

# Pacemaker Potentials for the Periodic Burst Discharge in the Heart Ganglion of a Stomatopod, *Squilla oratoria*

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**ABSTRACT** From somata of the pacemaker neurons in the *Squilla* heart ganglion, pacemaker potentials for the spontaneous periodic burst discharge are recorded with intracellular electrodes. The electrical activity is composed of slow potentials and superimposed spikes, and is divided into four types, which are: (a) "mammalian heart" type, (b) "slow generator" type, (c) "slow grower" type, and (d) "slow deficient" type. Since axons which are far from the somata do not produce slow potentials, the soma and dendrites must be where the slow potentials are generated. Hyperpolarization impedes generation of the slow potential, showing that it is an electrically excitable response. Membrane impedance increases on depolarization. Brief hyperpolarizing current can abolish the plateau but brief tetanic inhibitory fiber stimulation is more effective for the abolition. A single stimulus to the axon evokes the slow potential when the stimulus is applied some time after a previous burst. Repetitive stimuli to the axon are more effective in eliciting the slow potential, but the depolarization is not maintained on continuous stimulation. Synchronization of the slow potential among neurons is achieved by: (a) the electrotonic connections, with periodic change in resistance of the soma membrane, (b) active spread of the slow potential, and (c) synchronization through spikes.

## INTRODUCTION

Much valuable information is now available on the pacemaker activity of the myogenic vertebrate heart (Hutter and Trautwein, 1956). The origin of rhythmical burst activity, which appears frequently in the nervous system, seems to have been less well studied with modern techniques. In the crustacean heart ganglion periodic burst activities are continuously taking place (Maynard, 1955; Irisawa and Irisawa, 1957). This might then be a suitable material

for studying such a pacemaker. Progress has been hampered by the invisibility of pacemaker neurons in the hearts of decapods, which have been used almost exclusively as the experimental material. The present work has emerged from the finding that in the mantis shrimp, a stomatopod, the pacemaker cells are visible in the living preparation. Electrical activity from these cells was studied with intracellular electrodes. As will be shown, a slow potential change is continuously taking place in the soma or dendrites of the pacemaker neuron and this initiates periodic trains of spikes in axons.

#### METHODS

Somata in the pacemaker region of the heart ganglion of a stomatopod crustacean, *Squilla oratoria* de Haan, have been penetrated with intracellular electrodes to record membrane potential changes. The structure of the material is described in detail in the preceding paper (Watanabe, Obara, Akiyama, and Yumoto, 1967). The method of recording is also similar to that in the preceding paper.

Various authors disagree on the location of the pacemaker in the *Squilla* heart ganglion. Irisawa and Irisawa (1957) state, based on transection experiments, that the pacemaker is located at the thirteenth segment, which is near the caudal end of the heart. Shibuya (1961) and Watanabe and Takeda (1963) state, based on the electrophysiological determination of the direction of impulse propagation, that the pacemaker is in the rostral region. Brown (1964) states, based on the both types of experiments, that the bursts are initiated as frequently in the rostral as in the caudal end of the ganglion.

According to our own observation, the burst discharge invariably originates from the rostral region of the heart. This is based on the electrophysiological determination of the direction of impulse propagation. The observations were made on more than 100 isolated hearts and 5 hearts *in situ*. We observed, however, that a powerful secondary pacemaker exists at the caudal end of the heart. On transection of the heart at its middle portion, the direction of the impulse propagation in the caudal segment at once reversed from the rostrocaudal to the caudorostral direction. The same effect was also observed when an intentional injury was inflicted on the rostral region of the heart.

We conclude that under our experimental conditions in the *Squilla* heart the primary pacemaker is located in the rostral region of the heart, and if no injury is sustained by the preparation it always governs the whole heart. When the rostral pacemaker is injured, the secondary pacemaker in the caudal region of the heart at once takes over the driving function. In the present paper, all experiments were done on the primary pacemaker. Recordings were made from Gc 2-6. It was observed that the spikes start from Gc 1-5, although penetration of Gc 1-3 was difficult and we often had to estimate the starting point from records in Gc 4-6.

The pacemaker activity of a neuron was labile and tended to disappear while responses to stimulation persisted. It was especially difficult to maintain spontaneity with two electrodes in place. Often on insertion of the second electrode the spontaneous activity of the penetrated cell changed its type from pacemaker to follower. To

minimize the effect of injury, the tip of the microelectrode had to be invisible under the high magnification of an optical microscope. The resistance of the electrodes ranged between 50 and 150 M $\Omega$ .

To avoid the insertion of a second electrode we sometimes used a bridge circuit (Araki and Otani, 1955) to pass current through the cell membrane. With this technique, however, the electrode resistance often changed, and the balance of the bridge became uncertain to some extent.

Unless otherwise stated, the external solution was sea water. In some experiments, an artificial saline of the following composition was used: Na<sup>+</sup>, 450; K<sup>+</sup>, 15; Ca<sup>++</sup>, 10; Mg<sup>++</sup>, 20; Cl<sup>-</sup>, 525 (mmoles per liter solution). The pH was brought to 8.0 by adding a small amount (up to 1 %) of isotonic NaOH-H<sub>3</sub>BO<sub>3</sub> buffer or Tris-HCl buffer. The above formula is based on an analysis of serum (Lee and McFarland, 1962). The burst frequency is increased and the duration of the burst is decreased in the artificial saline compared with those in natural sea water. The experiments were performed at a room temperature of 18–22°C.

## RESULTS

### *Types of Spontaneous Activity*

When external electrodes pick up some spontaneous activity from the ganglion, the intracellular electrode always picks up some potential change from the penetrated neuron. This indicates that the excitation always spreads to all the neurons in the local system of the ganglion (Watanabe and Takeda, 1963).

The intracellular potential changes from the pacemaker cells vary extensively in their size and time course, and some sort of classification is necessary for their description. Mainly on the basis of the characteristics of the slow potential, we divide them into four types: (a) the "mammalian heart" type, (b) the "slow generator" type, (c) the "slow grower" type, and (d) the "slow deficient" type. It is stressed that the purpose of the classification is only for convenience of description. An actual potential change cannot be rigidly classified into one type. There are many intermediate forms. Furthermore, the type of activity of a neuron changes even during observation. This indicates that, at least under the present experimental conditions, a rigid correlation cannot be established between the type of activity and the anatomical location of cells.

Each of the four types is subdivided into two groups: spike initiators and spike acceptors. When the spike arises without distinct inflection from the preceding depolarization, the observed neuron is considered to be a spike initiator, and otherwise a spike acceptor. Whenever simultaneous recordings were made from two neurons, one a spike initiator and the other a spike acceptor, we found that the spike arose earlier in the spike initiator than in the spike acceptor.

**MAMMALIAN HEART TYPE** This type appeared only in fresh preparations. The activity could continue for hours, but once it ceased it rarely returned in its original form.

