The Spread of Excitation among Neurons in the Heart Ganglion of the Stomatopod, *Squilla oratoria*

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ABSTRACT Neurons in the heart ganglion of the mantis shrimp (a stomatopod crustacean) are functionally tightly linked together. The extracellular action potential from the whole trunk very often shows a complex form, but the response is all-or-none to the applied stimulus, indicating that the excitation in one neuron spreads very rapidly to all others. Application of isotonic $MgCl_2$ solution or repetitive stimulation sometimes separates the spike into its components. The resting potential of the soma membrane is 50 to 60 mv. External stimulation elicits a spike of 60 to 80 mv amplitude with a step on its rising phase. Hyperpolarization reveals one more inflection on the rising phase. These inflections divide the soma action potential into three parts, A_1, A_2, and B spikes in that order from the foot. The B spike disappears on increasing the hyperpolarization, but A_1 and A_2 remain, indicating that B originates from the soma membrane, whereas A_1 and A_2 originate from the two axons of the bipolar cell. Thus the impulse invades the soma from two directions, one from the stimulated side, the other from the other side *via* the "parallel axons" and the "side-connections;" the latter are presumed to interconnect the axons. When the parallel axons are cut, conduction takes place across the soma with a greatly reduced safety factor and a prolonged conduction time. Neuron-to-neuron transmission takes place in either direction.

INTRODUCTION

Crustacean hearts are known to be innervated by their heart ganglia, which are buried in the myocardial tissue and which generate the spontaneous discharge to drive the myocardium into rhythmic contractions (Alexandrowicz, 1932, 1934; Maynard, 1953, 1955). Because the heart ganglion is composed of a limited number of nerve cells, it has been regarded as a suitable material for examining the interaction among neurons, and a considerable number of papers have been published on its activity (Maynard,

1953, 1955; Matsui, 1955; Hagiwara and Bullock, 1957; Bullock and Terzuolo, 1957; Watanabe, 1958; and others). Most of these were, however, confined to the decapod heart ganglia, mainly because of the large size of the animals, while knowledge of other crustacean heart ganglia has been comparatively small (*cf.* Maynard, 1960).

This paper is concerned with the activity of the heart ganglion in the mantis shrimp, a stomatopod crustacean. Several works have already been published on this material (Irisawa and Irisawa, 1957, 1962; Shibuya, 1961), but they are mainly concerned with its spontaneous activity. The use of artificial stimulation and the intracellular electrode technique have revealed that the interaction among neurons in this ganglion is so strong that they act as a single unit. The results suggest that the neurons are linked to each other by junctions, probably of ephaptic nature, with a very high safety factor.

METHODS

All experiments were done on the isolated heart of the Japanese mantis shrimp, *Squilla oratoria* de Haan. The animals were obtained from Tokyo Bay, and kept in two circulatory sea water aquaria. Relatively large specimens (12 to 16 cm in length) were selected. After removing the dorsal carapace, the heart was dissected out together with the reproductive glands, which were then removed from the heart using a pair of microscissors.

The isolated heart is, in its general shape, a long tube woven of muscle fibers (*cf.* Alexandrowicz, 1934). The slack length of the tube is 6 to 8 cm, and the width 2 to 3 mm. The tube opens to the exterior through pairs of ostia on its dorsal surface (see Fig. 1). The ganglionic trunk runs longitudinally along the midline of the dorsal surface of the heart, and each of the somata of the ganglion appears on the trunk slightly caudal to a pair of ostia (except at the ends of the heart). The ganglionic trunk and the cell somata were visible under the dissecting microscope even in an unstained, unfixed preparation, especially when illuminated from the side.

In the experiments in Results, section A, the isolated heart was put in the experimental trough without further operation. In the other experiments, myocardium around a part of the ganglionic trunk was removed in such a way that the heart was divided into two parts which were connected to each other only by the thin isolated ganglionic trunk.

For experiments with intracellular electrodes (Results, sections C and D), the above operation was done at two sites of the heart, which divided the latter into three parts. The central part had an approximate length of 10 mm, and included only one cell soma at about the middle of its length (see Fig. 1). For mechanical support, a glass tube about 8 mm in length and 1.5 mm in diameter, with fused ends, was inserted into that part of the heart. The above procedure, incidentally, much improved the visibility of the ganglionic trunk.

For experiments with intracellular electrodes the soma was always chosen from the middle part of the heart, because of the relatively large size of the soma and the convenience of stimulating the ganglion at both ends of the heart.

The experimental trough was made from two lucite plates and was 10 cm long, 0.5 cm wide, and 0.3 cm deep. Many chlorided silver wire electrodes were buried in the floor of the trough, serving as extracellular stimulating and recording electrodes.

After the specimen was laid in the trough, vaseline partitions were made across the trough between the silver wire electrodes. The isolated parts of the ganglionic trunk were always brought just under the partitions. The compartments were filled with sea water.

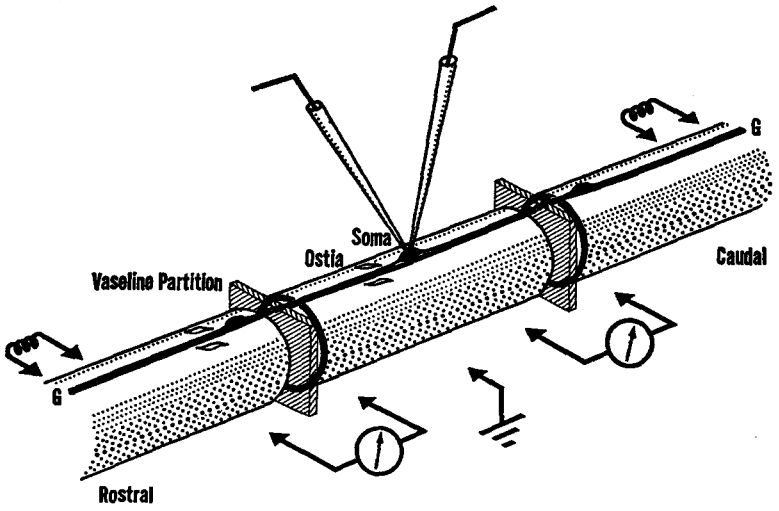

FIGURE 1. Diagrammatic representation of the general arrangement of the experiment. A part of the heart is shown with three ganglion cell somata on it. Microelectrodes are inserted in the middle soma. Myocardium is shown removed at two regions of the heart, leaving the ganglionic trunk (*G-G*) intact. Vaseline partitions insulate the middle from the remaining parts, and extracellular recordings are made across the vaseline partitions. The distance between the centers of the partitions is about 15 mm. Because of the thickness of the partitions (about 3 mm), the length of the central compartment was about 12 mm.

Intracellular electrodes were of the Ling-Gerard type with resistances of 10 to 40 MΩ. Negative capacity feed-back amplifiers were used at the head stage. A dual-beam oscilloscope with built-in DC amplifiers was used in most of the experiments, but later a four-beam oscilloscope was also used. For the extracellular recording, the signals were amplified before feeding to the oscilloscope input by AC amplifiers with various time constants. Experiments were done at room temperatures of 16–22°C.

RESULTS

A. The Electrocardiogram of the Whole Heart

When an isolated, but still intact heart was placed across a vaseline partition, and the recording was made from the pools at the two sides, potential changes

as shown in Fig. 2 *A* were observed. The spontaneous discharge was composed of a series of slow potentials, each being preceded by a rapid spike just at the foot of its rising phase. Thus the general shape of the potential is very similar to the records of Irisawa and Irisawa (1957), although the method of recording is different. Stimulation at an end of the heart evoked a similar slow potential preceded by a rapid spike in an all-or-none manner (Fig. 2 *B*).

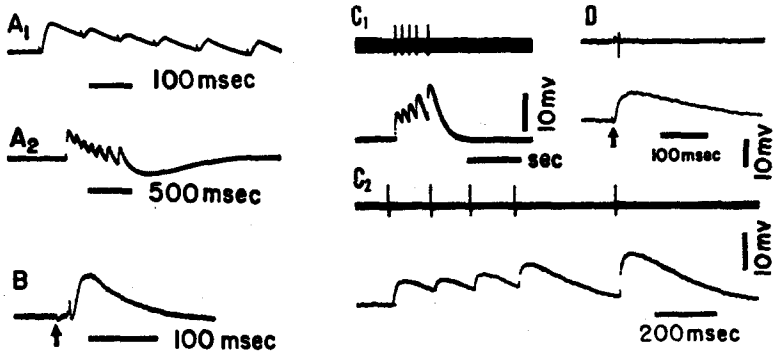

FIGURE 2. The electrocardiogram of the whole heart and its separation into components. A_1, A_2, and B, the electrocardiogram of the intact heart. A_1 and A_2, spontaneous firings. The records are somewhat distorted due to the time constant (about 1 sec.) of the AC amplifiers used, and the undershoot which appeared after the action potentials in A_2 is probably an artifact. B, response to external stimulation. C_1, C_2, and D, upper beam, extracellular records from the ganglionic trunk after the myocardium is removed from the recording region and the vaseline partitions are formed; lower beam, intracellular records from the myocardial tissue. The position of the microelectrode insertion is caudad to the position of the extracellular recording. C_1 and C_2, spontaneous firings. D, response to stimulation of the caudal end of the heart. Arrows in B and D show stimulus artifact.

When the myocardium was partly removed and replaced with vaseline, the rapid component became markedly bigger compared with the slow component. When the amount of the myocardium around the nerve was further reduced, the slow component decreased further and eventually disappeared, leaving only the rapid component. In Fig. 2 *C*, *D*, the responses are illustrated together with the muscle action potentials recorded intracellularly. In time course, the latter are very similar to the slow component of the whole heart action potential, and the spike potential from the nerve is, in its time course and temporal relation, very similar to the rapid component of the intact heart action potential. Thus, confirming the suggestion made by Irisawa and Irisawa, we conclude that the potential from the whole heart is composed of two different elements: a rapid one due to the spike in the nerve trunk and a slow one due to the muscle action potential.

