected current. The spike height shows a minimum around 1.7×10^{-9} A, which is about 2.5 times threshold. Further increase of the stimulus current increases the spike height. The same phenomenon is to be observed in Fig. 7 in which the minimum in spike height takes place at about four times threshold (Fig. 7 *F*), and a further increase in current intensity increases the spike height (Fig. 7 *G*). The reason for this phenomenon will be discussed in the following section.

Comparison of the Spike Amplitude on Direct and Axon Stimulation

Fig. 9 *A* shows one experiment indicating that the decrease in spike height occurs only when the soma membrane is directly excited. When the spike was initiated by rostral or caudal trunk stimulation, the depolarizing current increased the spike height, as seen in Fig. 9 A_2 and A_4. This is to be expected, since even at the peak of the spike the membrane impedance must remain finite. But when membrane was directly stimulated with a current pulse of the same intensity, the resultant spike showed a smaller amplitude, as seen in A_3.

The above experiment shows that the observed difference in spike amplitude depends on the difference in the site of spike initiation rather than on the effect of depolarizing current itself, which in fact tends to increase the spike height. Thus when the spike is initiated at the soma membrane, a smaller spike is recorded, even though the background stimulating current should have increased the spike height to some extent.

The above experiment further suggests that the decrease in spike height on strong current injection can be explained on the basis of the hypothesis described on p. 824. When a weak depolarizing current pulse is injected into a soma, the site of spike initiation is not at the soma but at the trigger zone in the axon. In other words even in the "direct" stimulation experiment the soma spike must be regarded as having been excited indirectly from invasion of the axon spike when the stimulus current is weak.

The decrease of spike height with stronger current pulses can now be explained as follows. As will be seen on p. 830 and in Fig. 11, the soma action potential is graded to a considerable extent (see also Fig. 7 *F* and *G*). When the basic depolarization is larger, the action potential increases its height. When weak stimulating current is applied at the soma, the axon fires first and its action current creates the A spike in the soma membrane. The A spike is superimposed on the electrotonic potential in the soma and its peak exceeds the threshold of the soma membrane. With this strong basic depolarization, the soma generates a large action potential. However, when the stimulating

current is strong enough to bring the soma membrane to the threshold by itself, the soma membrane is stimulated directly by the injected current. But at this moment the A spike does not appear at the soma membrane, and therefore the basic depolarization is just at the threshold value. Thus the elicited spike takes its smallest amplitude. The A spike can contribute to the soma potential only at the later stage of the soma action potential.

Since the increase of stimulus current intensity tends to increase the soma spike, the lowering of the spike height is a net result of the two counteracting