In Fig. 1 one example of the mammalian heart type spike initiator activity is presented. Corresponding to each burst in the extracellular records, a slow depolarization with superimposed spikes was observed. During the interburst period, the membrane was continuously depolarized. The first spike arose without a distinct inflection from the preceding depolarization, and then repolarized rapidly, but only to a level near the origin of the spike. The follow-

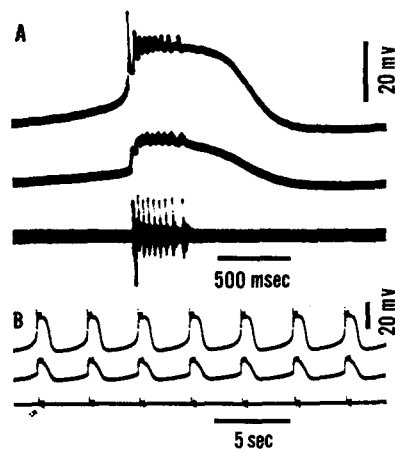


FIGURE 1. Spontaneous activity recorded simultaneously from two adjacent somata. Upper beam, Gc 5, cell 82. A mammalian heart type spike initiator. Middle beam, Gc 6, cell 83. A slow generator type spike acceptor. Lower beam, external recording. *A* and *B* came from the same preparation with different time and voltage scales. Distance between the somata was 3.9 mm. Cell numbers are the same as those of the preceding paper (Watanabe et al., 1967).

ing spikes had smaller peak values and less complete repolarization, and the average membrane potential formed a plateau. The duration of the plateau varied in different preparations, but was usually of the order of 1 sec. In some preparations the plateau persisted even after the burst of spikes was over, as seen in Fig. 1.

The designation of this type is, of course, based on the similarity in general appearance of the potential change to that of mammalian heart muscle, in spite of the accompanying train of spikes on the plateau of the slow potential. A marked difference between them is that in the *Squilla* heart ganglion the falling phase of the first spike does not continue monotonically to the plateau but instead there is a deep phase of relative hyperpolarization after the first spike. This suggests that in the heart ganglion the plateau is a phase of the slow potential which is a continuation of the interburst depolarization and which is more or less independent of the superimposed spike discharge, in contrast to the mammalian heart action potential, in which the plateau is a kind of after-potential of the initial spike.

The independence of the slow potential from the spike activity is best demonstrated by the appearance of the slow generator type of activity, but

some indication can also be seen in examples of the mammalian heart type activity. In Fig. 2 *A*, the rate of rise of the interburst depolarization increased once before the burst, but it again decreased to some extent, so that the slow potential was beginning to form a plateau before the spike discharge appeared. Such an upwardly convex curvature was often observed in the mammalian heart type activity, and it suggests that the distinction between the mammalian heart type and the slow generator type is not very clear, and that even in the former the whole activity can be interpreted as a superimposition of two distinct potential changes: slow potential and train of spikes.

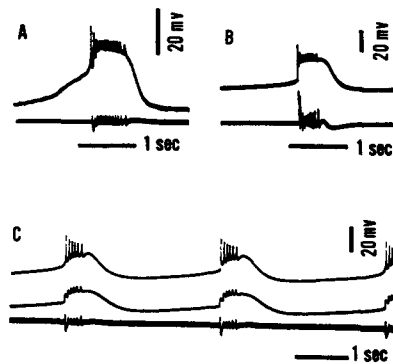


FIGURE 2. Various examples of the mammalian heart type spontaneous activity. In all records lower beams are external recordings. *A*, an upwardly convex prepotential. Gc 5, cell 80. *B*, a spike acceptor. Gc 5, cell 92. *C*, upper beam, Gc 4, cell 174. A mixed form of spike initiator and spike acceptor. Middle beam, Gc 5, cell 175. A slow generator type spike acceptor. Distance between somata was 4.8 mm.

The above description does not mean that the slow potential is not influenced by spikes. As shown in later sections, spikes can produce slow potentials under some conditions (Fig. 12–15). On the other hand, the slow potential is produced without any preceding spikes, and it has its own time course, even though the superimposed spikes can modulate its time course.

Fig. 2 *B* shows one example of the spike acceptor form of the mammalian heart type. Even though the spike arose with a sharp inflection, there was a certain amount of slow depolarization and a conspicuous plateau.

In Fig. 2 *C* an example is shown in which the foot of the initial spike changed back and forth between spike initiator form and spike acceptor form. Obviously such a change was due to critical competition with another pacemaker cell. The pattern of activity remained mammalian heart type, however.

**SLOW GENERATOR TYPE** When the slow component dominates the spontaneous potential change, the activity is classified as this type. In its extreme case, the slow potential is not accompanied by any spikes. Two such examples are shown in Fig. 3. In the preparation of Fig. 3 *A* recordings were made from three adjacent cells simultaneously. Every 5 sec, a slow rise and fall of the membrane potential were observed simultaneously in all three cells, with the largest amplitude in Gc 5. On every alternate phase of the depolarization,

a burst discharge of spikes was superimposed, but only in Gc 4 were the spikes of considerable size. On a rapid time base (not shown) it was seen that the spikes all started from Gc 4.

Comparison between Fig. 3  $A_1$  and  $A_2$  shows that the superimposed small spikes do not significantly change the time course of the slow potential in Gc 5 and 6. In Gc 4, however, the burst elicits a plateau in a later stage. The activity in Gc 4 belongs to the slow grower type, which is described in the next section.

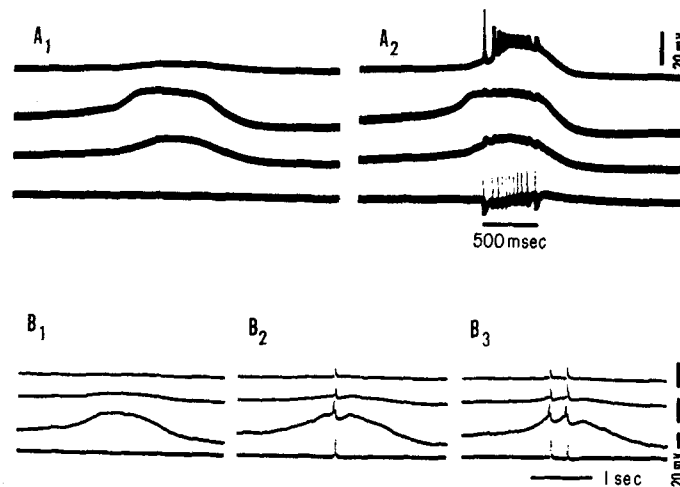


FIGURE 3. Two examples of the slow generator type of spontaneous activity. *A*, uppermost beam, Gc 4, cell 53. Second beam, Gc 5, cell 54. Third beam, Gc 6, cell 55. Lowest beam, external recording. The slow generator without spikes ( $A_1$ ) and the slow generator with spikes ( $A_2$ ) appeared alternately. The interval from  $A_1$  to  $A_2$  was shorter ( $4.98 \pm 0.05$  sec) than that from  $A_2$  to  $A_1$  ( $5.87 \pm 0.66$  sec). Distances, 6.2 mm between Gc 4 and Gc 5, and 6.4 mm between Gc 5 and Gc 6. *B*, uppermost beam, Gc 4, cell 101. Second beam, Gc 5, cell 100. Third beam, Gc 6, cell 99. Lowest beam, external recording. The interval between waves was about 10 sec, and spikes were superimposed irregularly on some of the depolarized phases. Distances, 3.4 mm between Gc 4 and Gc 5, and 5.0 mm between Gc 5 and Gc 6.

In Fig. 3 *B*, the focus of the slow potential is in Gc 6. Once in a while several spikes are superimposed on the depolarization. They were probably initiated in Gc 6.

The "slow generator without spikes" appeared only temporarily. In the case of Fig. 3 *A*, it appeared right after the penetration of the cells. Gradually every depolarized phase was accompanied by a number of small spikes. In the case of Fig. 3 *B*, this type of activity appeared in a later stage of the experiment. The spontaneity stopped once and after some time recovery commenced with this form of activity. In spite of its transient nature, the existence of this

type of activity indicates that the slow potential is independent of spike generation.

