Electrical Interaction of Paired Ganglion Cells in the Leech

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ABSTRACT The two paired giant ganglion cells (PGC's) found in each ganglion of the leech central nervous system fire synchronously in response to certain sensory input. Polarizing current passed into either of these cells is seen to displace the membrane potentials of both cells, the voltage attenuation between the two somata ranging from 2 to 5 times. This attenuation factor remains unchanged when the direction of the polarizing current is reversed, and remains about the same when the other cell of the pair is directly polarized. When one of the PGC's is partially depolarized with outward current, a repetitive train of impulses is generated. Each spike is followed by a spike in the other cell. Occasionally, a small subspike potential is seen in place of a follower spike. This potential appears to differ in shape and time course from synaptic potentials elicited by afferent input to these cells, and appears rather to be an electrotonic potential derived from the prejunctional impulse in the stimulated PGC. It is proposed that transmission between these cells is electrical, being accomplished by a flow of local circuit current across a non-rectifying junction or connection to the spike-initiating region of the other PGC.

INTRODUCTION

Electrophysiological studies have revealed that junctional transmission is commonly accomplished by presynaptic liberation of a transmitter chemical which alters the ionic conductance of the postsynaptic membrane (1–3). It is generally agreed now that in these junctions local circuit currents from the presynaptic action potential play a very minor role, if indeed any at all, in postsynaptic electrogenesis. A number of cases are now known, however, in which excitable cells influence each other by direct spread of electrical current from one cell to the other (4–12). In some of these cases the interaction is so pronounced that the cells behave as a functional unit, an impulse in one always producing an impulse in the other (4–7). A special case is seen in the rectifying electrical synapse of the crayfish lateral giant

axons with the giant motor fibers, where transmission occurs in one direction only (13). In other examples the amount of current passing between two cells is below threshold so that the cells can fire independently (8–12). In certain such cases it has been proposed that the subthreshold interactions may serve to loosely synchronize bursts of activity in a group of neurons.

The present paper describes electrotonic interaction between two prominent unipolar neurons in the segmental ganglia of the leech, *Hirudo medicinalis*. Sufficient current flows between these cells so that an action potential in one readily triggers an action potential in the other. The result is a synchronous discharge of the paired ganglion cells (PGC's).

MATERIALS AND METHODS

The Preparation The central nervous system of *Hirudo* consists of 21 segmental ganglia and the fused ganglionic masses in the head and tail, joined together by paired connectives. The entire central nervous system is enclosed in a tough transparent neurilemma, which contains longitudinal muscle fibers. The cord is further enclosed by a loose outer sheath having a heavily pigmented and coarse fibrous appearance. The lumen of the loose sheath forms the ventral sinus and hence serves to bathe the cord in blood.

At the center of each ganglion is a large neuropil. Clustered ventrally and laterally around the central mass of neuropil and fiber tracts are six distinct compartments of ganglion cells (see Fig. 1). Within each compartment (or "capsule") the pear-shaped unipolar cell bodies lie in an apparently clear fluid medium. Each ganglion cell sends its axon centrally through the compartment wall to join the central fiber tracts. The ganglion gives off two pairs of lateral roots. The anterior root innervates circular muscle, whereas the posterior root innervates the longitudinal muscles. Both roots also innervate the vascular system and viscera.

Lying in the ventral anterior compartment of the ganglion are the two large ganglion cells (the "colossal cells" of Retzius, 14) with which this paper is concerned (see Fig. 1). They can readily be distinguished because of their size (each *ca.* 70 micra) from the surrounding cell bodies. Gaskell (15) found that these cells gave a chromaffin reaction, and postulated that they contained adrenalin. Retzius' drawings indicate that each sends processes into both homolateral roots, and one branch into the posterior connective. There is no report of anatomical connection between the cells.

Methods A length of worm, several segments long, was cut open longitudinally along the dorsal line and pinned out under an appropriate Ringer saline. With the help of fine iridectomy scissors, a section of the cord including several ganglia was removed from the ventral sinus and transferred to a shallow recording chamber containing saline. The cord was held ventral side up by gently weighing down the lateral roots and connectives with short (2 mm) lengths of fine (0.25 mm) silver wire. Under these conditions the cord exhibits a bothersome degree of spontaneous movement arising from contractions of the longitudinal muscle fibers in the sheath; moreover,

electrical stimulation of the roots or the cord itself results in violent contractions. Adding 0.01 per cent tubocurarine chloride to the preparation effectively eliminated muscular responses to electrical stimulation, but did not completely eliminate the spontaneous movements. 5 per cent ethanol eliminated both spontaneous and evoked muscular activity. The addition of ethanol or tubocurarine did not have any apparent effect on the electrical activity of the ganglion cell. Either tubocurarine or ethanol was therefore used during the experiment.