A brief description of the intracellularly recorded muscle fiber potential

will be added here. The resting potential was about 50mv. On stimulation a depolarizing response of 10 to 20 mv amplitude and 500 msec. duration was elicited. It had several properties in common with the vertebrate or crustacean end-plate potentials: a slow falling phase, temporal summation, facilitation, and posttetanic potentiation. Detailed observations were, however, not performed in the present work (*cf.* Irisawa *et al.*, 1962).

B. Extracellular Recording from the Ganglion

SPONTANEOUS FIRING When the vaseline partitions isolated a portion of the ganglionic trunk, brief, roughly diphasic nerve action potentials could be recorded spontaneously from most of the preparations (Fig. 2 C_1, C_2; Fig. 3 *F*). They appeared usually in groups, or in bursts, at regular intervals. Each burst was composed of a varying number of spikes (from several to several tens). The frequency of burst occurrence was identical with the frequency of the heart beat, which was about 0.25 per second in isolated hearts.

It was characteristic of the *Squilla* heart ganglion that the height of the impulses was almost constant. In decapods, it is known that the nerve impulses from the heart ganglion are composed of spikes of many different amplitudes (Maynard, 1953, 1955). In the *Squilla* heart ganglion, it appears as if only one neuron were firing. This, however, is probably not true and the constant height is rather to be explained by the synchronized activity of many neurons in this ganglion (see Shibuya, 1961). The reasons for this conclusion will be given in the following sections.

From the polarity of each action potential it was possible to estimate the direction of impulse propagation. In the spontaneous discharge, impulses always propagated rostrocaudally, when the recording site was roughly in the middle portion of the heart (see Fig. 2 C_1, C_2; Fig. 3 *F*). It thus appears that the pacemaker is located in the rostral part of the ganglion. This conclusion is compatible with Shibuya's findings (1961), but not with those of Irisawa and Irisawa (1957). The latter authors concluded, on the basis of transection experiments, that the pacemaker is located in the thirteenth segment of the heart, which is certainly more caudal to the recording sites in most of the present experiments. The reason for this discrepancy is not entirely clear.

In a few experiments, attempts were made to locate the pacemaker more precisely. By checking the direction of impulse propagation at many sites along the heart, it was found that the pacemaker was near the rostral end of the heart, probably between ostia I and II. Assuming that Alexandrowicz's description (1934) is applicable to the present species, the pacemaker should be located in an area involving ganglion cells 1 to 3. The uncertainty in the above estimation originates from the difficulty of identifying and numbering

the ostia in the living preparations. Shibuya (1961) locates the pacemaker near cells 4 and 5.

HEART-END STIMULATION When artificial stimuli were applied at one end of the heart at regular intervals (usually one or two per second), the spontaneous discharge was suppressed. One obvious reason for this suppression is that the frequency of the artificial stimuli was usually higher than the

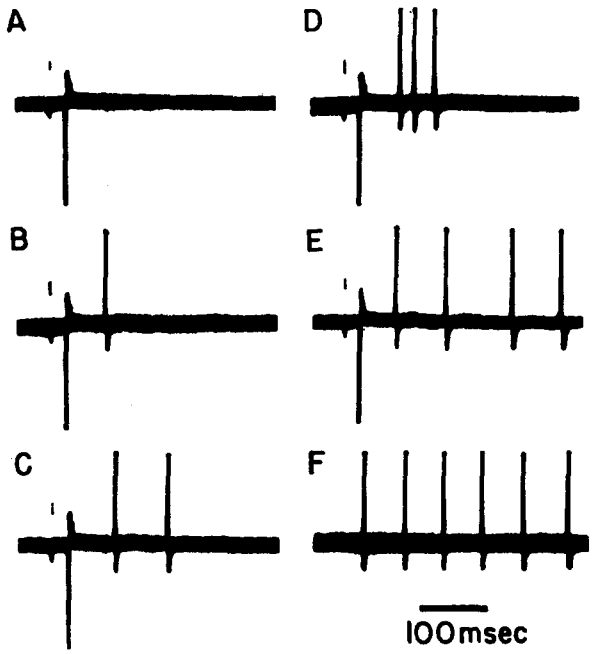

FIGURE 3. Extracellular spikes recorded from the isolated part of the ganglionic trunk. *A-E*, responses to stimulation at the caudal end. The records are arranged in order of the number of reflex impulses from the pacemaker. *F*, the spontaneous discharge. A part of a burst is shown. The rostrocaudally conducted impulses are represented as the upward-downward deflections, and the caudorostrally conducted impulses are represented as the downward-upward deflections. The same convention is adopted in all the records of the extracellular spikes in the present paper.

burst frequency (0.2 to 0.3 per sec.), so that the pacemaker was always in a state of refractoriness. In addition, the artificial stimuli must have some maintained effects on the pacemaker because even after the cessation of repetitive stimulation the spontaneous discharge did not recover soon. Whatever the reason, the spontaneous activity did not manifest itself during the course of stimulus experiments except that the occasional appearance of a reflex discharge in response to external stimulation (see below) gave some indication of it.

Fig. 3 *A-E*, illustrates the responses of the ganglion when a stimulus was

applied at the caudal end of the heart. The first impulse was the "direct" response, travelling rostrad. Following it, a train of impulses, which may be termed reflex impulses, travelled rostrocaudally. Apparently the direct response triggered the pacemaker activity in the rostral part of the ganglion. The number of reflex impulses was not constant. This lack of constancy might be explained by a spontaneous subthreshold activity at the pacemaker.

When the stimulus was applied to the rostral part of the heart, the response usually consisted of a varying number of impulses conducted rostrocaudally. Only rarely did impulses travel in the reverse direction. The shape of the action potential travelling rostrocaudally did not differ appreciably from that of the spontaneous discharge.

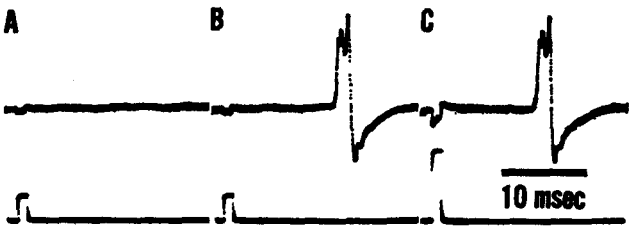

FIGURE 4. The all-or-none character of the extracellular spikes recorded from the isolated part of the ganglionic trunk. The lower beam monitors the stimulus voltage applied at the rostral end of the heart. *A*, stimulus intensity just under the threshold. *B*, just above the threshold. *C*, several times threshold.

The most remarkable property of this response was its all-or-none character (Fig. 4). When the stimulus intensity was below a threshold value, no response was observed in most of the experiments (see p. 782 for exceptions). Above the threshold, increase of stimulus intensity caused gradual decrease of the latency, but did not change the shape or magnitude of the response.

The above experiments suggest two possible explanations: (*a*) Only one neuron is active whereas other neurons are silent for some reason; *e.g.*, because of injury to the ganglion during the microdissection. (*b*) The neurons are all active, but because of some unknown mechanism the excitation in one neuron spreads rapidly into all other neurons.

We adopt the second alternative and reject the first. The reasons are summarized: (*a*) The action potential did not always show a simple smooth configuration. Very often it was separated into many peaks, as may be seen in Fig. 4. If this pattern of response originates from a single neuron, it is quite unlike that observed in other excitable cells. (*b*) The same all-or-none response could also be observed when the myocardium was removed only partially so that the chance of injury to the nervous element was minimized. Further, it was possible to observe the constant amplitude of the spontaneous discharge in the heart *in situ* (*cf.* Irisawa and Irisawa, 1957, Fig. 1). It would

be difficult to understand these consistent findings by assuming that injury or other unfavorable conditions killed all neurons but one in the ganglion so as to produce a single unit activity. (*c*) By applying isotonic magnesium chloride solution to the ganglion, or by repetitive stimulation, it was possible (though difficult) to separate the peaks (see below). (*d*) The most conclusive evidence comes from the intracellular recordings from two different neurons in the ganglion, both of which are active (*cf.* section D).

ATTEMPTS TO SEPARATE THE UNITS We tried to separate the components of the action potential, not only by changing the stimulus intensity very gradually, but also by changing several other conditions.

(*a*) Two stimuli were applied to the preparation successively. When their interval was changed it might have been expected that some of the action potential peaks might drop out because of possible differences in the refractory period among axons. It was found that the second impulse disappeared abruptly when the interval between the two stimuli came within a critical value around 10 msec. Separation of the peaks did not occur until then.

(*b*) Repetitive stimulation was applied to a part of the ganglion, in the possibility that the susceptibility to fatigue might be different among axons or their junctions. In some preparations separation of the peaks was observed, although disappearance of some of these peaks was not observed (Fig. 5 *A*) In other preparations, however, even the separation did not occur; the response simply disappeared abruptly.

(*c*) With an insulated silver wire of 100 μ diameter, a small area of the ganglion was stimulated near the recording site, in the expectation that some components of the action potential could be evoked before the spread of excitation took place through interneuronal junctions. However, even when the stimulating electrode was very near to the recording region, the action potential was very similar to that evoked by stimulation of the end of the heart.

(*d*) With the use of an intracellular electrode, it was possible to stimulate a single nerve cell. The action potential of the ganglionic trunk was very similar to that initiated by external stimulation.

(*e*) Isotonic magnesium chloride solution was found to block impulse conduction in the ganglion quickly and to some extent reversibly. When the solution was applied to a pool in which one of the recording electrodes was buried, the latency of the action potential increased gradually, and the action potential finally disappeared. In the intermediate period, the action potential changed its shape and the disappearance of one of the peaks was sometimes observed. Fig. 5 *B* represents one example which was observed during recovery after the external medium was again replaced by sea water.