When spikes accompany this type of slow potential, it can be classified further into spike initiator or spike acceptor, as usual. We did not record, however, a demonstrable example of the spike initiator (possibly Gc 6 in Fig. 3 *B* is an example), since usually it is not to be distinguished from the mammalian heart type spike initiator (see Fig. 2 *A*). Examples of the spike acceptor are easily obtained. Usually the spikes are not full sized, but only

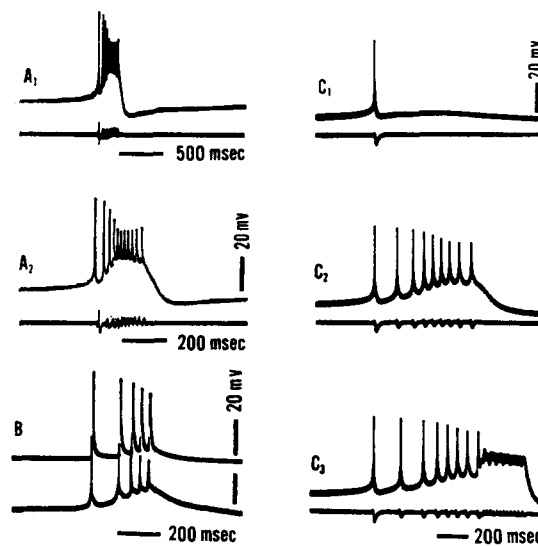


FIGURE 4. Examples of the slow grower type of spontaneous activity. *A*, upper beam, Gc 4, cell 26. Lower beam, external recording. Two records ( $A_1$  and  $A_2$ ) are shown with different time bases. Interval between bursts was about 9 sec. *B*, upper beam, Gc 4, cell 37. A slow deficient type spike acceptor. Lower beam, Gc 5, cell 36. A slow grower type spike initiator. Interval between bursts was about 15 sec. In the fresh stage of the preparation, the neuron showed a typical mammalian heart type activity, with an interval of about 5 sec. *C*, Upper beam, Gc, 5, cell 49. Lower beam, external recording. An irregular form. Three successive bursts are shown. The burst interval was not constant; from  $C_1$  to  $C_2$ , 3.3 sec, from  $C_2$  to  $C_3$ , 6.6 sec, and from  $C_3$  to the next, 8.2 sec.

small deflections, which are probably the electrotonic spread of action potentials in the axon (Fig. 3  $A_2$ , Gc 5 and Gc 6). On axon stimulation, a similar small spike is produced (see Fig. 14, Gc 6). Apparently the membrane is not electrically excitable, if excitability is defined only as ability to produce spikes. Spikes are usually produced in other neurons. This is an example of the dissociation of the pacemaker function. There will be further discussion on p. 857.

**SLOW GROWER TYPE** In Fig. 4, several examples of the slow grower type are presented. In this type, the interburst slow potential does not develop

very well, and the spikes start at a relatively small critical depolarization. The amplitude of the initial spike is large, but the plateau is at first small rising gradually to its peak only after an appreciable number of spikes have been produced. After the burst of spikes the plateau disappears rapidly. The interval between successive spikes is at first long, then gradually becomes shorter, whereas in the mammalian heart type the spike interval reaches a minimum after two or three discharges and then is prolonged.

The gradual buildup of the plateau suggests that in this type the plateau is mostly the result of the spikes. One indication is presented in Fig. 4 *C*. In this example the periodic burst activity was not regular. The number of spikes in a burst changed from 1 to about 20. The initial spike had almost the same shape in each burst. Slight differences in its after-potential determined the following time course. In Fig. 4 *C*<sub>1</sub>, the initial spike was followed by a small

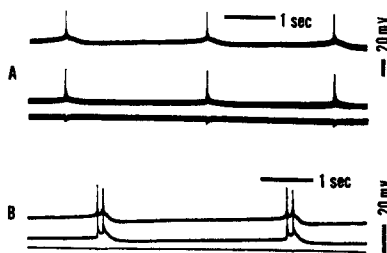


FIGURE 5. Two examples of the slow deficient type spontaneous activity. *A*, upper beam, Gc 4, cell 70. A spike initiator. Middle beam, Gc 5, cell 71. A spike acceptor. Lower beam, external recording. The distance between the somata was about 4 mm. *B*, upper beam, Gc 4, cell 93. A spike initiator. Middle beam, Gc 5, cell 92. A spike acceptor. In the fresh stage of the preparation, the neuron showed a mammalian heart type activity. The distance between the somata was 3.8 mm.

after-depolarization. In Fig. 4 *C*<sub>2</sub>, the after-depolarization produced the second spike, which added some amount of after-depolarization to the first one, leading to the third spike after a shorter interval. The later spikes were produced successively, with intervals still shorter than the previous ones, and the summated after-potentials formed the plateau. In *C*<sub>3</sub>, a larger plateau phase with a burst discharge of higher frequency followed.

The above interpretation is supported by the potential change shown in Fig. 3 *A*<sub>2</sub>, recorded from Gc 4. In this example, Gc 5 and Gc 6 were slow generators and Gc 4 was a slow grower. The focus of the slow potential was in Gc 5, Gc 4 receiving an electrotonic spread of the slow potential from Gc 5. If the potential attained threshold, a spike was initiated in Gc 4. In Gc 5 the time course of the slow potential changed very little, but in Gc 4 a conspicuous plateau appeared in the later stage of the burst. This must be the result of the repetitive spike activity.

As shown in later sections axon stimulation can produce slow potential in



pacemaker cells (Figs. 12 to 14). The same mechanism must exist in the spontaneously firing cell membrane, and the slow grower type can be interpreted on this basis.

**SLOW DEFICIENT TYPE** This type is characterized by the small amplitude and short duration of the plateau, and by the small number of spikes in a burst. Fig. 5 *A* is an example from a fresh preparation. There was only one spike in a burst.

This type often appeared in preparations following a temporary cessation of spontaneity. In Fig. 5 *B*, the activity of the preparation was of the mammalian heart type in the fresh state. During the experiment the spontaneous activity disappeared, and resumed with activity of the slow deficient type. Spontaneous activity in many preparations showed a similar time course. Apparently the plateau formation is more difficult to recover than the spontaneity itself.

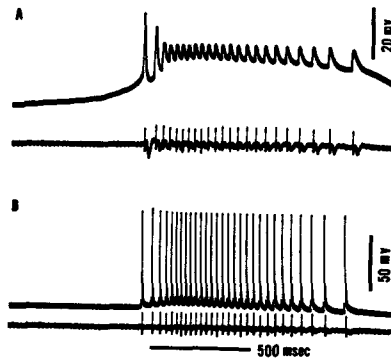


FIGURE 6. Spontaneous activities of a soma and one of its juxtaposed parallel axons. *A*, upper beam, Gc 5, cell 65. A mammalian heart type spike initiator. Lower beam, external recording. *B*, upper beam, the axon. Lower beam, external recording. Further descriptions in the text.

### *The Nature of the Slow Potential*

**AXON SPIKES** Successful penetration of the axon is far less frequent than that of the soma (seven examples during the period of experiment), but once the electrode slips into an axon, the recorded action potential is larger (50–90 mv) than that from the soma. The identification of the axon action potential is easily made from its rapid rising phase with no inflection (Watanabe and Takeda, 1963).