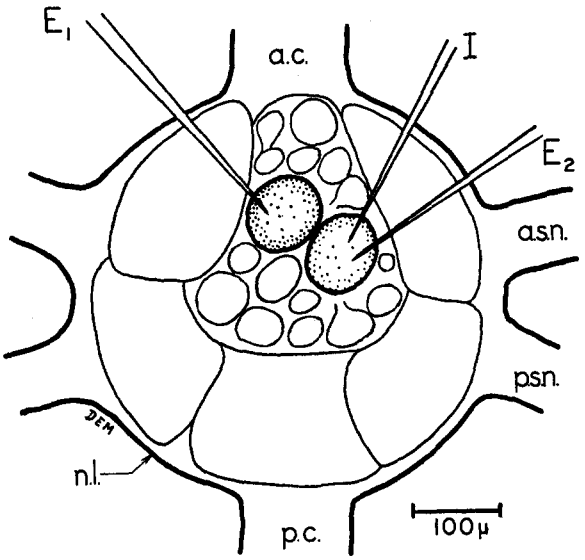

FIGURE 1. Diagrammatic representation of the paired giant ganglion cells lying in the anterior ventral compartment of an abdominal ganglion of *Hirudo*. Recording electrodes (E_1, E_2) and polarizing electrode (I) are shown in the experimental positions. For the sake of simplicity, only the cells of the anterior ventral compartment are drawn in. The remaining five compartments are shown in outline only. *a.c.*, anterior connective; *p.c.*, posterior connective; *a.s.n.*, anterior segmental nerve; *p.s.n.*, posterior segmental nerve; *n.l.*, neurilemma.

In small animals it was usually possible to penetrate the sheath with the electrodes. In some preparations, however, it was necessary to make a small tear in the sheath over the PGC's by using fine forceps.

Penetrations of PGC's were made under 50 × magnification and with the aid of darkfield illumination. Both recording and current-passing electrodes were of the glass capillary type, filled with 3 molar KCl, having a resistance of 15 to 40 megohms. Voltage recordings were obtained with the aid of two Amatniek negative capacitance feedback electrometer amplifiers. Current was passed from the isolation unit of a rectangular pulse generator through a 10^8 ohm stabilizing resistance and through the current electrode. A Keithley 603 differential electrometer amplifier, having an input impedance of 10^{14} ohms, was used to monitor the current across the 10^8 ohm resistor. The outputs of the voltage and current amplifiers were simultaneously dis-

played and photographed on the screen of a multiple trace cathode ray oscilloscope. The PGC which was polarized directly by means of the current electrode will be designated as cell *a*, whereas the other cell of that pair will be referred to as cell *b*.

When it was necessary to excite the PGC's synaptically by afferent stimulation, one of the lateral roots was sucked up into a pipette having a lumen slightly larger than the nerve. Stimulating current pulses were then passed between a silver–silver chloride electrode inside the saline-filled pipette and a similar electrode immersed in the bath just outside the pipette tip.

The saline used in these experiments is a modification after Gaskell (15), and is essentially a diluted Ringer-Locke solution: 6.90 gm NaCl; 0.30 gm KCl; 0.18 gm $CaCl_2$; 0.12 gm $NaHCO_3$; 100 ml 0.1 molar tris buffer, pH 7.4; and distilled water to a final volume of 1 liter. The experiments were performed at an ambient temperature of 20–25°C.

RESULTS

Resting and Action Potentials

When a recording electrode was inserted into one of the giant cell bodies a resting potential of 30 to 60 mv was observed. Sometimes impalement was followed by a transient train of impulses. These rarely exceeded 30 mv in amplitude, and were more often between 15 and 25 mv. On a very few occasions slightly overshooting spikes were noted immediately after impalement. With the passage of time, the recorded spikes frequently underwent a reduction in amplitude, occasionally to less than 10 mv. The lability of the recorded action potential is demonstrated in Fig. 2. A recording and a polarizing electrode were inserted into the soma of a PGC. When a sufficiently intense hyperpolarizing current pulse was applied to the cell, a rebound discharge was observed as the membrane potential returned toward its resting level. Along with an increase in the number of spikes, the amplitude of the recorded impulses became greater with increased intensity of the hyperpolarization. Together with the general failure to record overshooting spikes, this observation suggests that the impulses recorded from the cell soma are due to passive current spread from an active region of the neuron. This may also contribute to the long (10 to 15 msec.) duration of the spikes.