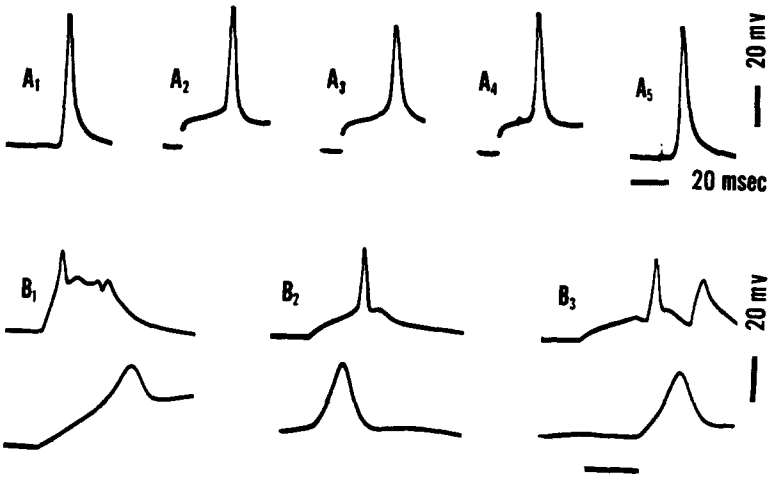

FIGURE 9. Properties of the directly evoked spike. *A*, a comparison of spikes evoked by current injection and by axon stimulation. A_1, caudal stimulation only. A_2, caudal stimulation with outward current pulse. A_3, outward current pulse only, which elicited an action potential. A_4, rostral stimulation with outward current pulse. A_5, rostral stimulation only. Pulse intensity was the same in A_2, A_3, and A_4. Gc. 4, cell 192. *B*, step formation in the directly evoked spike. Upper beam, the action potential evoked by injected current of about 50 msec duration. Gc 5, cell 220. Lower beam, same as the upper beam with five times expanded sweep. Time scale represents 50 msec for the upper beam and 10 msec for the lower beam. Current intensity is decreased from B_1 to B_3.

effects. Two cells among ten examples did not show a clear decrease in spike amplitude on stronger current application. Probably in such cells the two counteracting effects cancelled each other.

Comparison of the action potential size on weak current injection and on axon stimulation did not give consistent results. Usually axon stimulation gave a larger action potential, but sometimes it gave a smaller one. The cause of the inconsistency is that on current injection the stimulating current contributes to the height of the action potential to a variable extent. A constant result was obtained if the comparison was made while applying the same outward current. As shown in Fig. 9 *A* axon stimulation superimposed on a

direct stimulus always gave a larger action potential than the direct stimulus alone.

Spikes from the Falling Phase of Electrotonic Potential

When the intensity of stimuli is near threshold, the spike sometimes arises on the falling phase of the electrotonic potential (Fig. 9 B_3). This phenomenon can be interpreted in two ways. First, the soma membrane has a long response time, so that even after the stimulating current has subsided, the excitation process continues and finally produces an action potential after a long delay. Second, the site of spike initiation is not at the soma, so that the action potential arrives at the soma after some conduction time. The latter is the hypothesis adopted on p. 824.

These two alternatives are in fact not mutually exclusive. As for the first one, we sometimes observe a long lasting local response which may lead to an action potential, especially when there is the pacemaker activity (see Fig. 13 B_3, and Watanabe et al., 1967, Figs. 13 and 17). However, in the example shown in Fig. 9 B_3, almost no trace of the local response can be recognized. The spike arises quite sharply from the passively decaying electrotonic potential. This indicates that the observed spike is a conducted one, and that the first alternative is at most not enough for the explanation.

Thus we come again to the hypothesis previously adopted. Spikes on the falling phase of the electrotonic potential have been observed in a decapod heart ganglion, and explained by the same hypothesis (Hagiwara, Watanabe, and Saito, 1959).

The Inflection on the Rising Phase of the Action Potential on Direct Stimulation

On axon stimulation, the soma action potential shows an inflection on its rising phase (Fig. 4). On direct stimulation with a long and weak current pulse, the soma action potential also shows a step on its rising phase. The inflection is particularly clear if the spike takes place on the falling phase of the electrotonic potential (Fig. 9 B_3). With increased current intensity, the inflection gradually becomes obscured (Fig. 9 B_2).

The existence of the step on weak current stimulation strongly supports the hypothesis that the spike comes from the axon, because of the evidence that the A spike before the inflection originates from the initial segment (p. 820).

The disappearance of the inflection does not necessarily indicate that the soma membrane is directly stimulated. Because of depolarization of the soma, the safety factor for the axon-soma conduction becomes larger and the delay of soma spike production becomes smaller.

When the current intensity is strong enough to decrease the height of the

soma action potential, the step is never observed, in agreement with the hypothesis that the soma membrane is now directly stimulated (Fig. 9 B_1).

The Time Interval between Soma Spike and Axon Impulse

According to the hypothesis previously described, injection of weak current initiates a spike at the trigger zone in the axon. The spike then propagates in two directions, one towards the soma and the other towards the peripheral part of the axon. The interval between the intracellular soma action potential and the extracellular axon impulse will be called the apparent conduction time of the directly evoked spike. According to the hypothesis the apparent conduction time is the difference (t_1–t_2) between the two time intervals: the interval from the spike production at the trigger zone to that at the site of external recording (t_1), and the interval from the spike production at the trigger zone to that at the soma (t_2).