In the pacemaker region, another characteristic of the axonal activity is evident: axons do not produce any slow potential. Fig. 6 shows one example. A mammalian heart type activity is recorded from the soma of Gc 5. The activity of an axon from the same ganglion is recorded in *B*. The recording sites of *A* and *B* were very close to each other (less than 50  $\mu$ ). The record in Fig. 6 *B* must have come from a parallel axon, since the electrode was lateral to the cell soma.

The membrane potential of the axon did not show any slow potential

change before the burst of spikes started. During the burst, a slight elevation of the base line was noticeable, but this is to be ascribed to the summated after-potentials of spikes. The slow potential of the soma does not spread into the axon at all. The parallel axons are electrotonically isolated from the soma even when the distance between them is very small.

This isolation can be confirmed by passing current from the electrode in the soma and by recording potential change from the parallel axon. The experiment showed no electrotonic spread, no matter what the direction of the current, but with outward current above a critical intensity a conducted spike was produced in the axon. Exactly the same result was obtained when current was injected into the axon. The same result has also been obtained in the caudal part of the ganglion (Watanabe and Takeda, 1963).

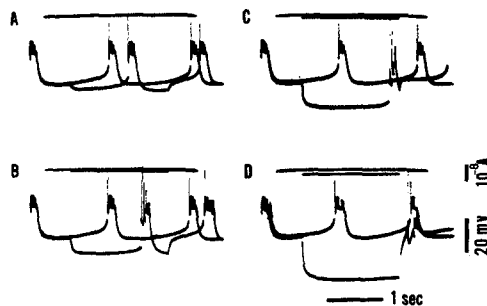


FIGURE 7. Effects of hyperpolarization on the mammalian heart type spike initiator activity. Gc 5, cell 218. Outside medium was the artificial saline. A separate current electrode was inserted into the soma. Upper beam, polarizing current. Lower beam, membrane potentials of the soma. Two successive sweeps were triggered by the initial spike of bursts and superimposed with and without current application.

The electrotonic isolation of the parallel axons from the soma is consistent with the histological finding (Watanabe et al., 1967), which indicates that the proximal part of the axon does not form side-junctions with the parallel axons. Since the side-junction is supposed to be a kind of ephapse, lack of side-junctions around the soma is a necessary condition for the above isolation.

On the other hand, it is known that the cell somata are electrotonically connected with each other across a distance of several millimeters (Watanabe et al., 1967), and the spontaneous slow potential spreads from one cell to another (Fig. 3). Thus we conclude that two systems exist in the rostral part of the ganglion. One of these, the axon system, is composed of a group of axons which are far from their somata. They are connected with each other via side-junctions so that spikes spread all over the system. The soma system is composed of a group of somata with their dendrites. They are coupled with each other via dendrodendritic electrotonic connections, so that the slow potential

spreads from one cell to another. The two systems exchange impulses but not the slow potentials, since they are electrotonically isolated.

Evidently the spontaneous slow potential originates from the soma system, and not from the axon system. In other words, the pacemaker potential is produced in the soma or dendrite membrane, or in both, but not in the axon membrane.

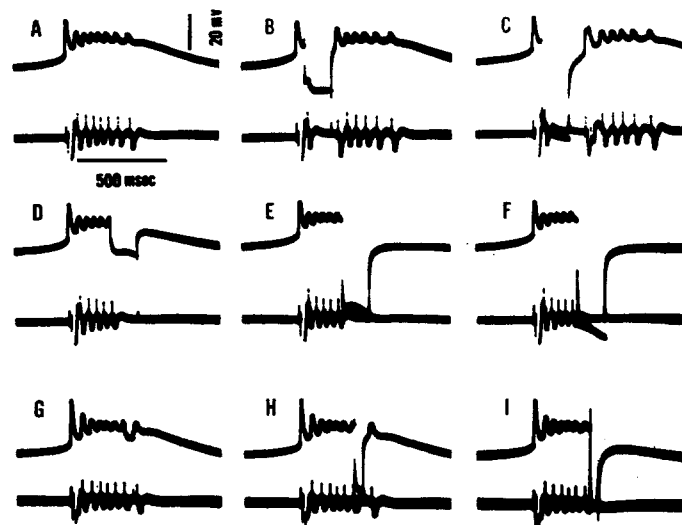


FIGURE 8. Effect of a brief hyperpolarizing pulse on the tail of the plateau. Upper beam, Gc 5, cell 58. A mammalian heart type spike acceptor. A bridge circuit was used to pass current. Lower beam, external recording. *A*, control. Interval between bursts was 3.5 sec. *B* and *C*, current pulses were applied at the initial stage of the plateau. *D* to *F*, current pulses were applied at later stage of the plateau. *G* to *I*, briefer pulses were used.

**EFFECT OF HYPERPOLARIZATION** Hyperpolarization impedes the development of the slow potential. When sufficiently intense it inhibits generation of the slow potential completely. Fig. 7 shows one example. With a hyperpolarizing current pulse of about  $3 \times 10^{-9}$  A, the pacemaker activity was completely suppressed during a pulse of about 1.8 sec (Fig. 7 *D*). With weaker currents a spontaneous burst appeared after some delay and took place with more or less sharp inflection from the base line, indicating that the first action potential was a conducted one (Fig. 7 *A-C*). The initiating point of the spike must have shifted to a region where the applied hyperpolarization was less effective.

This experiment then shows that the slow potential is not an electrically inexcitable synaptic potential, but is a kind of electrically excitable response of the membrane (Grundfest, 1959).

**ABOLITION OF THE PLATEAU** By applying a brief hyperpolarizing pulse at a certain phase of the plateau, the later part of the plateau can be abolished. Fig. 8 presents one experiment. Abolition was not possible with the strongest hyperpolarizing pulse employed at the initial stage of the plateau (Fig. 8 *B* and *C*). When applied at later stages, abolition was observed, but the pulse had to be so strong that the hyperpolarization attained a level far below the resting potential (Fig. 8 *E*, *F*, and *I*). Thus current strength necessary for abolition of the plateau is unusually high compared with that necessary in other materials (Weidmann, 1951; Tasaki, 1956; Tasaki and Hagiwara, 1957). The discrepancy may be due to some difference in structures of the materials rather than in their membrane characteristics. In the present material the pacemaker potential develops in each cell independently, although synchroni-

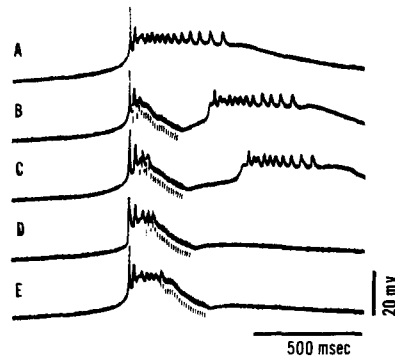


FIGURE 9. Abolition of the plateau by inhibitory fiber stimulation. Gc 5, cell 182. A mammalian heart type spike initiator. Outside medium was the artificial saline. *A*, control, interval between bursts was about 5.6 sec. *B* to *E*, shocks were applied to an  $\alpha$  nerve (an inhibitory regulator nerve) with a frequency of 100/sec for about 200 msec at different stages of the plateau.

zation mechanisms exist (see p. 855). Even when the hyperpolarization in one soma attains a critical value for abolition, the hyperpolarization is less in other somata because of the attenuation of the electrotonic potential.