Following the spike there was often a pronounced undershoot (Figs. 2, 4, 7, and 8), which may indicate that the PGC's did not have their normal resting potential under the experimental conditions. Since the saline is not based on direct analysis of leech body fluid, it may be at fault.

Synchrony of Discharge

When simultaneous voltage recordings are made from both PGC's, it is seen that either they are both silent, or a spike in one is normally closely

accompanied by a spike in the other (Figs. 3 and 4 B). This is true of synaptically evoked impulses as well as those due to direct depolarization of the cell membrane by applied current or injury. The spikes were not perfectly synchronized; one normally followed the other with a delay of 1 to 10 msec. In most cases the delay was about 2 msec. Occasionally a spike in the following cell was replaced by a smaller potential having 10 to 25 per cent

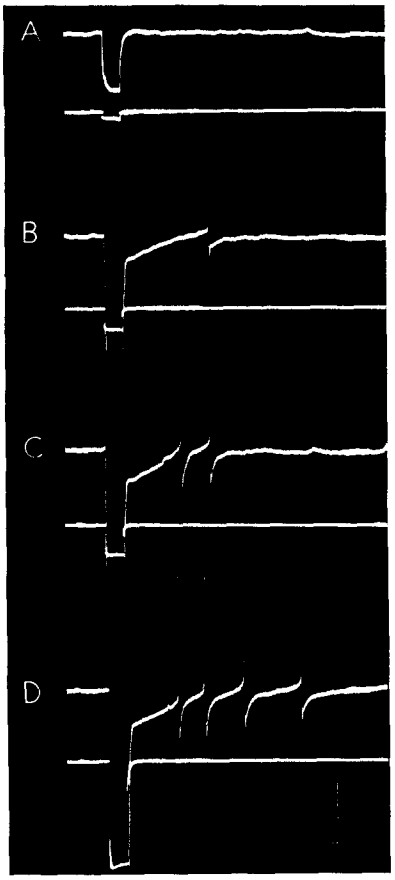

FIGURE 2. Lability of recorded action potential amplitudes during rebound excitation following hyperpolarizing pulses. Upper trace in each record was obtained by intracellular recording from the soma of a PGC. Lower trace in each record monitored the current that was passed into the same cell. Amplitude of hyperpolarization increased from record A to D. As the degree of hyperpolarization increased, the amplitude of the rebound-elicited spikes was increased, along with the number of spikes. Dashed line in record D represents the zero potential level determined by withdrawing the recording electrode from the cell. Calibrations: time, 200 msec.; voltage, 10 mv; current, 10^{-8} amp. In this figure and those that follow, both voltage and current calibration are indicated by a single vertical line.

the amplitude of the spike (Figs. 7 and 8). This small potential was found (see below) to be due to electrotonic currents arising from the impulse in the other PGC.

Intercellular Current Flow

It was possible to introduce into either of these cells (cell *a*) another electrode for passing current, as shown in Fig. 1. When a rectangular pulse of inward (anodal) current was delivered with the current electrode, a resulting hyperpolarization was recorded in that cell (Fig. 4 A, middle trace), and a some-

what attenuated hyperpolarization was simultaneously recorded from cell *b* (Fig. 4 A, upper trace). In the experiment illustrated, a current of 2×10^{-9} amperes hyperpolarized the soma membrane of cell *a* 24 mv while the potential of cell *b* was displaced about 8 mv. In this preparation the attenuation factor was therefore 3. This value is quite typical, but ratios ranged from 2 to occasionally as much as 5.

Outward (cathodal) current of equal magnitude passed into the same cell caused a depolarization and evoked a train of spikes (Fig. 4 B). These were closely followed by spikes in cell *b* superimposed on a small steady depolarization.

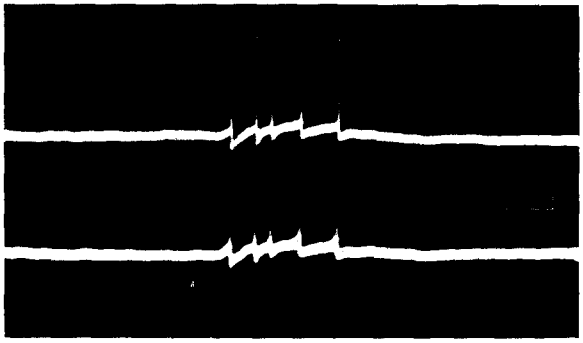

FIGURE 3. Simultaneous recording of spikes in both PGC's of one ganglion. In this preparation the head end of the worm along with the length of the nerve cord was dissected free from the remainder of the body. At approximately the time indicated by the arrow, the skin on the head region was pinched with forceps to elicit a train of spikes in the PGC's several segments away. Other noxious stimuli to the skin (*e.g.*, chemical stimulation with salt) will give similar responses. Note the synchrony of discharge. Calibrations: time, 500 msec.; voltage, 10 mv.