A check of the above hypothesis can be made by measuring the change of the apparent conduction time with the change of stimulus current intensity. When the current intensity is gradually increased, the hypothesis predicts the following events in the neuron. When the current intensity is weak and soma depolarization does not reach the threshold of the soma membrane, t_2 represents the conduction time of the spike from the trigger zone to the soma. Even when the current intensity becomes strong enough to stimulate the soma membrane directly, the soma will be invaded by the axon spike if the soma electrotonic potential reaches the threshold level after the axon spike arrives. With increase of the current intensity the soma action potential will be elicited earlier than the time of arrival of the axon impulse to the soma. Then the action potential amplitude will become smaller because of the lack of the local current from the axon spike, and at the same time the apparent conduction time will increase because of the decrease of t_2.

With further increase of the current intensity the soma action potential will be elicited even earlier, t_2 will become even smaller, and accordingly the apparent conduction time will become even larger.

An experimental test of the above prediction is presented in Fig. 10. As shown in Fig. 10 *A*, the latency of the soma spike decreases gradually with increase of stimulus current. In Fig. 10 *B*, the spike height and the apparent conduction time are plotted against the latency of the soma spike. When the latency is more than 30 msec, the action potential amplitude is about 56 mv. When the latency decreases to 30–25 msec, an abrupt fall of the action potential amplitude is observed. At the same time, the apparent conduction time starts increasing. The correspondence between the apparent conduction time and the spike amplitude is quite clear, with a correlation coefficient of −0.9. These results are exactly those to be expected from the hypothesis.

Direct Stimulation with a Brief Pulse

When the pulse duration of the injected current is brief (less than 10 msec), the effect of stimulation is different from that with a longer pulse. Fig. 11 shows one example. Even with a subthreshold stimulus, the electrotonic potential

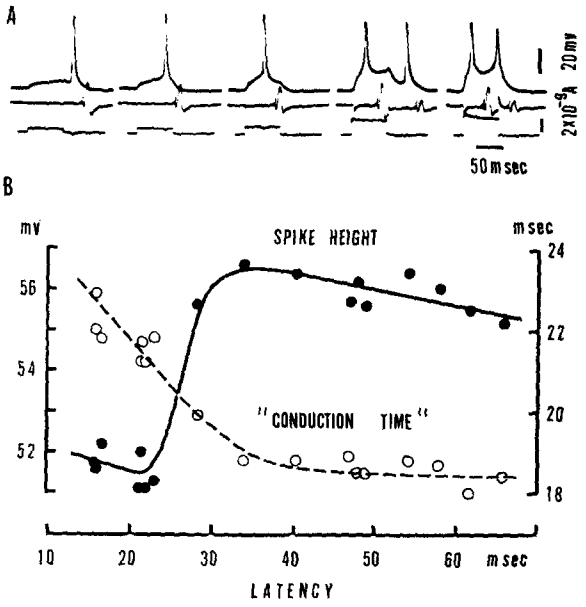

FIGURE 10. The relationship between the external and internal spikes on direct stimulation. Gc 4, cell 138. *A*, upper beam, intracellular recording; middle beam, extracellular action potential recorded across a petroleum jelly partition, which is set about 28 mm caudally from the penetrated cell; lower beam, injected current. *B*, their time relations and the spike amplitude. Filled circles with solid curve, spike height of the intracellular action potential, scale at the left. Open circles with a dotted curve, apparent "conduction time," which is measured between the rising phase of the intracellular action potential and the extracellular nerve impulse (the first rapid spike on the middle beam in *A*); scale at the right. Abscissa, latency of the intracellular spikes measured from the beginning of the current pulse.

has a prominent local response superimposed. The first spike arises at a critical depolarization of about 25 mv. With increased stimulus intensity the rate of rise and the amplitude of the spike are considerably increased. The following spikes probably originate somewhere in the axonal network, and we are not going to discuss them here.