According to the above suggestion, if many cells are simultaneously hyperpolarized, abolition should take place with a relatively small amount of hyperpolarization. With current injection this is technically impractical, but a similar effect can be produced by applying tetanic stimulation to an inhibitory regulator nerve to the ganglion. The effect of inhibitory nerve stimulation will be described in detail in a later paper. At this time it is sufficient to note that the inhibitory impulse produces an IPSP with an equilibrium potential near the resting level, and that the same inhibitory fiber innervates many adjacent cells at the pacemaker region. Fig. 9 presents an experiment in which a train of stimuli was applied to an inhibitory nerve when the spontaneous slow potential was at the plateau stage. When the inhibitory stimulation was applied early in the plateau, abolition did not take place and the plateau developed again after the stimulation. But when the stimuli were applied later the plateau could be abolished. Since the repolarization took place in every cell in the pacemaker region, the effect was spatially uniform,

and the abolition could be achieved with a smaller imposed potential change in the hyperpolarizing direction.

**MEMBRANE RESISTANCE** When the cell membrane is spontaneously producing the slow potential, the membrane resistance increases on depolarization. In Fig. 10 one experiment is shown. The outside medium was the artificial saline, which increased the burst frequency and decreased the duration of the plateau. Two electrodes were inserted into Gc 4. Repetitive application of the hyperpolarizing square pulse produced a continuous series of electrotonic potentials, which, however, did not reach a steady value. The increase in amplitude of the electrotonic potential indicates that a resistance change took place on depolarization.

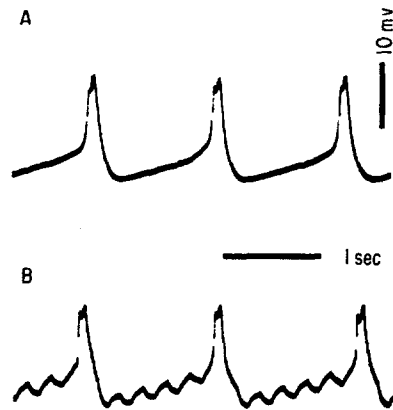


FIGURE 10. Periodic change in amplitude of the electrotonic potential. Gc 4, cell 143. Outside medium was the artificial saline. In a burst there were two spikes, which are not reproduced in the figure, but which made the over-all amplitude of the potential change about 40 mv. *A*, control, no current injection. *B*, current pulses of about 250 msec were injected periodically through a separate current electrode, resulting in a series of electrotonic potentials.

**DIRECT CURRENT INJECTION** Hyperpolarizing current increases the spike height and interburst interval. Depolarizing current decreases the spike height and interburst interval. Fig. 11 shows the interval change. When the current is applied, the first interval changes most prominently. Then the interval gradually returns towards a steady value (Fig. 11 *A*<sub>1</sub> and *A*<sub>2</sub>). The steady value of the interval is plotted against applied current intensity (Fig. 11 *B*). In this example about an 18% change took place on application of a current of  $10^{-9}$  A.

The number of spikes in a burst changes on current application, but the direction is not consistent. In this example hyperpolarizing current decreased the number of spikes (Fig. 11 *A*<sub>2</sub>), but depolarizing current did not increase the number (Fig. 11 *A*<sub>1</sub>). The tendency depended on the preparation. These diverse results can be explained by the fact that two counteracting tendencies are produced by current application. With increase of burst frequency the plateau duration and the spike number usually decrease (Brown, 1964). On the application of depolarizing current, therefore, the number of spikes tends

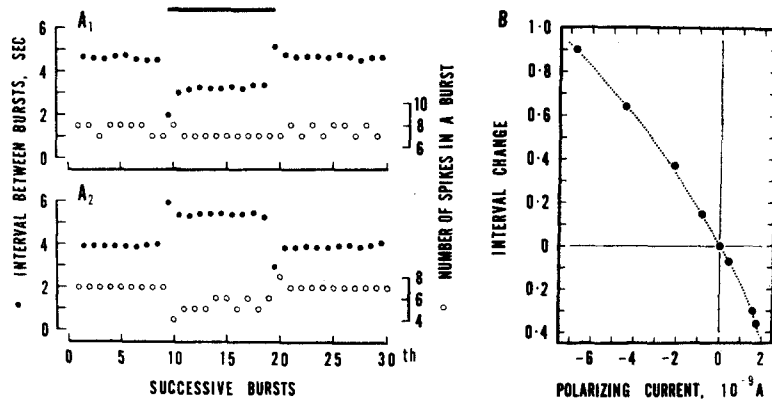


FIGURE 11. Effects of direct current application on the burst frequency. Gc 4, cell 58. The bridge circuit was used for current application. *A*, successive burst intervals (filled circles) and the number of spikes in a burst (open circles) are plotted. The bar at the top of the figure indicates the period of current application. *A*<sub>1</sub>, depolarizing current of  $1.54 \times 10^{-9}$  A was applied. *A*<sub>2</sub>, hyperpolarizing current of  $2.16 \times 10^{-9}$  A was applied. *B*, interval change at the final stage of current application is divided by the control interval and plotted against the intensity of the polarizing current.

to decrease. On the other hand the depolarizing current tends to increase the number of spikes since a smaller amount of the slow potential is enough to elicit spikes.

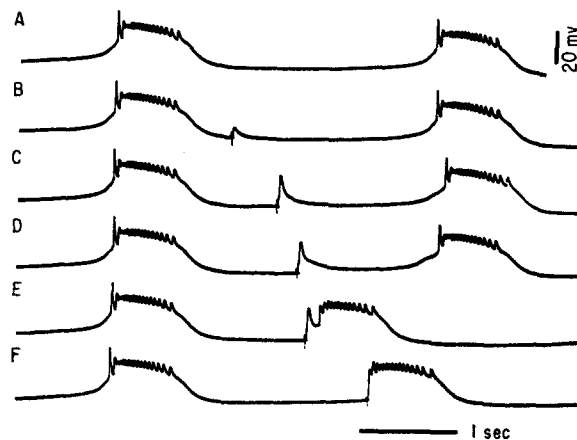


FIGURE 12. Effects of a single stimulus to the axon on the slow potential of a pacemaker cell. Gc 5, cell 75. A mammalian heart type spike initiator. *A*, control. *B* to *F*, single stimuli were applied to a caudal part of the ganglionic trunk.

#### *Initiation of the Slow Potential by Axon Stimulation*

**SINGLE STIMULUS** When the soma is producing the spontaneous slow potential, the effect of a single stimulus applied to the ganglion trunk is differ-

ent according to the phase of the spontaneous activity. Fig. 12 shows one example. When the stimulus was applied within 2 sec after the first spike of the preceding burst, the response was a small deflection of the beam which probably was an electrotonic spread from an axon spike (Fig. 12 *B-D*). The effect on the following burst was a slight prolongation of the burst interval, as most clearly seen in Fig. 12 *C*. When the stimulus was applied more than 2 sec after the first spike of the preceding burst, the stimulus evoked a burst. The elicited burst did not show the prepotential (the slow depolarization), and the initial part was quite unlike that of the natural burst, but in length