The possibility of recording artifacts was ruled out as follows. Either the recording electrode was withdrawn from a cell leaving the current electrode in place, or the current electrode alone was withdrawn. In either case the polarizing potentials disappeared. Under experimental conditions the two somata frequently appeared to be in contact with each other. If the cell bodies were gently pulled apart with the electrodes, the attenuation factor did not change significantly. The electrical connection between the PGC's is apparently somewhere other than at the somata.

Voltage Relationships of the PGC's

By inserting the current electrode first into one cell and then into the other, it was found that the spread of potentials between the two cells was typically similar in the two directions. This is illustrated in Fig. 5. The small differences that did occur in some preparations (as seen, for example, in Fig. 6)

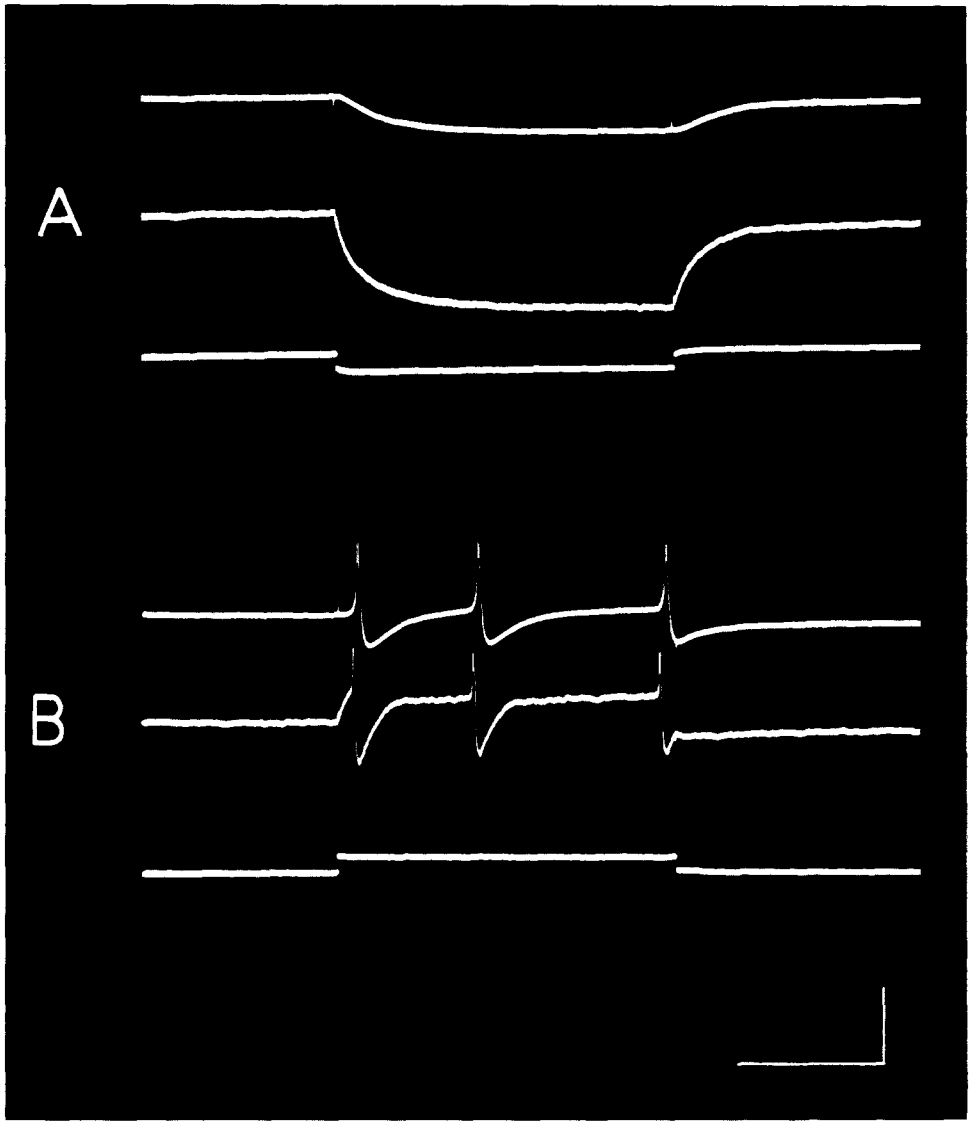

FIGURE 4. Passage of steady current between the paired ganglion cells.