Because of the membrane capacity, when the stimulating current pulse has a sufficiently brief duration, the spread of the electrotonic potential towards the trigger zone can be kept so low that the depolarization at the trigger zone

is below the threshold value. Under such conditions we can stimulate the soma membrane directly. In Fig. 11, the high value of the firing level indicates that the soma membrane is directly stimulated by a current pulse of this duration.

Small Prepotentials on Rostral Stimulation

As described under Methods, rostral stimulation was applied with the cut end of an insulated silver wire. The response to the stimulation was sometimes similar to that with caudal stimulation, as seen in Fig. 4 *B*. However, we often observed a small prepotential of about 2–8 mv which preceded the main action potential. In Fig. 12 one example is presented. In this preparation the amplitude of the small prepotential was about 7 mv in Gc 4 and about 5 mv in

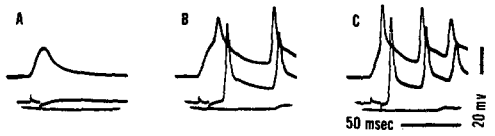

FIGURE 11. Direct stimulation with a brief pulse. Upper beam, Gc 4, cell 178. Current pulse is applied to this cell. Middle beam, Gc 5, cell 179. The distance between the somata is 3.5 mm. Lower beam, extracellular records from the ganglion trunk.

Gc 5. The prepotentials were different from the usual A spike, which had an amplitude of about 20 mv. Even when the small prepotential appeared before the main spike, the A and B spikes could still be recognized, although the inflection sometimes became more obscure. In Fig. 12 *A*, the action potential recorded on the upper beam is electronically differentiated and displayed on the lower beam to show the inflection point. On rostral stimulation (A_1), the small prepotential appeared first, but the spike showed another inflection which divided the A and B spikes. The whole action potential is thus composed of three components, demonstrating that the small prepotential and the A spike are different in origin.

The small prepotential appeared in an all-or-none fashion, independently in the individual cells. In Fig. 12 *B*, the stimulus intensity was increased from B_1 to B_3. With a weak stimulus the small prepotential appeared in Gc 4 in an all-or-none manner (B_2). With a stronger stimulus the small prepotential also appeared in Gc 5, again in an all-or-none manner.

A full sized spike, evoked by rostral stimulation, is blocked very easily by repetitive stimulation or by applying hyperpolarizing current, when the small prepotential can be seen in isolation. Fig. 12 *C* shows an example of block of the full sized spike in the course of repetitive stimulation at 2 sec intervals. When the spike did not appear, the extracellular recording failed to pick up any spike, showing that the small prepotential is a highly local event, in con-

trast with the usual action potential, which always spreads throughout the local system (Watanabe and Takeda, 1963).

The effects of hyperpolarization of the soma membrane are shown in Fig. 13 *A*. The intensity of the rostral stimulation was kept constant. With hyper-