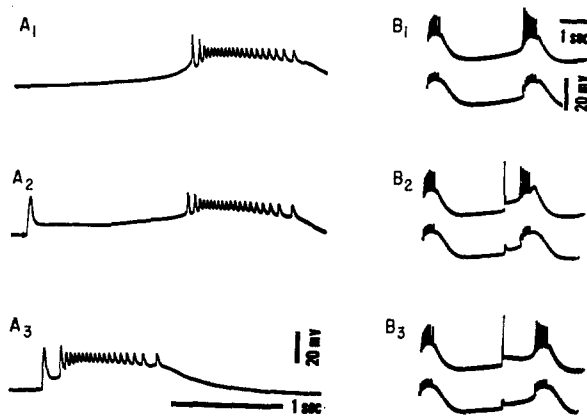


FIGURE 13. Slow potentials evoked by a single stimulus to the axon. *A*, Gc 5, cell 65. A mammalian heart type spike initiator. *A*<sub>1</sub>, control. The burst interval was about 6.2 sec. *A*<sub>2</sub> and *A*<sub>3</sub>, single stimuli were applied at the caudal part of the ganglion at 4.77 sec (*A*<sub>2</sub>) and 4.91 sec (*A*<sub>3</sub>) after the initial spike of the preceding burst. *B*, another example. Upper beam, Gc 4, cell 174. Lower beam, Gc 5, cell 175. *B*<sub>1</sub>, control. *B*<sub>2</sub> and *B*<sub>3</sub>, single stimuli were applied at the caudal part of the ganglion about 2.7 sec after the initial spike of the preceding burst.

and shape the plateau was very similar to the natural one. The elicited burst set the rhythm of the following bursts.

When the stimulus is applied at a critical interval, the response is often a single spike with a long lasting after-depolarization which can continue into the prepotential of the following burst. Fig. 12 *D* shows one example, but more prominent examples are collected in Fig. 13. In Fig. 13 *A*, the critical interval for evoking the burst was 4.9 sec (*A*<sub>3</sub>). At 4.8 sec, a stimulus caused a spike with longlasting after-depolarization (*A*<sub>2</sub>). Fig. 13 *B* shows an example with simultaneous recording from Gc 4 and Gc 5. The long lasting depolarization was observed in both cells (*B*<sub>2</sub> and *B*<sub>3</sub>).

The above results indicate that a single spike can evoke a slow potential or a burst, if the stimulus is applied at a sufficient time after the preceding burst.

**REPETITIVE STIMULI** When the axon is repetitively stimulated, the slow potential is evoked even at a short time after a preceding burst. Fig. 14 shows one example. A single stimulus, applied about 3 sec after the initial spike of the preceding burst, produced an abortive spike (Fig. 14 *B*). No slow potential was produced. In Fig. 14 *C*, six stimuli were applied to the axon at intervals of 0.1 sec. The third and the fifth stimuli were not effective because they col-

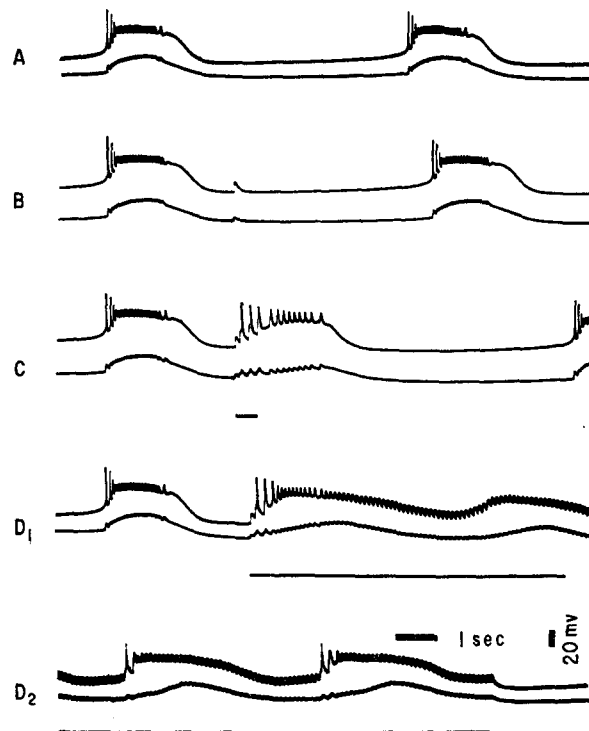


FIGURE 14. Slow potentials evoked by repetitive stimuli to the axon. Upper beam, Gc 5, cell 104. A mammalian heart type spike initiator. Lower beam, Gc 6, cell 105. A slow generator type spike acceptor. Distance between the somata was 5.7 mm. Stimuli were applied at a caudal part of the ganglion with a frequency of 10/sec. Period of stimulation is indicated by bars below the records. *A*, no stimulus. *B*, a single shock. *C*, six shocks. *D*<sub>1</sub> and *D*<sub>2</sub>, a continuous record with a period of stimulation of about 12 sec.

lided with impulses evoked in Gc 5. The number of effective stimuli was therefore four. After the stimuli the base line continued to rise and formed a plateau with many small spikes on its top. The evoked burst set the rhythm of the following slow potential.

Fig. 14 *D* shows an example in which the period of repetitive stimulation was further extended. The evoked slow potential showed an undulatory time course, with a peak interval of about 5 sec. The interval was smaller than the spontaneous burst interval of about 7 sec (Fig. 14 *A*). The experiment indi-



cates that a series of spikes can evoke the slow potential, but cannot maintain the plateau. The membrane has its own rhythmical property, which can be evoked and modified, but cannot be suppressed, by spikes.

Experiments on the effect of axon stimulation were all performed in sea water. In this medium excitation of the regulator fibers in the ganglionic trunk hardly affects the activity of the local system. Strong repetitive stimuli applied to accelerator nerves rarely produce any effect in sea water, although in the artificial saline the effect is immediately evident. The lack of after-effects after tetanic stimulation (Fig. 14  $D_2$ ) also supports the interpretation that an accelerative effect from the regulator fiber excitation has nothing to do with the observed slow wave, since the accelerative effect in the artificial saline has a prominent and long lasting after-effect (Watanabe et al., in preparation).

#### *Synchronization of the Slow Potential among Neurons*

When the recording is made from two somata simultaneously, slow potentials are observed in each of them with good synchronization. Since electrotonic connections allow transmission of the slow potential from one soma to another, at least a part of the synchronization can be explained on this basis. However, the rates of depolarization during the interburst period in two adjacent cells are often too close to be explained by the electrotonic connection only. For example, in Fig. 13  $B_1$ , the rate of depolarization at the middle stage of the interburst period is 2.9 mv/sec in Gc 4 and 2.2 mv/sec in Gc 5. The ratio is about 1.3, which is certainly out of the range of the attenuation ratios observed in resting cells (Watanabe et al., 1967). In this preparation, a third electrode insertion was made into Gc 5, after the records of Fig. 13  $B$  had been taken. The rate of depolarization was then 0.64 mv/sec in Gc 4 and 1.32 mv/sec in Gc 5. The ratio was 2.1. The attenuation ratio of the electrotonic potential was measured by injecting current into Gc 5, and it was found to be 3.7.

These discrepancies indicate that slow potential synchronization cannot be explained simply by the passive spread of the slow potential which is produced in a single neuron. The slow potentials are actively produced in many neurons in the pacemaker region, and some mechanism causes them to synchronize. In the following, some possible mechanisms of control will be analyzed experimentally.

**SYNCHRONIZATION THROUGH SPIKES.** Since the spike potential can evoke slow potentials (see Figs. 12 to 14), the synchronization of the slow potential can be achieved through spikes. This is especially important for the plateau formation. In Fig. 3  $A_1$ , Gc 3 (upper beam) showed only a very small depolarization. In Fig. 3  $A_2$ , spikes were elicited, and Gc 3 showed a large plateau, which must have been elicited by spikes.

