

Electrical Transmission among Neurons in the Buccal Ganglion of a Mollusc, *Navanax inermis*

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ABSTRACT The opisthobranch mollusc, *Navanax*, is carnivorous and cannibalistic. Prey are swallowed whole by way of a sudden expansion of the pharynx. The buccal ganglion which controls this sucking action was isolated and bathed in seawater. Attention was focused upon 10 identifiable cells visible on the ganglion's rostral side. Two cells were observed simultaneously, and each was penetrated with two glass microelectrodes, one for polarizing the membrane and the other for recording membrane potential variations. The coupling coefficients for direct current flow and action potentials of several identified cells were tabulated. Attenuation was essentially independent of the direction of current flow, but depended upon the relative size of the directly and indirectly polarized cells. The attenuation of subthreshold sinusoidally varying voltages increased with frequency above about 1 Hz. The coupling coefficient for spikes was lower than for DC due to greater high frequency attenuation. There is considerable similarity in the spontaneous PSP's of all cells, which is not due to the electrical coupling but to input from a common source. The 10 cells were not chemically interconnected but some were electrically connected to interneurons which fed back chemically mediated PSP's. The feedback can be negative or positive depending upon the membrane potential of the postsynaptic cell. We conclude that electrical coupling among the 10 cells plays a minor role in sudden pharyngeal contractions but that the dual electrical-chemical coupling with interneurons may be important in this respect.

INTRODUCTION

Direct electrotonic coupling between nerve cells has been demonstrated in a variety of vertebrate and invertebrate species (Bennett et al., 1967 *b*). In molluscan ganglia interactions of this type are encountered infrequently however (Tauc, 1959, 1969; Waziri, 1969). The following report is concerned with electrical coupling among several identifiable neurons in the *Navanax* buccal ganglion, and describes some of its electrophysiological characteristics.

METHODS

Specimens of *Navanax inermis*, a tectobranch mollusc found in California coastal waters (MacFarland, 1966), were obtained from Pacific Biomarine Supply Co. (Venice, Calif.) and ranged in length from about 7 to 25 cm. An outstanding feature of this very slow moving creature is its predatory behavior (Marcus, 1961; Paine, 1963), usually aimed at other opisthobranchs and members of its own species. Prey are swallowed whole by way of a sudden expansion of its relatively large pharynx. The buccal ganglion is located medially on the ventral surface of the pharyngeal mass and has direct control over this sucking action. Formed by two halves, the ganglion communicates with the rest of the nervous system via two cerebrobuccal connectives, and appears to innervate the pharynx symmetrically via 11 trunks.

In the present study the isolated buccal ganglion with its nerves and connectives was mounted with insect pins on paraffin in a small Lucite chamber filled with seawater at room temperature (22–25°C). Attention was focused upon 10 identifiable cells visible in the rostral side of the ganglion (Fig. 1). Four cells were identified repeatedly on the right side: (a) the laterally placed giant G-R (diameter about 600 μ); (b, c) two small cells (diameters about 100 μ) C-RV and C-RD, located near the commissure connecting the two halves of the ganglion; and (d) a medium sized cell M-R (diameter about 300 μ) located more lateral than C-RV and C-RD, dorsal and/or medial to the giant cell. Six cells were identified on the left side: (e) a laterally placed giant G-L; (f-h) three small cells C-LV, C-LM, and C-LD; (i and j) two medium sized cells M-LD and M-LV. In each case R and L denote the right and left side of the ganglion, respectively, while V, M, or D denote the relative ventral, medial, or dorsal positions, respectively.

Two cells were observed simultaneously. Each cell was penetrated either with two independent glass microelectrodes or with double-barreled microelectrodes filled with 2.5 M KCl or a saturated solution of K₂SO₄. One electrode polarized the mem-

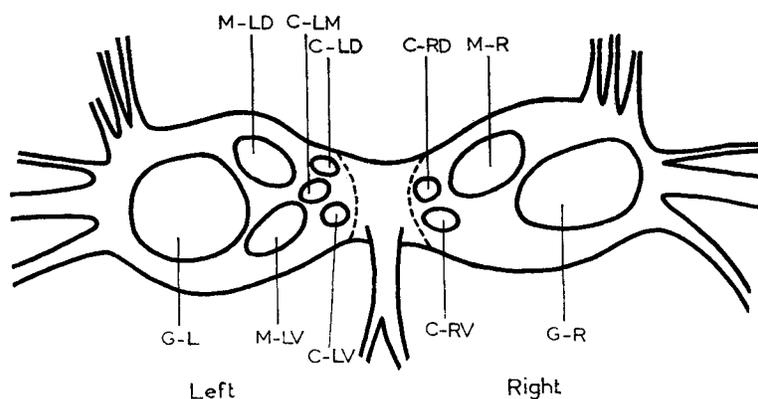


FIGURE 1. Schematic diagram of rostral face of *Navanax* buccal ganglion. Identifiable cells are labeled G, M, or C with secondary letters L and R referring to left and right halves of ganglion, and D, V, and M to relative dorsal, ventral, or medial position of like-sized cells in each half. The ganglion is about 2 mm in lateral extent.