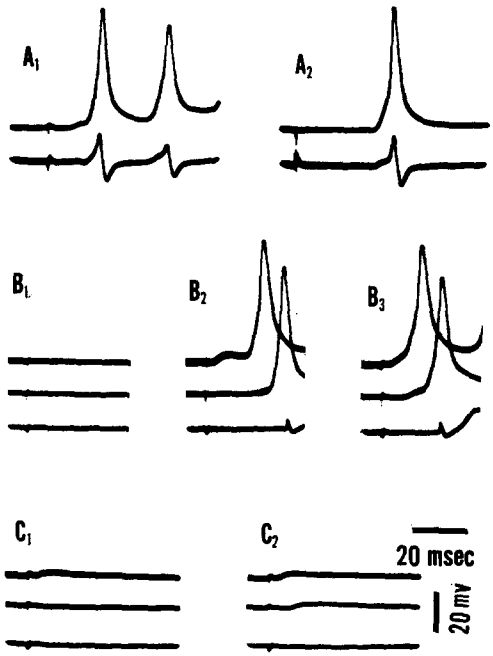

FIGURE 12. Small prepotentials in response to rostral stimulation. All records are from the same preparation. *A*, upper beam, Gc 5, cell 205; lower beam, the electronically differentiated records of the intracellular potential. A_1, rostral stimulation. A_2, caudal stimulation. *B*, rostral stimulation. Upper beam, Gc 4, cell 204. Middle beam, Gc 5, cell 205. Lower beam, extracellular records across a petroleum jelly partition, set at 21.5 mm caudally from the Gc 5. B_1, subthreshold stimulus. B_2, superthreshold stimulus for the small prepotential in the Gc 4. B_3, superthreshold stimulus for the small prepotentials in Gc 4 and Gc 5. *C*, rostral stimulation. Small prepotentials without spikes. Three beams as in *B*. C_1, weaker stimulus. C_2, stronger stimulus. Stimuli were applied repetitively at 2 sec intervals. The distance between the somata was 4.6 mm. The distance between the rostral stimulus electrodes and the Gc 4 was 1.5 mm.

polarization of about 8 mv, the full sized action potential disappeared, and the extracellular trunk impulse was simultaneously abolished. With a slight increase of hyperpolarizing current, the small prepotential also disappeared in an all-or-none manner, indicating that the threshold for the small prepotential was increased on hyperpolarization. The experiment suggests that the origin of the small prepotential is at a part of the membrane which is electrically continuous with the soma membrane. No trace of an increase in

amplitude of the small prepotential could be obtained in the course of hyperpolarization.

A change in threshold occurs with spontaneous changes of the membrane potential level. In Fig. 13 *B*, the membrane potential of the soma was spontaneously fluctuating because of some residual pacemaker activity. Rostral stimuli of a constant intensity usually elicited isolated small prepotentials, but when the membrane potential drifted to a depolarized level, the small

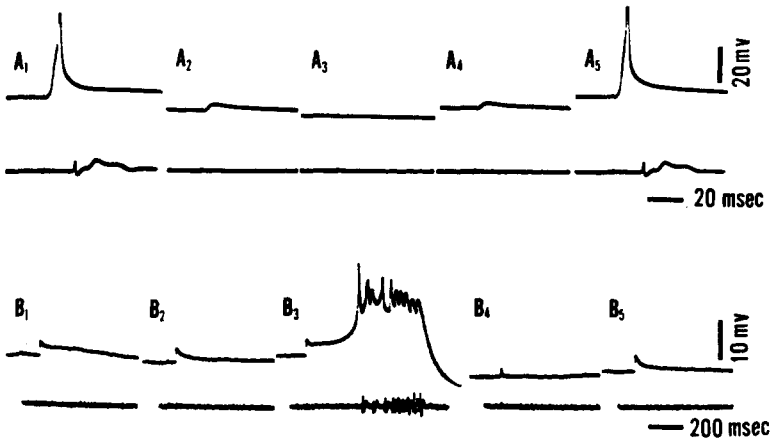

FIGURE 13. The small prepotentials and the state of the local system. All records were obtained with rostral stimulation. *A*, effects of hyperpolarization. Gc 4, cell 208. Two electrodes were inserted into the soma. A_1 and A_5, no hyperpolarization. A_2, A_3, and A_4, hyperpolarization was applied with various current intensities. The strength of rostral stimulation was constant. The distance between the rostral stimulating electrodes and the soma was 3.5 mm. *B*, the effect of spontaneous potential fluctuation on the threshold for rostral stimulation. A different preparation. Gc 6, cell 169. Successive records with a stimulus interval of 3.4 sec. The distance between the rostral stimulating electrodes and the soma was 240 μ.

prepotential was followed by a burst discharge on a slow wave of depolarization (Fig. 13 B_3). After that the membrane potential took a high value, and the rostral stimulation failed to elicit the small prepotential (B_4). On recovery of the membrane potential, the small prepotential reappeared. The experiment again shows that the threshold for the small prepotential depends on the membrane potential of the soma. Since the stimulus interval was about 3.4 sec in the above experiment, the effect of refractoriness may safely be ignored.