In Fig. 15, another example is presented. A slow depolarization of about

40 mv amplitude appeared periodically, with an interval of about 9 sec. The pacemaker activity of the penetrated cell was of the type slow generator without spikes. A train of abortive spikes also appeared periodically, but with an

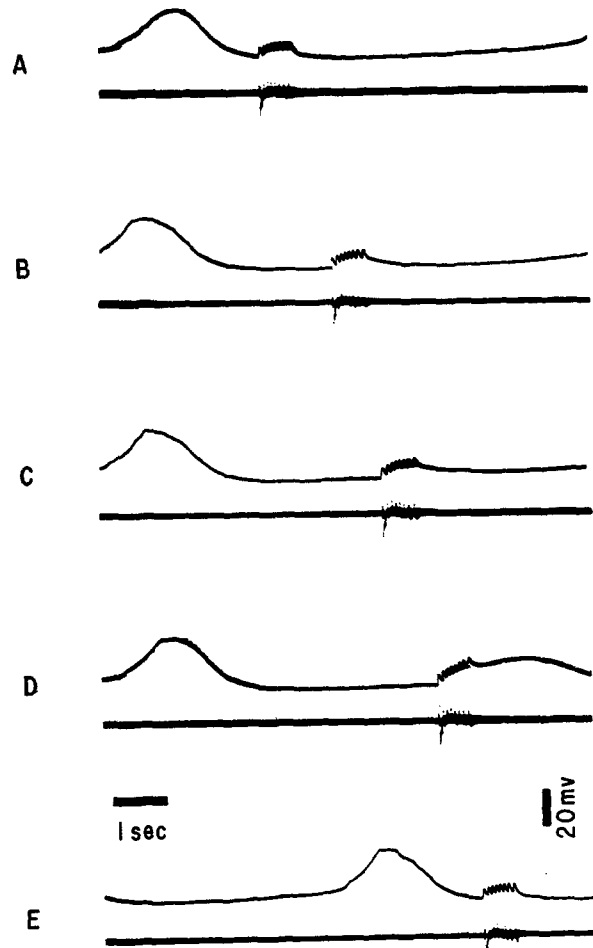


FIGURE 15. Interaction of two pacemakers through spikes. Upper beam, Gc 6, cell 106. A slow generator type spike acceptor. Lower beam, external recording. All records were spontaneously produced (no stimuli). The original continuous record was cut into 9 sec units and vertically arranged to show the periodicity of the slow depolarization. In *D* and *E* the periodicity is disturbed when impulses are received from another pacemaker.

interval of about 10 sec. The two rhythms were independent for a while (*A-C*), but when the train of spikes arrived at the soma about 5 sec after the preceding depolarization, the spike train was followed by a slow depolarizing wave (*D*). The rhythm of the slow depolarization was shifted (*E*) by the "extra" slow depolarization evoked by the spike train, but the rhythm of the spike train

was not. In this ganglion, then, a second pacemaker, which was not influenced by the slow potential of the penetrated cell, produced the train of spikes and sent it to the penetrated cell. The spikes can produce the slow potential most effectively when they arrive at the soma some time after the preceding depolarization (Fig. 12). Unless the slow potential was produced within the cell, the spikes could not have changed the rhythm of slow potential in the penetrated cell.

In this example the synchronization of the two pacemakers could be achieved only temporarily, because the second pacemaker had a lower frequency than the first one. If the second pacemaker had possessed a higher frequency, it would have dominated the first pacemaker completely, and the penetrated cell would have been a simple follower with a prominent slow potential.

**SLOW GENERATOR SPIKE INITIATOR COUPLING** The spread of the slow potential through electrotonic connections is a fundamental means of achieving synchronization of the slow potential among neurons. Probably the significance of the electrotonic connection can best be demonstrated by a form of the pacemaker which is made up of two neurons: a slow generator and a spike initiator. Fig. 16 presents one example. In this preparation, Gc 5 was a spike initiator of the slow grower type ( $A_1$ ). Gc 6 was a spike acceptor of the slow generator type ( $A_2$ ). When two potential changes were simultaneously recorded and superimposed, we found that the amplitude of the slow potential in Gc 6 was far larger than that in Gc 5 at the initial phase of depolarization ( $A_3$ ). The prepotential in Gc 5 could be interpreted as a mere electrotonic spread from the prepotential of Gc 6, since the ratio of depolarization rates was more than 5. However, the threshold of the soma membrane of Gc 6 was apparently very high, and Gc 6 did not initiate spikes. With a rapid time base (Fig. 16 *B*), a definite delay was found between spikes in Gc 5 and Gc 6. Thus Gc 6 was a focus of the slow potential, but the spikes started from Gc 5.

In this example, different functions of the pacemaker were allocated to two neurons. The frequency of the heartbeat was determined by the rhythm of the slow potential, and the slow generator controlled it. The intensity and duration of each heartbeat were determined by the spike frequency and the duration of each burst, and the spike initiator controlled them.

**ACTIVE SPREAD OF SLOW POTENTIAL** As shown in Fig. 3 *A*, synchronization of the slow potential can be achieved without spikes, and the amplitudes of the slow potentials in adjacent cells are sometimes too close to each other to be explained by the electrotonic connection only. One factor which contributes to the spread of the slow potential is the periodic resistance change of the membrane. As shown in Fig. 10, membrane resistance of a pacemaker neuron increases on depolarization. When a pacemaker neuron produces a

slow depolarization, it spreads to adjacent neurons, and depolarizes the latter; then the spread becomes more efficient because of less attenuation in the depolarized phase.

However, the observed attenuation is sometimes even smaller, and we are forced to assume that the slow potential itself can propagate from one cell to another actively. Such an assumption is not unreasonable, since we could find examples in which the electrotonic potential spreads from one cell to another with augmentation rather than with attenuation. Fig. 17 shows one example.

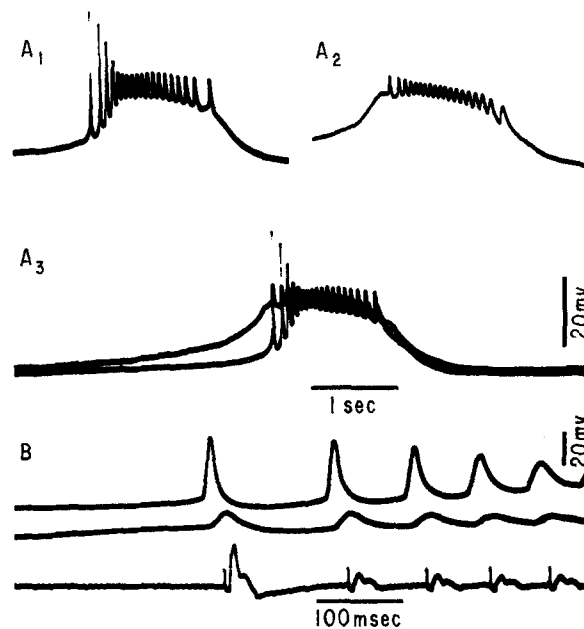


FIGURE 16. A slow generator spike initiator couple. All records are from the same preparation. Spontaneous activities.  $A_1$ , Gc 5, cell 97. A slow grower type spike initiator.  $A_2$ , Gc 6, cell 98. A slow generator type spike acceptor.  $A_3$ , activities of the two cells were simultaneously recorded and superimposed.  $B$ , upper beam, Gc 5. Middle beam, Gc 6. Lower beam, external recording. Time base is expanded ten times. Burst interval, 7.9 sec. Distance between somata, 5.1 mm.