The above experiments indicate that the spread of excitation takes place

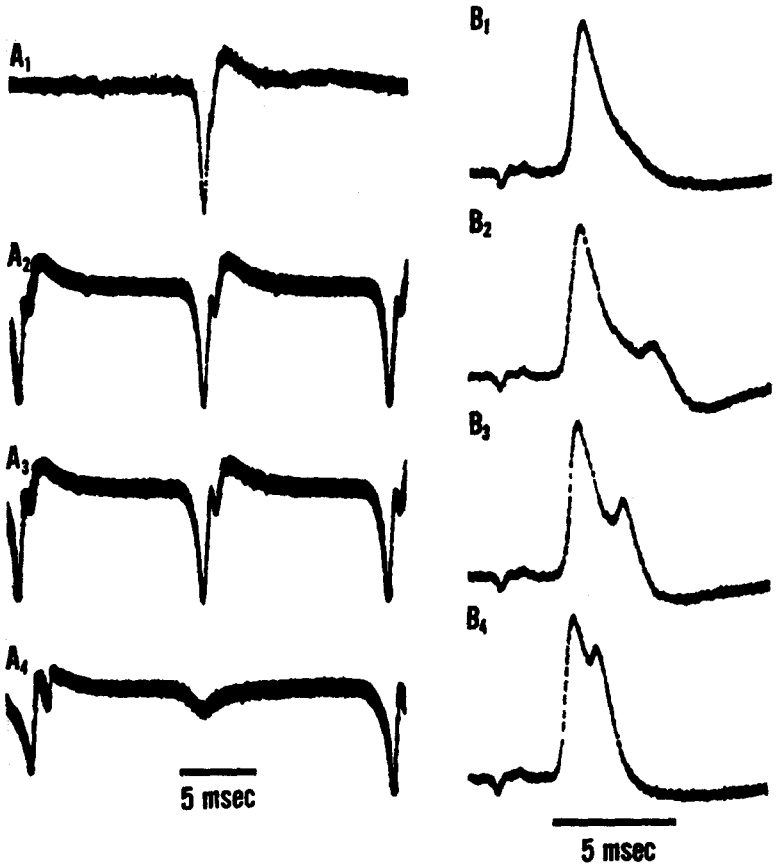

FIGURE 5. Separation of peaks of the extracellularly recorded action potentials. *A*, change of the spike configuration elicited by repetitive stimulation. A_1, response to a single stimulus. A_2-A_4, responses to repetitive caudal stimulation at a frequency of 80/sec. The whole sequence took place within 15 sec. *B*, change of spike configuration which appeared during the recovery from a block caused by the application of isotonic magnesium chloride solution. By replacing the external medium with sea water, the action potential recovered. Its configuration was at first simple (B_1), but within a few seconds another peak returned and was gradually superimposed on the first spike (B_2-B_4).

with very high safety factor. As a matter of fact, it was almost impossible to separate the action potential into units contributed by individual neurons. On the basis of these experiments, we conclude that the junctions responsible for this spread of excitation must be numerous and must be distributed along the axon.

Extrinsic Fibers

In the course of the experiments we encountered several preparations in which very small spikes could be recorded below the threshold of the usual

all-or-none action potential described above. Fig. 6 shows one example. The amplitude of the small spike was less than one-tenth that of the usual action potential. The conduction velocity of the small spike was considerably smaller than that of the usual all-or-none action potential (Fig. 6 *A*, *B*).

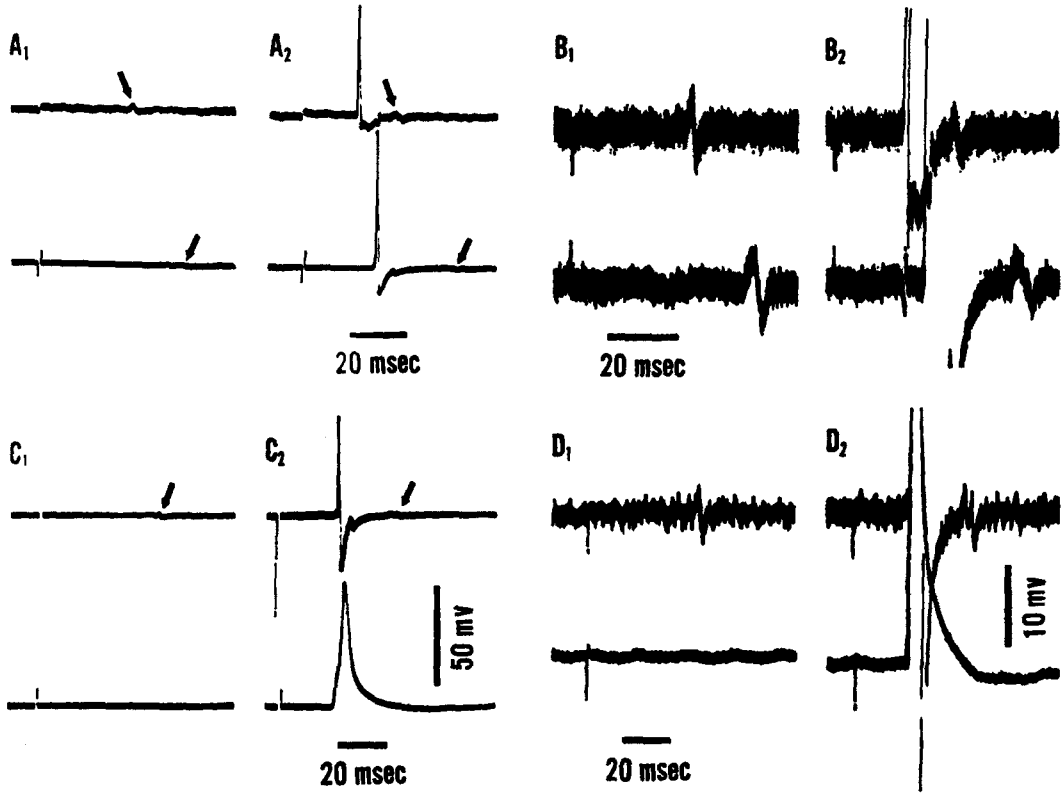

FIGURE 6. The action potentials of the extrinsic fibers and the local systems. Rostral stimulation. In *A* and *B*, beams record the extracellular spikes of the ganglionic trunk from two sites which are 16 mm apart. *A*, low amplification; *B*, high amplification. A_1 and B_1, stimulus intensity is superthreshold to the extrinsic fibers, but subthreshold to the local system. A_2 and B_2, stimulus intensity is superthreshold to both. In *C* and *D* the upper beam shows the extracellular spikes whereas the lower beam shows the intracellular action potentials from the soma. *C*, low amplification; *D*, high amplification. C_1 and D_1, stimulus intensity is superthreshold to the extrinsic fibers, but subthreshold to the local system. C_2 and D_2, stimulus intensity is superthreshold to both. Arrows in *A* and *C* indicate the position of the action potentials of the extrinsic fibers.

Spontaneous discharge of the small spike was also observed, with a rhythm independent of that of the usual action potential. From these observations it may be concluded that the small spikes originate from a system in the ganglion which is independent of the system giving rise to the usual action potential.

Histologically there are two systems in the ganglionic trunk: the local

system and the extrinsic fibers (Alexandrowicz, 1934). We suggest that the usual action potentials originate from the local system and the small spikes originate from the extrinsic fibers. The main reason for this supposition comes from the intracellular records from the ganglion cell soma. As shown in Fig. 6 *C* and *D* when the usual action potential was observed extracellularly, the intracellular electrode always picked up an action potential, which will be described in detail in the following sections. On the other hand, when only the small spikes were observed extracellularly, the intracellular electrode picked up no spikes. Because the ganglion cell soma belongs to the local system, and because in this system the excitation spreads among neurons freely, the units for the small spikes must belong to the extrinsic fibers.

C. Intracellular Recording from the Soma

HEART-END STIMULATION The resting potential of the soma was 50 to 60 mv, inside negative. Stimulation evoked action potentials of 60 to 80 mv amplitude with a step or sharp change of slope on the rising phase (Figs. 6, 7, and 10). Although stimulations at the rostral and caudal ends were both effective, the shape and magnitude of the action potential were different according to the stimulus site. Usually caudal stimulation gave an action potential with a lower peak value and a clearer step. When two shocks were applied at the ends in such a way that the two action potentials collided at the soma, the action potential had the highest peak value and the shortest duration without any step on its rising phase (Fig. 7 A_3).

Usually a slight reversal of membrane potential was observed. In some cells, however, rostral stimulation gave a slight overshoot whereas caudal stimulation did not.

A short after-depolarization followed the spike, without clear distinction from the latter, so that the total duration of the action potential was about 10 msec. Following this, there was sometimes a slight after-hyperpolarization. The action potential appeared to be all-or-none, without exception.

Fig. 7 *A* illustrates the time relation between the externally recorded spikes and the intracellular action potential. The intracellular spike arose approximately midway in time between the two extracellular spikes, but because of the slow rising phase it attained its peak only after another 3 to 5 msec. In some of the experiments the peak even followed the second extracellular spike.

A simple explanation of the above time relation would assume that the second extracellular spike is not triggered by the soma spike. In other words the firing of the cell soma is supposed not to be essential for the impulse propagation, but the axons which run parallel to the soma (for brevity they will be called *parallel axons*, see Fig. 8) are thought to play the essential role. This situation is somewhat like that in vertebrate spinal ganglion cells (Svae-

tichin, 1951; Ito, 1957), in which, too, the soma excitation is not an essential process for the conduction in the neuron. Similar behavior has also been reported for the supramedullary cells of the puffer (Bennett *et al.*, 1959) and some of the neurons in a ganglion of *Aplysia* (Tauc, 1962). In the present material, however, the routes of invasion of impulses into the soma must be multiple, because the shape of the action potential is different according to the stimulus site.