A. Experimental arrangement as shown in Fig. 1. One current electrode and one recording electrode were in the PGC represented by the middle trace. During passage of current pulse (lower trace), this cell was hyperpolarized. At the same time the other cell of this pair (upper trace) underwent a similar, but somewhat attenuated, hyperpolarization.

B. In the same preparation the direction of the current was reversed so as to depolarize. Again the potential shift in the upper trace cell was an attenuated facsimile of the potential shift in the PGC being directly displaced. Note the synchrony of spike discharge. After withdrawal of the current electrode to a point just outside the neuron, the recorded responses vanished. Calibrations: time, 100 msec; voltage, 20 mv; current, 10^{-8} amp.

are to be expected, since the cells are not of precisely the same size, and therefore do not serve as completely symmetrical resistances.

Occasionally the attenuation factor increased markedly after the current electrode was withdrawn from one cell and was inserted into the other PGC. Such an increase in attenuation was found to be associated with a lowered membrane resistance, presumably due to injury to the membrane

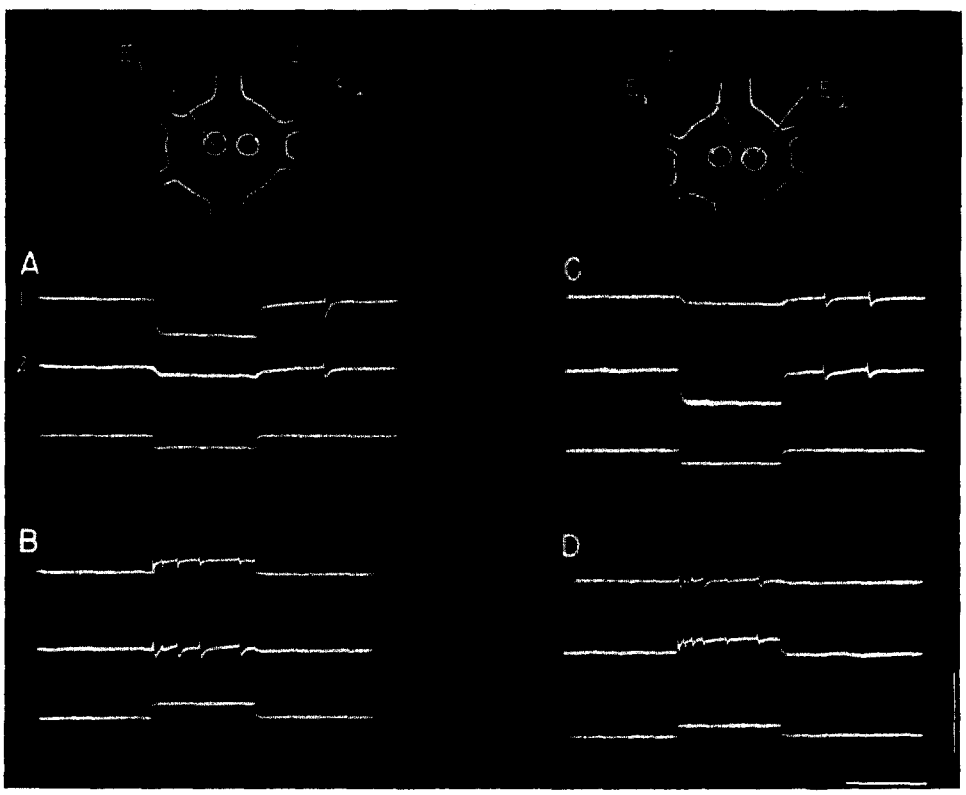

FIGURE 5. Reciprocal nature of electrotonic potential changes in the PGC's. The diagrams indicate the experimental arrangements used to make the recordings below each drawing. Calibrations: time, 50 msec.; voltage, 50 mv; current, 2.5 × 10^{-8} amp.

by insertion or removal of the current electrode. In one example, the attenuation ratio with the current electrode in cell *a* (Fig. 5) was 1.7 while the effective resistance, determined by dividing the cell *a* potential change by the applied current, was 4.3 × 10^6 ohms. After the current electrode was removed from *a* and inserted into soma *b*, the resistance calculated at *b* was 4.8 × 10^6 ohms, while the attenuation ratio was 4.0. On returning the current electrode to soma *a*, it was found that the effective resistance of

a was now only 2.8×10^6, and that the attenuation ratio had increased to 3.7.