In this preparation, a polarizing current pulse was injected into Gc 4 and its electrotonic spread was observed from Gc 5. At this stage of the experiment, the ganglion was not spontaneously producing spikes, but the membrane potential of Gc 5 was spontaneously fluctuating within 2 or 3 mv. When the current pulse was applied at the hyperpolarized phase, the usual attenuation of the electrotonic potential was observed between Gc 4 and Gc 5 (Fig. 17  $A_1$ ). However, when the current pulse was applied at the depolarized phase of the membrane potential in Gc 5, the electrotonic potential fell only after a long lasting plateau (Fig. 17  $C$ ), or it did not fall but continued to depolarize

to lead a burst discharge (Fig. 17 *A*<sub>2</sub>). At such times the membrane potential in Gc 4 had already returned to the original level, and thus the voltage gradient between the two cells was reversed.

The above phenomenon indicates that the applied current pulse triggered the slow potential in Gc 5. Undoubtedly such a phenomenon only appears when the recipient cell is in the depolarized state and is almost ready to produce its own slow depolarization. Thus the active spread of the electrotonic potential takes place only when the membranes of the different somata have similar properties and their intrinsic rhythms have frequencies close to each

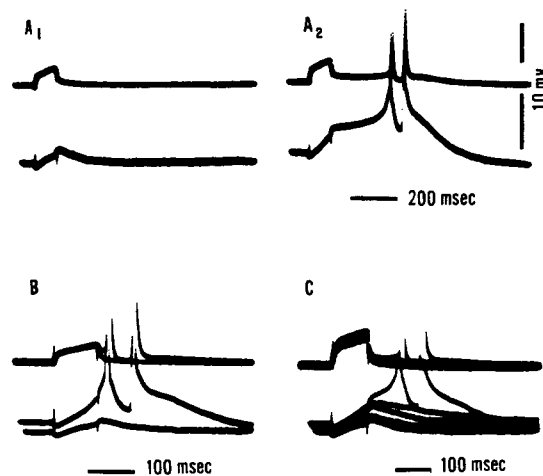


FIGURE 17. Active spread of the electrotonic potential. Upper beam, Gc 4, cell 41. Lower beam, Gc 5, cell 42. Polarizing current pulse of about  $1.5 \times 10^{-9}$  A intensity and 0.1 sec duration was injected into Gc 4 with the bridge circuit. The membrane potential of the Gc 5 was at a hyperpolarized level in *A*<sub>1</sub>, and at a depolarized level in *A*<sub>2</sub>. In *B* responses under the two conditions are superimposed. In *C* several traces with various membrane potential levels of Gc 5 are superimposed, showing that various responses were obtained with current pulses of the same intensity. Voltage calibrations in *A*<sub>2</sub> are applicable to all the records. Distance between the somata 3.4 mm.

other. When such conditions are fulfilled in adjacent somata in the pacemaker region, the spontaneously produced slow potential can also trigger the slow potential in adjacent cells, and the synchronization of slow potentials can thus be achieved.

#### DISCUSSION

It was found that the slow potential is continuously produced in somata in the pacemaker region, and that the periodic burst discharge in the ganglion is generated from the slow potential.

The occurrence of slow potentials in the crustacean heart ganglia has been

reported several times. With surface electrodes Matsui (1955) found slow potentials with superimposed spikes from the lobster heart ganglion. With this recording method it is difficult to analyze the nature of the potentials in detail, but at least some of them must be slow potentials similar in nature to those described here, although some of them may have been summated synaptic potentials. With intracellular electrodes Hagiwara and Bullock (1957) and Bullock and Terzuolo (1957) recorded a type of spontaneous slow potential from follower cells (followers with spontaneity) of heart ganglia in decapods. Watanabe (1958) also recorded slow potentials from follower cells of a lobster heart ganglion after anesthetizing the pacemaker cells. These potentials must be similar in nature to those described in this paper and in fact their shapes are similar to those of the slow deficient type activity of the *Squilla* heart pacemaker. In decapods, however, recordings were made from follower cells and their slow potentials may be regarded as "pathological" (Hagiwara, 1961). In the present material, the recordings were made from "physiological" pacemakers, and the pacemaker potential was consistently more stable and prominent.

Spontaneous slow potentials are most commonly observed in nervous systems of higher orders. Since, however, the recordings are usually made with extracellular electrodes, their nature is still not well analyzed. Sometimes the slow potential seems to evoke burst discharges. Adrian and Buytendijk (1931) observed a slow rhythmical potential change in an isolated goldfish brain and advanced the hypothesis that the recorded slow potential is due to a gradual depolarization and recovery of the nerve cells, and that the depolarization triggers the rhythmic burst discharge in the respiratory motor axons. Apart from the effector, which is heart muscle in the present material, their hypothesis is in perfect agreement with what we have observed in the *Squilla* heart ganglion.

Much more work is necessary for elucidating in detail the nature of slow potentials. The results obtained in this paper can be summarized as follows:—(a) The slow potentials are not single, or summated, synaptic potentials. (b) They can develop without spikes. (c) Axon stimulation or direct stimulation affects the slow potential, but it has to be applied at a proper phase of the spontaneous activity. (d) The slow potential has several characteristics which are also found in the mammalian heart action potential, e.g. abolition with hyperpolarizing pulse (Weidmann, 1951), and periodic change of the membrane resistance (Weidmann, 1951).

The last fact suggests that we can construct a theory of the pacemaker potential in terms of the ionic theory, as has been done for the mammalian heart action potential by Grundfest (1961) and by Noble (1962). One cannot exclude, however, other possibilities, as for example, some contribution of

metabolism, an effect of an accumulation of some substance within or without the cell membrane, or both of these.

The relationship of spikes to the slow potential indicates that the structure of the neuron is not homogeneous in this material but is composed of at least two components, one for the slow potential and another for the spikes. A reasonable assumption is that the axon generates spikes but not slow potentials whereas the soma and dendrites are responsible for producing slow potentials, although spikes can also be elicited in soma and dendrites with higher threshold and smaller amplitude (see the discussion in Bullock and Horridge, 1965, p. 128). This is supported by the observations that bursts in the axon do not show any slow potential (Fig. 6), and that the soma spikes are smaller than the axon spikes. Possibly similar structural heterogeneity is also responsible for such compound type action potentials as the burst discharge found in the hippocampal neurons (Kandel and Spencer, 1961), and the action potential in some smooth muscle (Irisawa and Kobayashi, 1962). Oikawa (1962) could produce a compound action potential by injecting TEA inside a part of the squid giant axon, to create an artificial heterogeneity in an excitable cell.

Synchronization exists among slow potentials in the pacemaker neurons, and several ways have been found to achieve this: (a) the electrotonic connections, (b) periodic change in membrane resistance (Fig. 10), (c) the active spread of the slow potential (Fig. 17), and (d) the generation of the slow potential through spikes (Fig. 14). In this particular material, probably the last factor is most important for the synchronization, because the spike spreads into all the neurons in the local system. It is possible, however, that in some other materials the spread of slow potential plays a major role, as was found in follower cells in the lobster heart ganglion (Watanabe, 1958).

We wish to express our gratitude to Drs. H. Grundfest and M. V. L. Bennett for correcting the English in the manuscript and making suggestions.

This study has been supported by a grant from the Ministry of Education in Japan.

*Received for publication 30 March 1966.*

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