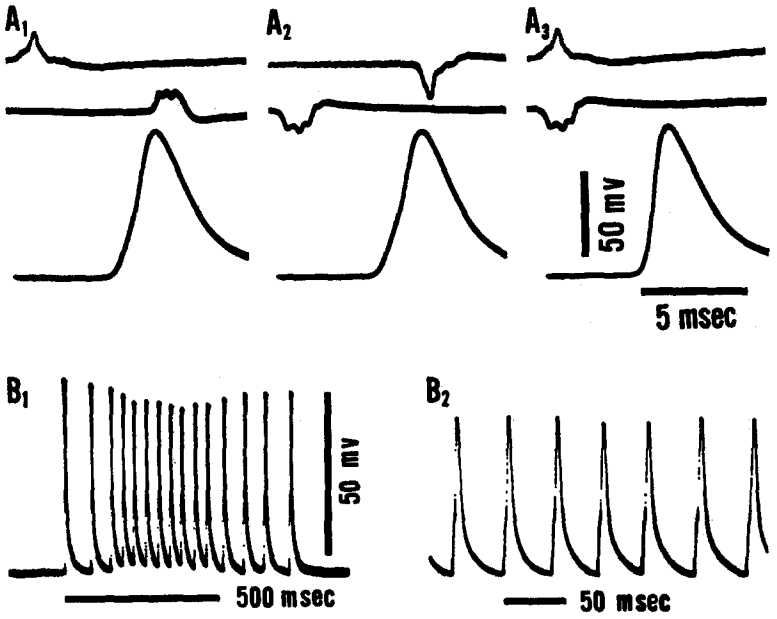

FIGURE 7. Examples of the intracellular soma action potentials. *A*, response to stimulation. The upper two beams are the records of the extracellular trunk potentials. Uppermost beam, across rostral partition. Middle beam, across caudal partition. Lowest beam: intracellular action potential from the soma between the two partitions (see Fig. 1). A_1, rostral stimulation. A_2, caudal stimulation. A_3, stimulation was applied at both ends of the heart so that the impulses collided at the soma. *B*, spontaneous discharge. B_1, the whole sequence of a burst. B_2, a part of a burst.

A simple hypothetical schema of the neuronal network around the soma has been devised on the basis of the above and the following experiments, as shown in the upper part of Fig. 8. The essential assumption of the schema is the insertion of many junctions (which will be called *side-connections*) between axons which allow impulses to pass with a high safety factor. The introduction of the side-connections is necessitated by the all-or-none nature of the response, but the same schema explains many other experimental findings. Thus, in the case of external stimulation, the impulses travel along parallel axons as well as along the soma axon, keeping pace with each other. At the soma, however, the impulse in this neuron is delayed because of the

slow rising phase of the soma potential. The impulses in the parallel axons continue to propagate with their constant velocities, and they invade the axon at the other side of the soma through side-connections. Impulse conduction across the soma may also take place by itself (see p. 792), but in normal conditions it has no practical use, because of the rapid propagation of excitation through parallel axons and side-connections, bypassing the soma.

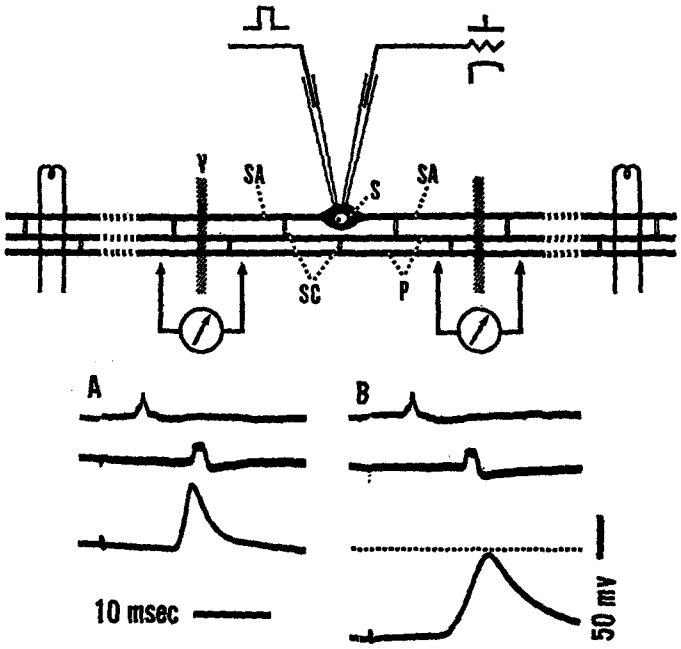

FIGURE 8. Upper part, a hypothetical schema of the neuronal network around the soma. *S*, soma. *SA*, soma axon. *P*, parallel axons. *SC*, side-connections. *V*, vaseline partition. The dotted regions of the axons imply that the stimulating electrodes are far from. the recording sites. Lower part, constancy of the conduction time between the two partitions regardless of the soma membrane potential. Electrode arrangement the same as in Fig. 1. *A*, before polarization. *B*, membrane hyperpolarized. Dotted line indicates the resting level.

The lower part of Fig. 8 shows the absence of an effect of hyperpolarization of a soma on the conduction time of the part of the ganglionic trunk including this soma. Even when the peak of the intracellular potential was less than the level of the resting potential, and the soma therefore did not fire (see discussion of Fig. 10 in the text), the conduction time did not change at all. This experiment confirms the conclusion that the conducted impulse bypasses the soma along parallel axons, and that the second extracellular spike is not triggered by the soma action potential.

In one preparation the soma axon was cut by a needle under visual control.

Conduction still persisted after the operation, supporting the view that the soma axon is not essential for impulse propagation.

Spontaneous Activity

Fig. 7 *B* shows the intracellular records during spontaneous activity. The potential change was similar to the response to repetitive stimulation delivered at the rostral end of the heart. During the activity the base line was elevated slightly as the result of summation of the tails of action potentials, and the peak of the spike was decreased when the following spike came during the relative refractory period.

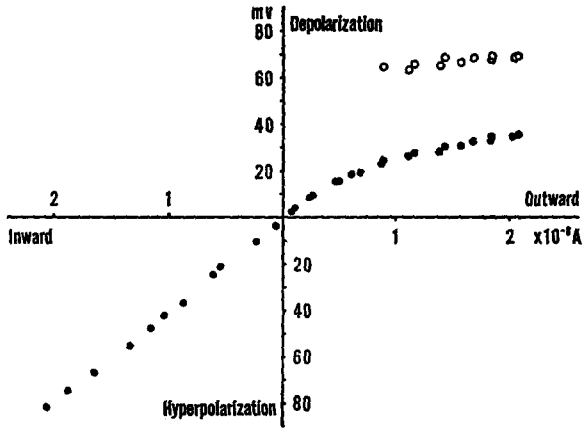

FIGURE 9. I–V curve of the soma membrane. Solid circles indicate the final level of the electrotonic potential elicited by 30 to 50 msec. current pulses. Open circles indicate the peak of the action potentials when the outward current exceeds a critical value.

The I-V Curve of the Soma Membrane

In five cells the relation between the membrane potential and the current applied through the second intracellular electrode was plotted (Fig. 9). The resistance near I = 0 was 3 to 5 MΩ, which is of the same order of magnitude as that of lobster cardiac ganglion cells (Hagiwara *et al.*, 1959). Assuming that almost all current passes through the soma membrane (which is not the case in the lobster cardiac ganglion), membrane constants were tentatively calculated as follows: Specific membrane resistance, $8.5 \times 10^3 \Omega$ cm^2; time constant, 10 msec.; specific membrane capacitance, $1.3 \mu f/cm^2$.

The I-V curve shows a considerable amount of rectification when the depolarization exceeded 10 mv. In some cells, the resistance increased slightly with strong hyperpolarization.

The Effect of Hyperpolarization on the Action Potential

Fig. 10 shows the effect of hyperpolarization on the soma action potential elicited by rostral stimulation. At first the action potential amplitude increased (*B*), indicating a reduced membrane impedance at the peak. Increase of the polarizing current revealed an inflection in the rising phase about 40 mv above the resting level. Thus at this level of hyperpolarization the action potential had two steps, which divided the action potential into three parts (*C-E*). They will be called A_1, A_2, and B spikes, respectively, starting from the foot. On further increase of current, the B spike gradually decreased in amplitude, was delayed, and eventually disappeared (*F*). The

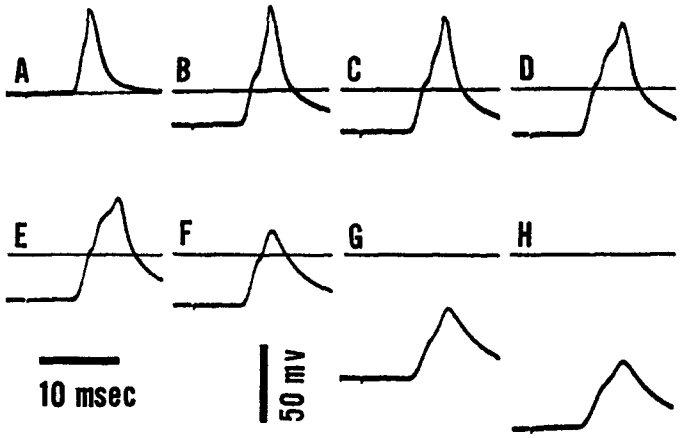

FIGURE 10. The effect of hyperpolarization on the spike recorded intracellularly from the soma. Rostral stimulation. One beam registered the initial level of the membrane potential.

remaining two parts, A_1 and A_2, however, did not disappear even with hyperpolarization of about 100 mv. Sometimes the distinction between A_1 and A_2 became obscure, because of the gradual disappearance of the inflection, when strong hyperpolarization was applied. Even in such cases, a delay or decrease of A_2 was never observed.