A linear relationship exists between the potential changes in the two cells. In the example shown in Fig. 6 the attenuation factor was 2.7 when current was passed in the first cell, and 2.1 when current was introduced into the other cell. The attenuation factor remains constant as the current direction is reversed. This lack of current rectification is consistent with the observa-

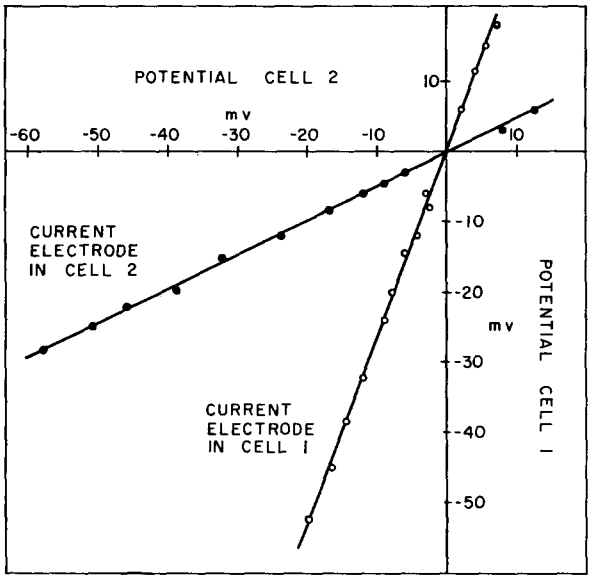

FIGURE 6. Voltage-voltage data obtained in a single experiment. First one cell of the pair (cell 1) was directly polarized to various levels. These potential shifts were plotted against the concomitant potential changes in cell 2, resulting in the open circles. The polarizing electrode was then inserted into cell 2 in order to obtain the data indicated by solid circles. Note the absence of rectification.

tion that electrical transmission of impulses between these cells (see below) proceeds equally well in both directions.

Electrical Transmission of Impulses between PGC's

Since the recording electrodes were not at the site of spike initiation and propagation, it was not possible to determine the magnitude of the critical potential for spike formation or the true size of the conducted impulses themselves. Nor can it be said how close to the site of junction the impulses conduct. However, it was possible to determine several aspects of spike transmission between the PGC's.

It has already been shown that an impulse in one cell does not always lead to an impulse in the other PGC. When the second cell fails to fire, one sees in place of the 10 to 30 mv spike a small subspike potential having 10 to 25 per cent of that amplitude (Figs. 7 and 8). This small potential bears some resemblance to synaptic potentials which result from the action of transmitters at synapses. It is therefore necessary to consider the possibility that these potentials, too, are chemically triggered. If this were the case, each of the two PGC's of a pair would have to have chemically transmitting

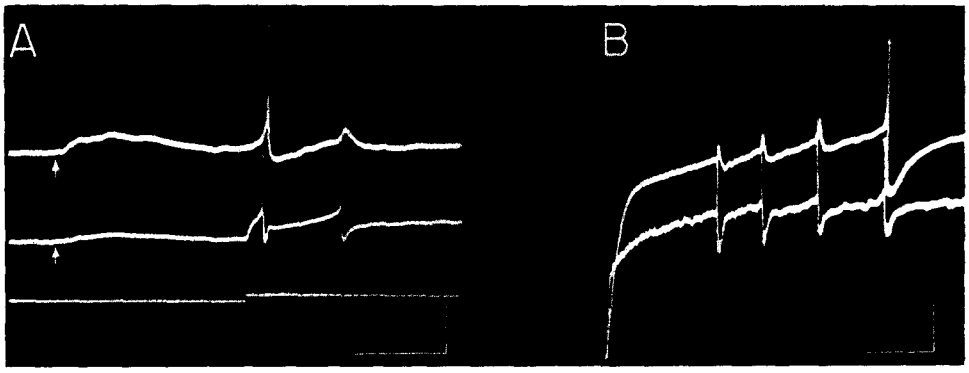

FIGURE 7. A. The nonidentity of the classical postsynaptic potential and the subspike potential associated with spike activity of the PGC's. The soma represented by middle trace (cell *a*) is impaled with a polarizing electrode in addition to a recording electrode. Bottom trace shows hyperpolarizing current already on at beginning of record, and off just past the center of the trace. Cessation of the polarizing current results in a rebound discharge in cell *a*. The first spike electrotonically triggers a spike in cell *b* (upper trace), whereas the second cell *a* spike elicits only a small subspike electrotonic potential in cell *b*. In order to compare the subspike potential with a p.s.p., cell *b* was synaptically excited by stimulation of afferent fibers. The arrows indicate the faint stimulus artifacts associated with the 0.5 msec. pulse applied to one of the segmental roots of the ganglion. Because of the hyperpolarizing current, the resulting postsynaptic potential does not reach the critical level, and so is uncomplicated by generation of a spike. This potential is probably due to asynchronous presynaptic discharge, and thus is not strictly comparable to the single electrotonic subspike potential. Nevertheless, it appears that there are differences in the rates of rise and decay between the two kinds of potentials. Calibrations: time, 50 msec.; voltage, 10 mv; current, 5×10^{-9} amp.