These experiments show that the small prepotential arises in some excitable membrane with which the soma membrane is electrically continuous. It seems unlikely that the small prepotential is a postsynaptic potential from the regulator nerves, which form synapses on the local system. Electrotonic coupling between the local system and the regulator nerves can be excluded

in the case of the inhibitor fibers and is very improbable in the case of the accelerator fibers (Watanabe, Obara, and Akiyama, in preparation). Furthermore, with repetitive rostral stimulation a summated small prepotential can be elicited, but it has neither inhibitory nor acceleratory effect on the pacemaker potential, in contrast to regulator nerve stimulation, the effect of which will be described in a later paper. In fact, the effect of the regulator nerves can be almost completely blocked by using sea water as the extracellular medium, because of its high magnesium content. The small prepotentials can be produced in sea water. It is thus concluded that the origin of the small prepotential is inside the local system.

Since in the pacemaker region the neurons in the local system are electrotonically coupled, it is possible that the small prepotential is a postsynaptic or a postephaptic potential generated by another neuron in the local system. This assumption seems to be improbable, however, because we have not obtained even one example in which one of the cells is stimulated without eliciting an all-or-none action potential in other cells, as well as an impulse in the ganglionic trunk recorded externally (see Fig. 11). The small prepotential could only be observed when a part of the nerve trunk was locally stimulated by the cut end of the insulated silver wire electrode.

Thus it is probable that the small prepotential is generated in a part of the neuron in which the recording electrode is inserted. We assume that it is a response of a dendrite of the cell from which the potential is observed. Since the dendrites have a very small size (usually less than 5 μ), their action currents generate very small electrotonic potentials in the soma membrane. Probably the dendrite action potential first stimulates the axon membrane, which has a lower threshold compared to that of the soma membrane, and the axon spike then invades the soma to cause a full sized soma spike.

DISCUSSION

Passive properties of the pacemaker neurons in the *Squilla* heart ganglion have been described. Although they are more or less similar to the properties of other nerve cells, there are several unique features. In the following discussion, some comparison will be made with the properties of other nerve cells.

The Excitability of the Individual Neurons

On axon stimulation, the soma action potential shows a step on its rising phase at a level of 20–25 mv above the resting level. The simplest interpretation is that the initial part (A spike) is an electrotonic spread of the initial segment action potential, and that with 20–25 mv depolarization the soma membrane is excited and its action potential makes up the main part, or the B spike. According to this interpretation the threshold of the soma membrane

is 20–25 mv. The firing level on weak current injection is about 17 mv in Gc 4 and about 7 mv in Gc 5. The difference indicates that the soma membrane has a higher threshold than that of the axon membrane. This property is observed in many other nerve cells, for example, the toad or cat motoneurons (Araki and Otani, 1955; Coombs, Curtis, and Eccles, 1957), the stretch receptor cell of a lobster (Edwards and Ottoson, 1958), although there are some exceptional neurons, for example, the supramedullary cells of the puffer (Bennett, Crain, and Grundfest, 1959).

In the motoneurons of toads and cats, direct stimulation produced a spike in the initial segment before the soma-dendritic membrane was excited (Araki and Otani, 1955; Frank and Fuortes, 1956; Coombs, Curtis, and Eccles, 1957). In the *Squilla* heart ganglion, weak direct stimulation initiates a spike in the trigger zone, but strong direct stimulation initiates a spike in the soma. This is a unique feature of this ganglion, and deserves some discussion.