The fact that the B spike is eliminated by membrane hyperpolarization indicates that it originates from the soma membrane, or a part of the neuron membrane near the soma, as does the B spike, or SD spike, in the vertebrate motoneuron (Brock *et al.*, 1953; Fuortes *et al.*, 1957). The critical depolarization for the generation of the B spike is 30 to 40 mv.

The inflection of an unpolarized action potential is the dividing point between A_1 and A_2 spikes. From the fact that even when the peak of the A_1 spike is under the resting level A_2 does not disappear, it is concluded that the

A_1 spike does not elicit the A_2 spike from the soma. Thus A_1 and A_2 come to the soma independently, from outside the soma.

From the histological evidence we assume that the A_1 and A_2 spikes originate each from one of the two axons of this bipolar neuron. When, *e.g.* the rostral end of the heart is stimulated, an impulse invades, mostly electrotonically, from the rostral axon to the soma to form the A_1 spike. Another impulse invades from the caudal axon *via* parallel axons and side-connections to form the A_2 spike. The two spikes add, and at the top of the summated potentials the B spike is superimposed as the electrogenic response of the soma membrane.

Here it is emphasized that the terms A_1 spike and A_2 spike do not imply any active responses of the soma membrane. Rather, the A_1, A_2 spikes are considered to be potentials spread electrotonically from the axons.

When the soma action potential was produced by the collision of two impulses conducted from the two ends of the heart, the lower inflection was absent even in the unpolarized action potential, and hyperpolarization only eliminated the top part of the action potential. This experiment indicates that the rostral and caudal axons near the cell soma fired simultaneously, so that the A_1 and A_2 spikes fused. Only the B spike disappeared when the peak of the summated $A_1 + A_2$ spikes fell below the threshold level for that response.

Direct Stimulation

When the membrane was depolarized by an outward current pulse (Fig. 11), the action potential was initiated at a rheobasic current of about 10^{-8} A and at a critical depolarization of 25 to 40 mv. Extracellular recordings showed that impulses propagated in both directions from the stimulus site. The directly elicited spike did not manifest any step on its rising phase. This may be correlated with the fact that the conducted spikes arise in the two axons simultaneously.

With a long depolarizing pulse, the action potential was sometimes repetitive. The following spikes were, however, considerably smaller than the first one, indicating that a rather strong inactivation process was going on. In many of the cells the response consisted of only one spike from the stimulus site, presumably because in these cells the inactivation process was too strong to allow the generation of the second spike. A reflex discharge from the rostral end of the ganglion occurred often, which had to be distinguished from the locally evoked spikes. As the external recordings showed the direction of spike propagation, the origin of the spikes could be identified very easily.

At a critical current strength excitation also took place at the end of the current pulse and the response sometimes appeared as a double peak in the falling phase of the electrotonic potential (Fig. 11 *E*). The external recordings

show that each component of the double peak corresponded to one of the impulses conducted in the two directions. In Fig. 11 *E* the first peak corresponded to the caudorostrally conducted impulse and the second peak corresponded to the rostrocaudally conducted impulse. With stronger current the second response almost disappeared in the intracellular records (*F*). In

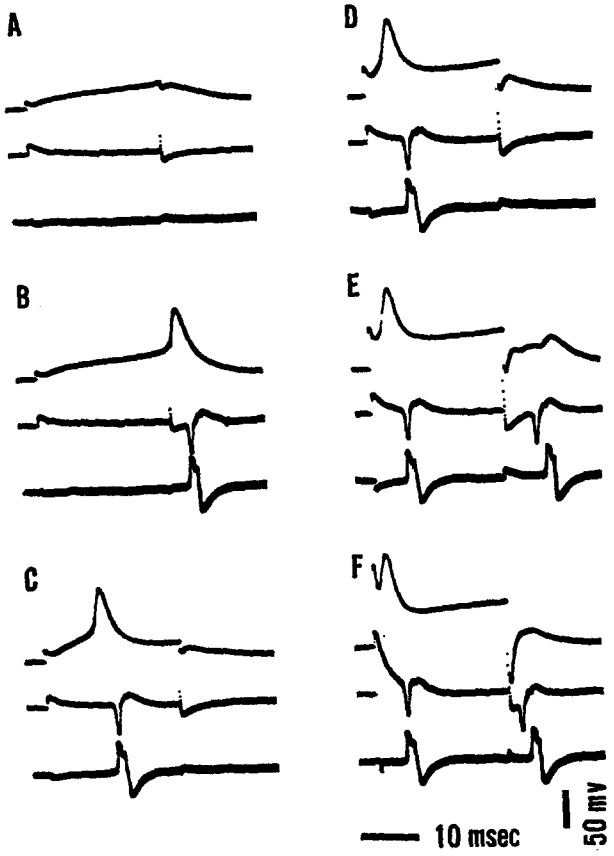

FIGURE 11. Direct stimulation of a cell. An outward current pulse of about 20 msec. duration was applied to the soma membrane. Upper beam, change in membrane potential. Middle beam, extracellular spike recorded at the rostral partition. Lower beam, extracellular spike recorded at the caudal partition. Further description in text.

the extracellular records, however, impulses were still seen to propagate in both directions, but the latencies were shorter. In such a case the spike occurred far from the soma and could not invade the soma because of a strong inactivation.

Separation of the peaks was never observed in the first action potential. The separation manifest after the end of the current pulse was presumably due to the reduced safety factor caused by the inactivation process of the

soma membrane. The appearance of the peaks in the falling phase of the electrotonic potential in the soma record indicates that the spike originated at a point in the axon at some distance from the soma and that it was conducted to the latter mostly electrotonically to create the first peak. This corresponds to the A_1 spike. Because of the inactivation process of the membrane around the soma, the A_1 spike fails to elicit the A_2 spike directly in the axon at the other side of the soma. The long delay between the two peaks suggests that the A_1 spike, which arose in the rostral axon, went around the soma by way of the parallel axons and side-connections, and invaded the soma through the caudal axon, again mostly electrotonically.

The separation of a spike into a double peak was observed in the lobster cardiac ganglion by Bullock and Terzuolo (1957) and Hagiwara *et al.* (1959). These authors supposed that the peaks are due to the asynchrony of the firings in two axons of the neuron. This interpretation is also applicable to the present data. In the *Squilla* heart ganglion, however, the number of the peaks could not be reduced to one, in contrast to the case of the lobster, even by the finest adjustment of the stimulus strength. This is to be expected, since in the *Squilla* heart ganglion an action potential occurring anywhere in the local system spreads to all other parts; the reentry of an impulse from the other axon is unavoidable.

Extra Spike Generation from the Region of the Soma

A slightly hyperpolarized cell sometimes produced one extra spike in response to a single external stimulus. Fig. 12 shows one example. When the hyperpolarization attained a critical level the B spike was delayed and a hump appeared on the falling phase of the spike in an all-or-none manner. Extracellular records indicated that, following the usual rostrocaudally conducted impulse, an extra spike started from the soma region and propagated in both directions. A further increase of hyperpolarization eliminated the B spike, and at the same time the extra spike vanished.

A similar phenomenon was also observed in a monopolar ganglion cell of *Aplysia* by Tauc (1957). His explanation was that because of the delay in generating the soma potential by hyperpolarization the axon recovered from refractoriness when the soma fired. An essentially similar explanation can be applied to the present data.

When the cell was slightly hyperpolarized, the occurrence of the B spike was retarded for more than 1.4 msec. due to a reduced safety factor. This lapse of time was enough for the rostral axon to recover to a level of excitability at which the B spike could elicit an action potential in the rostral axon. The new impulse resulted in the hump in the cell potential during the falling phase of the first spike. The caudal axon did not fire simultaneously, because the time for recovery was insufficient. (When two axons fired simultaneously,

the two impulses were recorded with a very small time interval, as may be seen in Fig. 11, first response.) The new impulse in the rostral axon propagated rostrally and caudally through side-connections and parallel axons. Sometimes this new impulse invaded the soma again from the caudal axon and formed a second peak on the falling phase of the action potential.

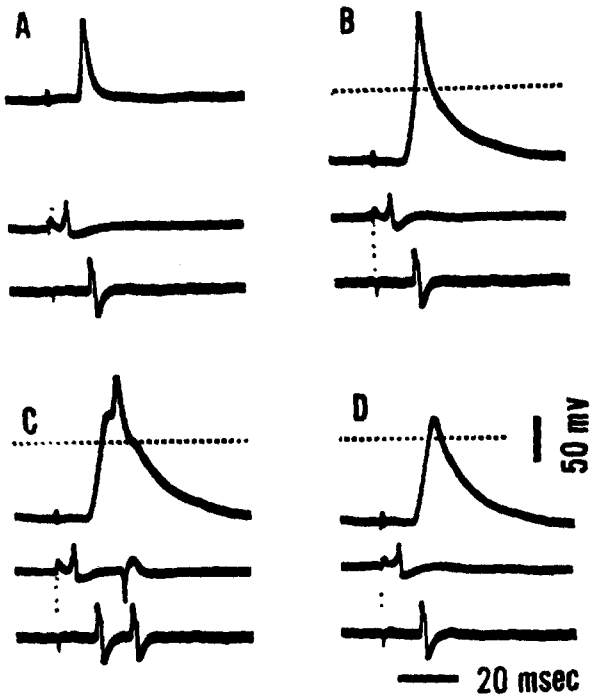

FIGURE 12. Generation of extra spike from the region of the soma by a critical hyperpolarization. Three beams as in Fig. 11. The same preparation as in Fig. 11. Rostral stimulation. *A*, no polarization. *B*, membrane hyperpolarized by about 70 mv. *C*, polarization slightly increased. Extra spike appeared. *D*, polarization increased slightly further. B spike vanished and the extra spike also disappeared. The broken lines indicate the initial resting potential.

The extra spike production sometimes took place in an unpolarized soma, especially during the refractory period. Its origin was similar: the decreased safety factor and the delayed occurrence of the B spike in relation to the spikes in the two axons.