B. Different preparation. Polarizing electrode in cell *a* (lower trace). Cell *a* was discharging at a steady rate (prior to photograph) possibly owing to injury depolarization. A hyperpolarizing current pulse (off screen) inhibited discharge until current ceased. As cell *a* membrane potential returned to previous level, spike discharges resumed. The first three spikes resulted in subspike electrotonic potentials recorded in soma *b* (upper trace). During this time the electrotonic potentials did not attain the critical spike-generating level at the spike-initiating region. The fourth cell *a* spike triggered a spike in cell *b*, indicating that as the cell *b* membrane returned to its previous potential level, the electrotonic potential was able to attain the critical level for spike initiation. Calibrations: time, 100 msec.; voltage, 10 mv.

endings on some portion of the other, and an impulse in either one would elicit a synaptic potential in the other. In addition, a low resistance electrical pathway would have to exist between the two cells in order to allow the spread of current from one cell to the other which has been demonstrated above. Since the unrectified spread of current from one cell to the other is sufficient to explain the observed subspike potentials, chemical transmission between the PGC's is an unnecessary postulate. Moreover, comparisons in the same preparation of postsynaptic potentials due to afferent input (presumably transmitter induced) and subspike potentials due to activity in the twin PGC indicate some differences in time course and shape (Fig 7). Most important is the small negativity following the positive (upward) electrotonic postjunctional deflections. This small downward deflection is correlated with a similar, but larger, undershoot in the prejunctional PGC spike (Figs. 7 B and 8 A). It seems reasonable to conclude that the small subthreshold potentials which occasionally replace spikes during transmission

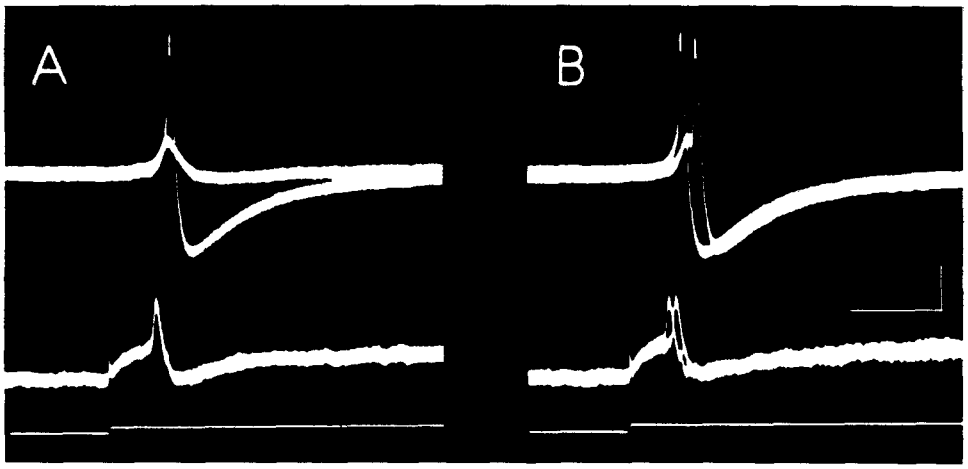

FIGURE 8. Spike initiation from subspike electrotonic potential. Polarizing electrode in cell *a* (middle trace); current pulses monitored with bottom trace. Each figure shows two superimposed sweeps.

A. Depolarizing current step elicited an impulse in cell *a* which is recorded as a much attentuated electrotonic spike in the soma of that cell. In one of the two sweeps the cell *a* spike gave rise to a small electrotonic potential in cell *b* (upper trace), whereas in the other sweep the cell *a* spike was followed by a spike in cell *b*. The cell *b* spike clearly arose out of the electrotonic subspike potential.

B. Two sweeps in which the cell *a* spikes were not quite superimposed. One cell *b* electrotonic potential gave rise directly to a spike, while the other first elicited a momentary local response which in turn gave rise to an action potential. Calibrations: time, 50 msec.; voltage, 5 mv; current, 2.5×10^{-8} amp.

from one PGC to the other are the result of current spread from the propagating membrane of the active cell.

Current originating in the active membrane of one PGC must depolarize to a critical level the spike-initiating region of the other PGC if the second cell is to fire. If this threshold level is not attained, only the passive subspike potential will be recorded in the soma of *b*. This is demonstrated in Fig. 7 B.