It always takes some time to produce an action potential by injecting a square current pulse into a ganglion cell, because of the time constant and the response time of the soma membrane. During this period, the electrotonic spread of the stimulating current can elicit a spike in the initial segment and the spike can invade the soma. In motoneurons, the membrane of the initial segment emerges from the cell body as the axon hillock, so that geometrically the initial segment is a direct continuance of the cell body. Such a structure makes the attenuation of electrotonic spread very small, and the invasion of the IS spike very rapid.

In the *Squilla* heart ganglion, however, the arrangement of the membrane seems different from that in nerve cells such as the cat motoneuron, in that the high threshold membrane extends outside the soma, and therefore invasion by the IS spike takes some time, which allows the soma action potential to develop before the IS spike arrives at the soma on strong direct stimulation. A direct approach to determining the site of the trigger zone is to pick up the extracellular field near the soma (Edwards and Ottoson, 1958). Preliminary experiments indicated that in Gc 5 the trigger zone is located caudally about 0.5–1 mm away from the cell. The value seems to be acceptable, since Edwards and Ottoson found a distance of 0.5 mm from the cell in the lobster stretch receptor. In the molluscan ganglion cell Tauc (1962) found that on soma stimulation the spike is initiated at a point about 1.5 mm away from the soma.

Communications among Cells

In the pacemaker region, cells are electrotonically coupled and the activity of all-or-none impulses spreads from one cell to all. The spread of impulses is also found in caudal cells of the ganglion, but the electrotonic coupling, if any, is too small to be measured. In both instances impulses probably spread by

way of ephapses since the transmission goes both ways, there is only small indication of fatigue (Watanabe and Takeda, 1963), and changing the extracellular divalent ion concentrations has little effect (Watanabe et al., in preparation). We could not find any communication through synapses within the local system of this ganglion, although synapses do exist between the regulator fibers and the local system (Watanabe et al., in preparation). The small prepotentials on rostral stimulation were probably the spikes of dendrites rather than postsynaptic potentials.

In the lobster heart ganglion, spikes do not propagate across electrotonic connections although the spread of the slow potential can be observed (Watanabe, 1958). In the caudal cells of the *Squilla* heart ganglion, only the spread of spike potentials is observed. In the pacemaker cells, both exist. One might ask whether their routes are the same. The physiological data presented in this paper cannot solve the problem. However, histological data suggest that they are different, although the answer is not conclusive.

The spike spread must occur by way of the side-junctions, because it is found in both rostral and caudal parts of the ganglion. As pointed out in the histology section, side-junctions do not exist around the somata in the pacemaker region, even though the adjacent somata are connected by electrotonic connections. A tentative explanation for the above inconsistency is to assume that the structure of the electrotonic connection is too fine to be found by routine light microscope observation.

Alexandrowicz (1934) describes many dendrites and collaterals from the axon, especially at its proximal portion. It is possible that they form the routes for the electrotonic connection. Good physiological support for the above schema is supplied by a spontaneously active preparation. There slow potential does not spread into axons close to the soma, but does spread into adjacent cells several millimeters away (Watanabe et al., 1967).

Dendritic Spikes

In contrast to the findings in decapod heart ganglia (Hagiwara and Bullock, 1957; Bullock and Terzuolo, 1957; Watanabe, 1958; Hagiwara, Watanabe, and Saito, 1959), no evidence of synapses within the local system was found. The origin of small prepotentials is within a part of the membrane which is electrically continuous with the penetrated neuron. The small prepotentials are probably the activity of the dendrites. Potentials with similar properties are found in other nerve cells, e.g. the hippocampal neurons (Spencer and Kandel, 1961), chromatolyzed motoneurons (Eccles, Libet, and Young, 1958), and their origins are presumed to be sites somewhere in the dendrite arborizations.

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