EFFECTS OF BLOCKING THE PARALLEL AXONS To examine whether the impulse can propagate across the soma from one of its axons to another without the help of the parallel axons, attempts were made to cut the parallel axons so that the only route for the impulse propagation would be across the soma. Under the dissecting microscope the parallel axons could be recog-

nized as a bundle which was more or less separated from the soma and its axons. The procedure was to thrust the tip of an empty micropipette into the bundle repeatedly with the help of a micromanipulator and the dissecting microscope.

In almost all cases, a marked prolongation of the conduction time was observed after the operation. In one example shown in Fig. 13 *A*, the conduction time between two vaseline partitions was about 5 msec. before the

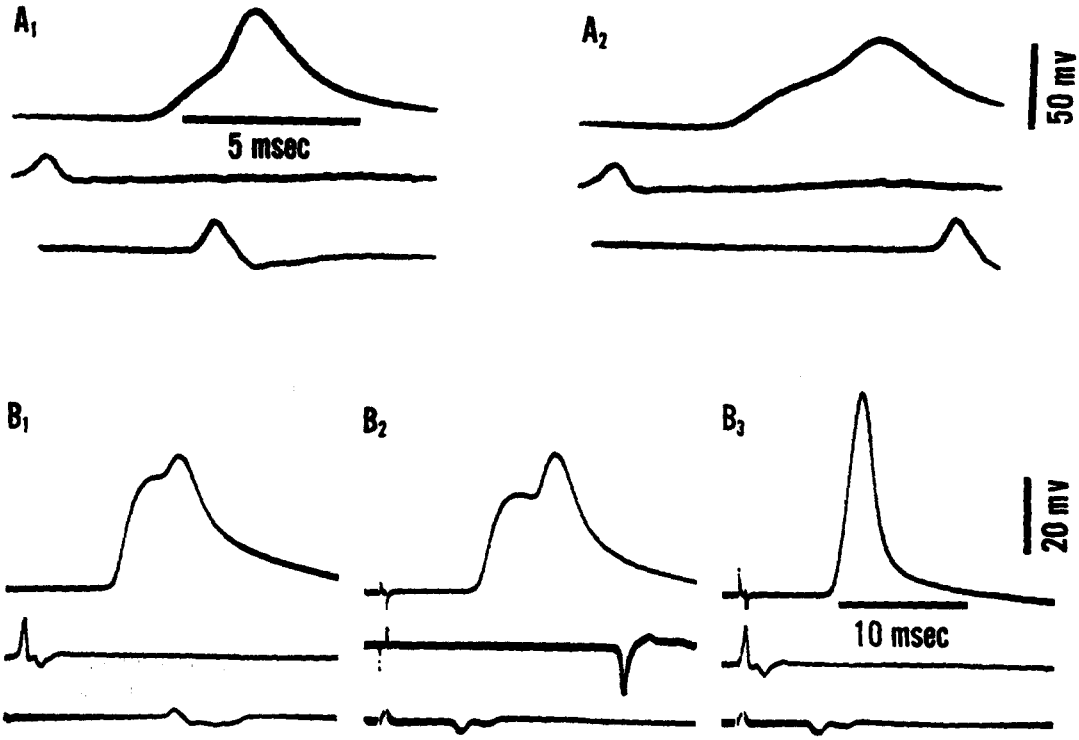

FIGURE 13. Conduction across the soma after cutting the parallel axons. Three beams as in Fig. 11. *A*, rostral stimulation. A_1, before operation. A_2, after operation. *B*, another axon after operation. B_1, rostral stimulation. B_2, caudal stimulation. B_3, both ends of the heart were stimulated to cause a collision at the soma.

operation and about 10 msec. after the operation. The intracellular record shows that the soma action potential also changed its shape; the step became much more conspicuous, the peak was lowered and delayed, so that the general shape of the action potential looked like a superposition of two independent spikes, which were identified as A_1 and A_2 spikes as before. The time of onset of the A_1 spike, however, did not change.

It seems unlikely that such changes in the soma action potential were due to injury to the soma itself during the operation, because an action potential

of the standard size and form could be obtained by collision of two impulses elicited with stimuli delivered with an appropriate interval at the two ends of the heart (Fig. 13 B_3). The most likely interpretation seems to be that the A_1 spike elicits the A_2 spike by local current from one of the soma axons to the other. Because the soma membrane has a low excitability, the conduction takes place in almost "saltatory" fashion, without causing excitation of the soma membrane to generate the B spike.

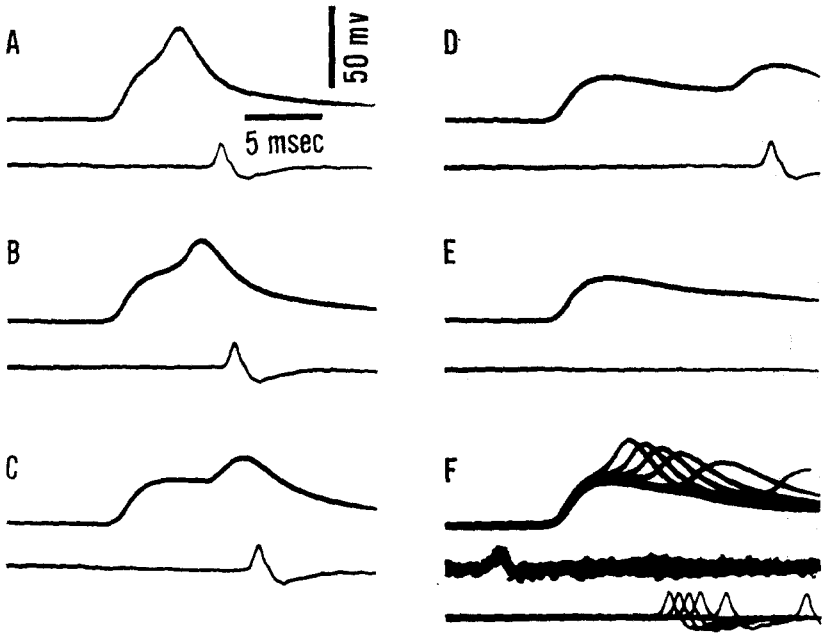

FIGURE 14. Correspondence between the intracellular A_2 spike and the extracellular spike at the distal partition. Parallel axons were cut. Rostral stimulation. In *A-E*, upper beams indicate the intracellular records and the lower beams indicate the extracellular spike recorded across the caudal partition. *A*, response to a single stimulus. *B-E*, response to repetitive stimuli at a frequency of 2 per sec. *F*, a superimposed record of the sequence between *B* and *E*, with the record of extracellular spike at the rostral partition (shown on the middle beam), which did not change during the above sequence.

An experiment which confirms the above explanation is shown in Fig. 14. When stimuli were delivered to the rostral end of the heart with a repetition rate of 2 per sec. or more, the A_2 spike gradually delayed and eventually disappeared (Fig. 14 *B-E*). The extracellular spike, picked up at the caudal partition, always followed the A_2 spike, and when the latter disappeared, the former also disappeared (*E*). This strict correspondence implies that the conduction of the impulse took place across the soma, and not through some surviving parallel axons. Thus it is concluded that the conduction across the soma can occur without the help of any parallel axons. In an uninjured

preparation, however, it does not have any functional significance, because of the rapid impulse conduction along the parallel axons and the invasion of the impulse from the parallel axons into the soma axon.

During the above experiment we failed to observe any soma activity (B spike). Because the amplitude of the action potential was more than 50 mv, and because the critical depolarization determined by hyperpolarization experiments (Fig. 10) was 30 to 40 mv, the B spike should have appeared at the top of the action potential. It is possible that the absence of the B spike was due to an increase of its threshold caused by slight injury during the operation to block the parallel axons. Since, however, we tried to keep some distance between the soma and the site of operation, we prefer an alternative explanation. According to it, the absence of the B spike was due, first, to an increase of its critical depolarization, because the soma membrane was depolarized for a considerable length of time with the A_1 spike, so that inactivation to the soma membrane took place. The second factor is that the depolarization of the soma was smaller after operation than that before operation. On the other hand, the determination of the critical depolarization by hyperpolarizing experiments (Fig. 10) may not be directly comparable, because the soma membrane received a strong hyperpolarization for some time before firing.

Reentry of the Axon Spike into the Soma

With external stimulation, the soma is invaded by impulses from the two soma axons. The impulse from the soma axon at the side opposite to the stimulus site propagates against the main direction of the impulse conduction in the ganglion. In principle, we should be able to record this "backfiring" impulse by setting two external electrodes near the soma, but actually the side-connections appear to be so numerous that the backfiring impulse takes place only in a short stretch of the soma axon. Its existence can only be surmised by the analysis of the intracellular records.

The following experiments, however, demonstrate the reentry of the impulse into the soma directly under a special condition. In Fig. 15, the cell was penetrated with two intracellular electrodes after an operation as described above to damage the parallel axons. On rostral stimulation, two spikes were observed extracellularly at the caudal partition (*A*, lower beam). The first one (S_1) was small and hyperpolarizing the soma membrane did not change its latency. The second one (S_2) was larger and changed its latency with changes in the soma membrane potential (*B-D*, lower beam).