Fig. 8 illustrates a preparation in which the safety factor for electrotonic spike initiation was apparently close to unity, resulting in frequent failure to record normal-sized spikes in soma *b* in response to impulses in neuron *a*. The lowest trace in records A and B shows an outward current step of 5×10^{-9} amperes being passed through a current electrode in soma *a*. This depolarized neuron *a* (middle trace) sufficiently to cause an impulse soon after onset of the current pulse. In one of two consecutive superimposed sweeps in Fig. 8 A the cell *a* spike was followed by a spike in neuron *b* (upper trace). In the other sweep a similar cell *a* spike failed to cause a spike in cell *b*; but in this case the electrode in soma *b* recorded in its place a subspike electrotonic potential originating with the spike in neuron *a*. The electrotonic potential at the impulse-initiating region of neuron *b* in this preparation was apparently very close at its peak to the threshold for spike initiation. In the two superimposed sweeps of Fig. 8 B each spike in cell *a* was successful in initiating a spike in cell *b*; however, one of the electrotonic potentials first gave rise to a local response before the spike "took off."

Earlier in this paper it was shown that for direct current an attenuation factor of 2 to 4 exists when current is experimentally applied to the PGC soma with a polarizing electrode. This ratio would certainly be smaller still if one could repeat the experiment with polarizing and recording electrodes nearer the site of electrical junction. The action potential is undoubtedly greater in amplitude in the neurite, close to the electrical junction, than is apparent when recorded from the soma. Moreover, with the shorter "cable distance" to the spike-initiating membrane of cell *b*, the current from the presynaptic spike must undergo less high frequency attenuation during electrotonic transmission than is apparent in recordings obtained from the postsynaptic soma.

In summary, transmission between the PGC's seems to consist of these steps:

Cell *a* spike potential → electrotonic currents spread to cell *b* across junction → subspike electrotonic potential in cell *b* → cell *b* spike potential.

If the subspike potential does not sufficiently depolarize the spike-initiating region of cell *b*, the final step will be blocked, and in that case no postsynaptic

spike will be generated. In preparations which are in good condition, one-to-one transmission is, however, the rule.

DISCUSSION

Although the PGC's might be described as electrically syncytial, it remains to be seen whether or not actual cytoplasmic continuity exists between these cells. The answer will most likely have to come from histological instead of physiological evidence. Hagiwara *et al.* (9) have suggested that "intercellular routes" may connect the electrically interacting cells of the lobster cardiac ganglion; however, histological confirmation of continuity is still lacking. On the other hand, a membrane-limited one-way electrical junction was demonstrated in the crayfish by Furshpan and Potter (13), and at the segmental septa of crayfish and earthworm giant axons (4–6). Watanabe and Bullock (8) have expressed doubt as to the proposed continuity between lobster cardiac ganglion cells. More recently, Furukawa and Furshpan (16) have demonstrated an electrical inhibitory junction on the goldfish Mauthner cell with apparent cellular boundaries. It is not unlikely that in the PGC's of the leech transmission occurs by way of unpolarized membrane-limited junctions between collateral processes in the neuropil.

Electrical interaction of the PGC's differs quantitatively from that of both the lobster cardiac ganglion cells and the fish supermedullary neurons. Watanabe and Bullock (8) have shown that the interaction in the cardiac ganglion is limited to slow subthreshold currents which have their effect primarily by feedback modulation of the burst frequency of the pacemaker cell. The frequency loss and the attenuation of the currents reaching the pacemaker cell are sufficient to preclude direct postjunctional generation of spikes. Similarly, the electrotonic interconnections of the supramedullary cells are thought to serve primarily to loosely synchronize the activity of these neurons. The relay type of transmission seen in the PGC's is, on the other hand, more like that which occurs between the segments of giant axons, and between the lateral giant fibers of the earthworm (7).

For what functional reasons do the PGC's fire in synchrony? Unfortunately, neither the afferent nor the efferent connections of the PGC's are known. The PGC's were excited when noxious stimuli were applied to the skin (Fig. 3), or when the segmental roots were stimulated (Fig. 7). The response of these cells to stimulation diminished as the point of stimulation occurred at greater distances. Their one-to-one transmission suggests that these cells may be concerned with bilateral coordination of an effector system having a rapid action. Gaskell (15) concluded from histological and pharmacological evidence that these cells were providing excitatory adrenergic innervation to the "hearts"—peristaltic blood vessels running longitudinally, one along

each side. Direct stimulation of the PGC's (unpublished experiments), however, did not have any noticeable effect on the peristaltic rate. At present the physiological role of the paired giant ganglion cells remains unknown.

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Note During the preparation of this manuscript there appeared a preliminary report by Hagiwara and Morita (*Fed. Proc.*, 1962, **21**, 361) describing a similar investigation of these cells. A more detailed account of their work is in press.

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