Hyperpolarization increased the delay of the S_2 spike as well as that of the intracellular A_2 spike (*B-C*, upper beam). At a certain level of hyperpolarization, however, S_2 became fixed in latency, but changed in amplitude, until finally the polarity of the spike was reversed on gradual increase of

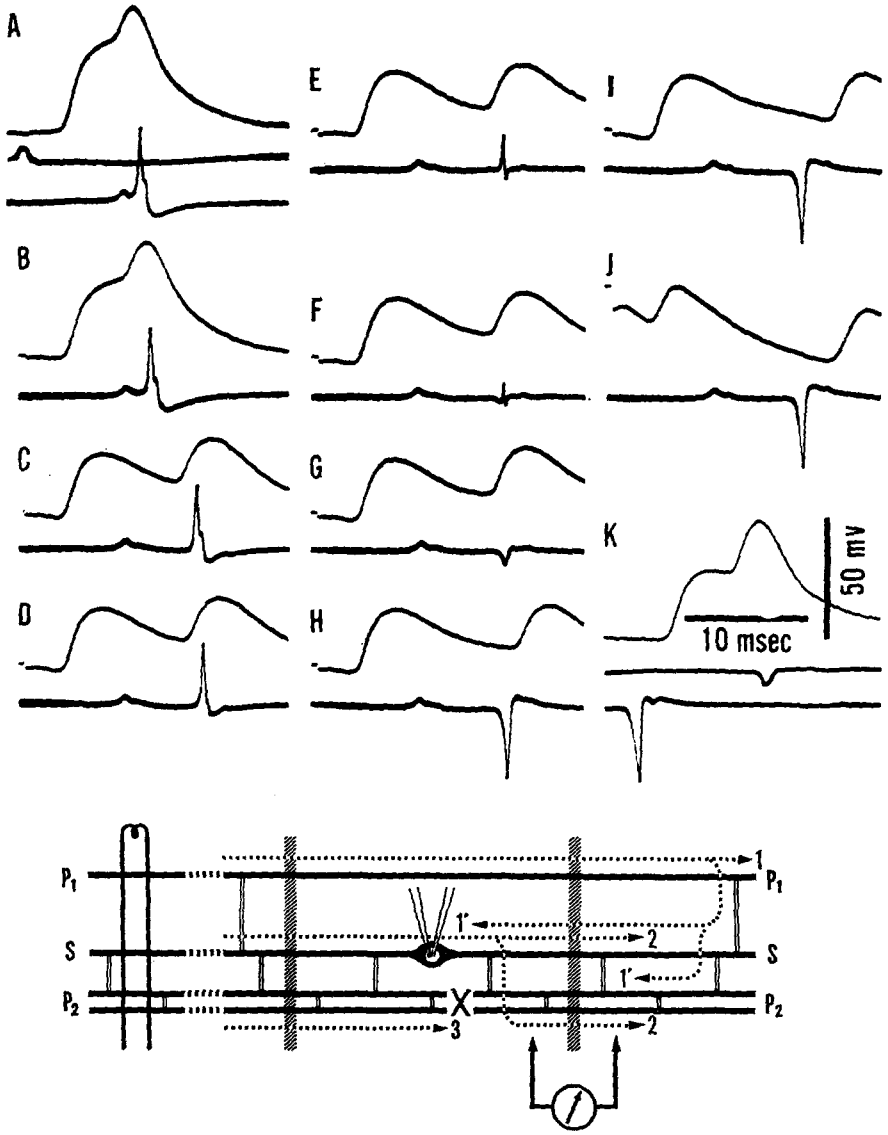

FIGURE 15. The demonstration of reentry of the spike into the soma from the side opposite the site of stimulation. *A-J*, rostral stimulation. *K*, caudal stimulation. In each record the upper beam indicates the intracellular soma potential, the middle beam (present only in *A* and *K*) indicates the extracellular spike across the rostral partition, and the lower beam indicates the extracellular spike across the caudal partition. From *B* to *J* the membrane was hyperpolarized with current pulses of gradually increasing strength. Diagram, supposed routes for the impulses. *S-S*, soma and its axons. P_1-P_1, a part of the parallel axons which escaped destruction. P_2-P_2, the main part of parallel axons which have been blocked. Numbers mark the impulses caused by rostral stimulation. Impulse 1 is marked as 1′ after crossing the side-connections to invade the S-P_2 bundle. For further details, see text.

the hyperpolarizing current (*C-H*, lower beam). On the other hand, the delay of the intracellular A_2 spike further continued to increase so that the foot of the A_2 spike appeared only after the reversed S_2 spike (*H-I*, upper beam). Finally the latency of the A_2 spike also became fixed (*I-J*). Thus at the final stage of the hyperpolarization the time relation between S_2 and A_2 was very similar to that elicited in response to caudal stimulation (compare Fig. 15, *J* and *K*).

On the basis of schema in Fig. 15, the above experiment can be explained as follows. Although the main part of the parallel axons, P_2, had been cut by the operation, a small fraction, P_1, escaped destruction, and conducted the S_1 spike (impulse 1). An impulse traveled across the soma (impulse 2), spread into all axons in P_2, and generated the big S_2 spike. These two impulses collided at some point due to side-connections. Although the exact site of the collision cannot be determined from the present data, except that it occurred caudad to the caudal partition, it is likely that the site was on the S-P_2 bundle rather than on the P_1 bundle, because the conduction across the soma was slow and impulse 2 was delayed accordingly. So the collision probably occurred between impulse 2 and the caudorostrally traveling impulse which was evoked by impulse 1 and transmitted across a side-connection at the caudad side of the caudal partition. The latter is designated impulse 1′.

When the soma membrane was hyperpolarized, the generation of A_2 and that of S_2 were further delayed (*B-C*), and the point of collision of the two impulses (1′ and 2) gradually approached the soma along the P_2-S bundle. The reversal of the polarity of S_2 spike (*G-J*) indicates that the point of collision was between the soma and the caudal partition. When the hyperpolarization completely suppressed the generation of the A_2 spike from the A_1, the backfiring S_2 spike determined the time of onset of the A_2 spike (*I-J*).

Simultaneous Recordings from Two Somata

Fig. 16 illustrates a simultaneous recording from two neighboring somata. Their thresholds were identical, again indicating the all-or-none behavior of this ganglion. The action potentials had similar shapes and magnitudes. The time interval between the two action potentials is the conduction time of the impulse across one segment, which amounted to about 4 msec. and was almost the same when the direction of the impulse was reversed.

When the electrode in the rostral cell was switched to pass depolarizing current, an action potential could be observed from the other cell. The axon impulse was recorded extracellularly from between the two somata with the same shape and magnitude as were obtained with rostral stimulation. The situation could be reversed by switching the current and potential electrodes; thus the action potential was recorded from the rostral cell by

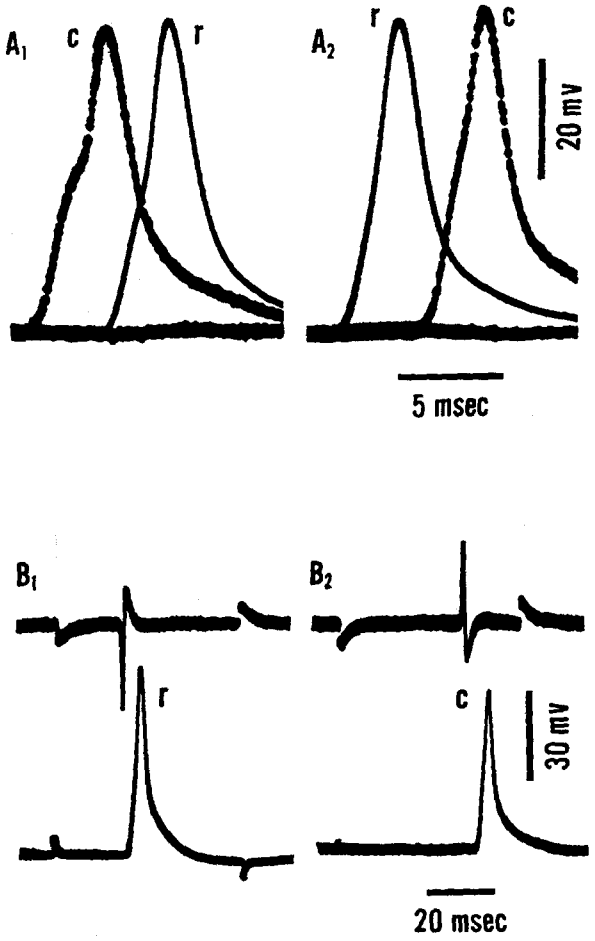

FIGURE 16. Relations between two neighboring somata. *A*, simultaneous recording from the two somata. A_1, caudal stimulation. A_2, rostral stimulation. *c*, action potential from the caudal cell. *r*, action potential from the rostral cell. *B*, same preparation. Upper beam, extracellular spikes recorded between the two somata. Lower beam, intracellular records. B_1, the caudal cell was directly stimulated. B_2, the rostral cell was directly stimulated.

direct stimulation applied to the caudal cell. The axon impulse reversed its sign, and the shape and magnitude were the same as with caudal stimulation. These results show that the impulse travels from one neuron to the other in either direction.

Spread of electrotonic potential was not observed between the two somata.

D. Intracellular Recording from the Axon

In several cases, the microelectrode happened to pick up spike potentials which were very different from the action potentials observed in cell somata

and in heart muscle fibers. In Fig. 17 one example is presented together with the usual soma potential. In this particular case the distance between the two recording electrodes was less than 100 μ, so that it does not seem possible to explain the difference between the two potentials by supposing that they originated from two different parts of the same neuron. We conclude that they came from two different neurons. This conclusion is supported by an experiment in which current of a considerable intensity was supplied through the electrode to the soma membrane. No electrotonic potentials could be recorded with the other electrode even when the current was strong enough to evoke anode-break excitation.

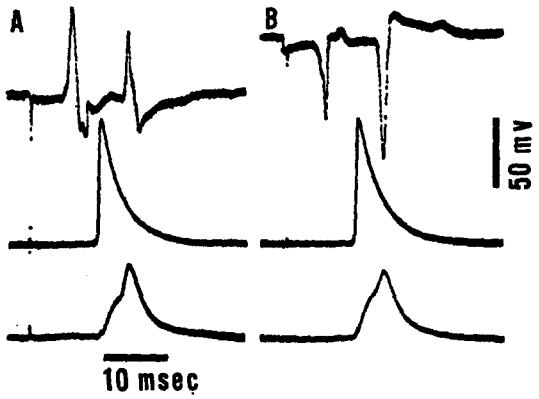

FIGURE 17. Simultaneous recordings of the action potentials from the soma and the axon. *A*, rostral stimulation. *B*, caudal stimulation. Upper trace, action potentials recorded extracellularly at the two vaseline partitions. The two signals were mixed electronically by feeding them into the two push-pull inputs of the amplifier. Thus the trace does *not* indicate any repetitive activity; the interval between the two spikes merely indicates the conduction time of the trunk action potential between two partitions. Middle trace, action potentials from the axon recorded intracellularly. Lower trace, action potentials from the soma recorded intracellularly.

It is concluded that the potential in the middle beam in Fig. 17 came from one of the axons. The axon potential had a very rapid rate of rise with no inflection. The rate of decay was slower, which made the total duration more than 10 msec. The shape and magnitude were almost the same regardless of the stimulus sites. The threshold for the axon spike was exactly the same as that for the soma action potential and for the extracellular axon potentials as well.

DISCUSSION

All extracellular and intracellular records presented under Results can be understood from the point of view that in the *Squilla* heart ganglion neurons

are tightly linked through side-connections (Fig. 8). Although their exact location, number, and nature are not clear, that such connections exist seems to be an inescapable conclusion.

Thus the neurons form a kind of neuronal network. Similar systems are also found in the abdominal cord of the crayfish (Wiersma, 1947; Watanabe and Grundfest, 1961), of the earthworm (Bullock, 1945; Rushton, 1946; Kao and Grundfest, 1957; Wilson, 1961), and of some polychaetes (Bullock, 1953). The characteristics of such systems are more or less similar: the spread of excitation, with a high degree of safety factor when a part of it is injured, and with repetitive firing or extra spike generation under some unfavorable conditions, such as fatigue, injury, or refractoriness. Probably the principal usefulness of such systems is the increased safety factor. On the other hand the flexibility of such systems is decreased accordingly.

The present experiments do not supply any direct information about the nature of the side-connections. But the results seem to suggest that transmission takes place electrically rather than chemically, because (*a*) transmission is possible in either direction, (*b*) the fatigue of transmission by repetitive stimulation is very small, and (*c*) the delay of transmission seems very small considering that the shape of the extracellular spike was almost the same regardless of the distance between the site of impulse generation and the site of recording (compare, *e.g.*, the first and second spikes on the lower beam of Fig. 12 *C*).

The histological work done by Dr. A. Irisawa with the electron microscope (personal communication) throws some light on this problem. In axons of this ganglion there are structures composed of two plasma membranes which are very closely apposed to each other. Their appearance is similar to that of the septal junctions in giant fibers of the earthworm abdominal cord (Hama, 1959). Although the results are still preliminary, they lend support to the supposition of electrical transmission, because it is suggested that in "chemical" synapses the synaptic clefts should be wider to insure a low resistance between the synaptic cleft and the external medium (*cf.* Furshpan and Potter, 1959; Hama, 1961, 1962).

In the heart ganglion of the lobster the interaction among neurons takes place in two ways: synapses and electrical connections (Watanabe, 1958; Hagiwara *et al.*, 1959; Watanabe and Bullock, 1960). In making a comparison, it can be said that in the *Squilla* heart ganglion the electrical connections among neurons have developed to the extent that the independence of the individual neurons is almost abolished. On the other hand, we failed to record any synaptic activity from the soma at the middle part of the *Squilla* heart. This does not, of course, exclude the possibility that synaptic transmission occurs in hearts *in situ*. Especially we may expect that the pacemaker cells receive synapses from the extrinsic fibers. According to Shibuya's

brief report (1957) each extrinsic fiber has a different effect on the heart beat. On the other hand the cell soma in the central part of the heart seems not important for conductile function, although it probably serves as the trophic center of the neuron. Hence even if synapses did exist on the soma, they could not change the pattern of the activity in this ganglion.

We wish to express our gratitude to Professor H. Grundfest for reading the manuscript and making suggestions.

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

Received for publication, September 4, 1962.

REFERENCES

ALEXANDROWICZ, J. S., The innervation of the heart of the Crustacea. I. Decapoda, *Quart. J. Microscop. Sc.*, 1932, **75**, 181.

ALEXANDROWICZ, J. S., The innervation of the heart of the Crustacea. II. Stomatopoda, *Quart. J. Microscop. Sc.*, 1934, **76**, 511.

BENNETT, M. V. L., CRAIN, S. M., and GRUNDFEST, H., Electrophysiology of supramedullary neurons in *Spheroides maculatus*. I. Orthodromic and antidromic responses, II. Properties of the electrically excitable membrane, III. Organization of the supramedullary neurons, *J. Gen. Physiol.*, 1959, **43**, 159.

BROCK, L. G., COOMBS, J. S., and ECCLES, J. C., Intracellular recording from antidromically activated motoneurones, *J. Physiol.*, 1953, **122**, 429.

BULLOCK, T. H., Functional organization of the giant fiber system of the *Lumbricus*, *J. Neurophysiol.*, 1945, **8**, 55.

BULLOCK, T. H., Properties of some natural and quasi-artificial synapses in polychaetes, *J. Comp. Neurol.*, 1953, **98**, 37.

BULLOCK, T. H., and TERZUOLO, C. A., Diverse forms of activity in the somata of spontaneous and integrating ganglion cells, *J. Physiol.*, 1957, **138**, 341.

FUORTES, M. G. E., FRANK, K., and BECKER, M. C., Steps in the production of motoneuron spikes, *J. Gen. Physiol.*, 1957, **40**, 735.

FURSHPAN, E. J., and POTTER, D. D., Transmission at the giant motor synapses of the crayfish, *J. Physiol.*, 1959, **145**, 289.

HAGIWARA, S., and BULLOCK, T. H., Intracellular potentials in pacemaker and integrative neurons of the lobster cardiac ganglion, *J. Cell. and Comp. Physiol.*, 1957, **50**, 25.

HAGIWARA, S., WATANABE, A., and SAITO, N., Potential changes in syncytial neurons of lobster cardiac ganglion, *J. Neurophysiol.*, 1959, **22**, 554.

HAMA, K., Some observations on the fine structure of the giant nerve fibers of the earthworm, *Eisenia foetida*, *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 61.

HAMA, K., Some observations on the fine structure of the giant fibers of the crayfishes (*Cambarus virilus* and *Cambarus clarkii*) with special reference to the submicroscopic organization of the synapses, *Anat. Rec.*, 1961, **141**, 275.

HAMA, K., Some observations on the fine structure of the giant synapse in the stellate ganglion of the squid, *Doryteuphis bleekeri*, *Z. Zellforsch.*, 1962, **56**, 437.

IRISAWA, H., and IRISAWA, A., The electrocardiogram of a stomatopod, *Biol. Bull.*, 1957, **112**, 358.

IRISAWA, H., IRISAWA, A., MATSUBAYASHI, T., and KOBAYASHI, M., The nervous control of the intracellular action potential of the *Squilla* heart, *J. Cell. and Comp. Physiol.*, 1962, **59**, 55.

ITO, M., The electrical activity of spinal ganglion cells investigated with intracellular microelectrodes, *Japan. J. Physiol.*, 1957, **7**, 297.

KAO, C. Y., and GRUNDFEST, H., Postsynaptic electrogenesis in septate giant axons. I. Earthworm median giant axon, *J. Neurophysiol.*, 1957, **20**, 553.

MATSUI, K., Spontaneous discharges of the isolated ganglionic trunk of the lobster heart (*Panulirus japonicus*), *Scient. Rep. Tokyo Kyoiku Daigaku, Sect. B*, 1955, **7**, 257.

MAYNARD, D. M., Activity in a crustacean ganglion. I. Cardio-inhibition and acceleration in *Panulirus argus*, *Biol. Bull.*, 1953, **104**, 156.

MAYNARD, D. M., Activity in a crustacean ganglion. II. Pattern and interaction in burst formation, *Biol. Bull.*, 1955, **109**, 420.

MAYNARD, D. M., Circulation and and heart function, *in* The Physiology of Crustacea, (T. H. Waterman, editor), New York, Academic Press Inc., 1960, **1**, 161.

RUSHTON, W. A. H., Reflex conduction in the giant fibres of the earthworm, *Proc. Roy. Soc. London, Series B*, 1946, **133**, 109.

SHIBUYA, T., On the impulse of regulator nerves of the *Squilla* heart, *Zool. Mag. Japan*, 1957, **66**, 128 (in Japanese).

SHIBUYA, T., On the pace maker mechanism of the heart of the squill, *Squilla oratoria* de Haan, *Japan. J. Zool.*, 1961, **13**, 221.

SVAETICHIN, G., Analysis of action potentials from single spinal ganglion cells, *Acta Physiol. Scand.*, 1951, **24**, suppl. **86**, 23.

TAUC, L., Stimulation du soma neuronique de l'Aplysie par voie antidromique, *J. physiol., Paris*, 1957, **49**, 973.

TAUC, L., Site of origin and propagation of spike in the giant neuron of *Aplysia*, *J. Gen. Physiol.*, 1962, **45**, 1077.

WATANABE, A., The interaction of electrical activity among neurons of lobster cardiac ganglion, *Japan. J. Physiol.*, 1958, **8**, 305.

WATANABE, A., and BULLOCK, T. H., Modulation of activity of one neuron by subthreshold slow potentials in another in lobster cardiac ganglion, *J. Gen. Physiol.*, 1960, **43**, 1031.

WATANABE, A., and GRUNDFEST, H., Impulse propagation at the septal and commissural junctions of crayfish lateral giant axons, *J. Gen. Physiol.*, 1961, **45**, 267.

WIERSMA, C. A. G., Giant nerve fiber system of crayfish, a contribution to comparative physiology of synapse, *J. Neurophysiol.*, 1947, **10**, 23.

WILSON, D. M., The connections between the lateral giant fibers of earthworms, *Comp. Biochem. Physiol.*, 1961, **3**, 274.