brane and the other recorded membrane potential variations. The smaller C cells were more conveniently studied with the double-barreled electrodes after the connective sheath of the ganglion had been opened. The intracellularly recorded signals were lead off by unity gain cathode followers with negative capacitance compensation, and subsequently amplified for recording on a magnetic tape unit. Illustrations were reproduced on film or an ink recorder from the magnetic tape recordings.

RESULTS

A. Attenuation of Direct Current Flow Recording from two cells at a time the coupling characteristics between 25 of 45 possible pairs were observed. Fig. 2 A illustrates the response of the two giant cells, G-R and G-L, whose somas are in different halves of the ganglion, to a step of hyperpolarizing

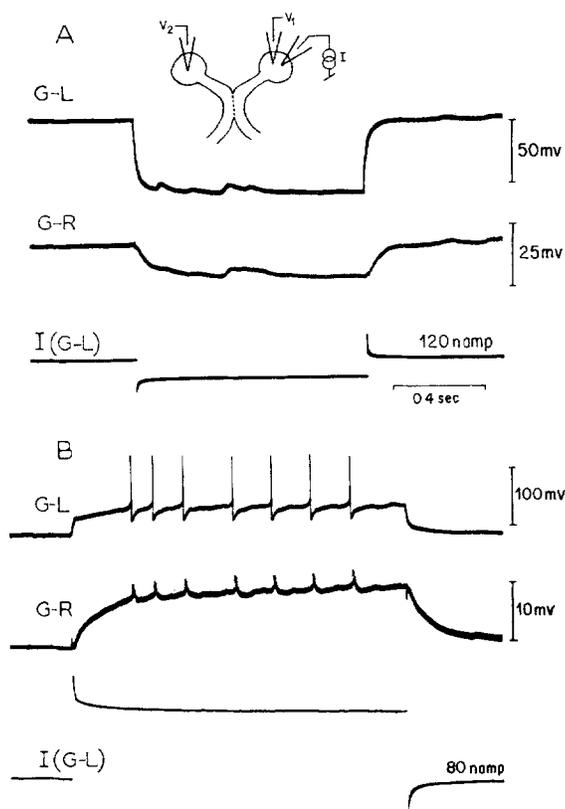


FIGURE 2. A, hyperpolarizing current step I of 120 namp applied to left giant cell, G-L, caused polarization changes in left, G-L, and right, G-R, giant cells, respectively. B, depolarizing current step I of 80 namp applied to G-L produced a depolarization in this cell sufficient to initiate spike activity. The G-R cell shows an attenuated step depolarization, upon which are superimposed brief deflections that are almost coincident with the spikes in G-L. Resting potential in both cells is about -60 mv. In Figs. 2-4 inset schematically shows placement of current and voltage electrodes in the two giant cells that presumably are connected along their axonal processes.

current (I) applied to G-L. The rate of rise of the hyperpolarization induced in each cell is a function of the resistance-capacitance characteristics of the membranes involved and is slower in G-R. A mathematical analysis of an equivalent electrical network has been made by Bennett (1966). Fig. 2 B illustrates the response of the same two cells in another animal to a step of depolarizing current sufficient to produce spiking in the directly polarized cell. Action potentials occurring spontaneously were never observed in the giant cells. Relatively large currents had to be injected in order to initiate spikes in these cells and this probably was due to the marked current drain provided by the large soma. Approximately coincident in time with each spike, the indirectly polarized giant cell shows fast, positive-going voltage deflections superimposed upon the step polarization. These deflections persisted after the ganglion was bathed in seawater containing about three times the normal magnesium (150 mM/liter in place of 50 mM/liter), an environment which quickly and reversibly suppressed all other synaptic potentials observed.

For all observed cell pairs the *ratio* of the steady-state change produced in the directly polarized neuron (primary change V_1) to that recorded in the other (secondary change V_2) depended little on the direction and intensity of the current flow. In Fig. 3 the amplitude of the polarization change produced in the giant cells is plotted as a function of the applied current. A delayed

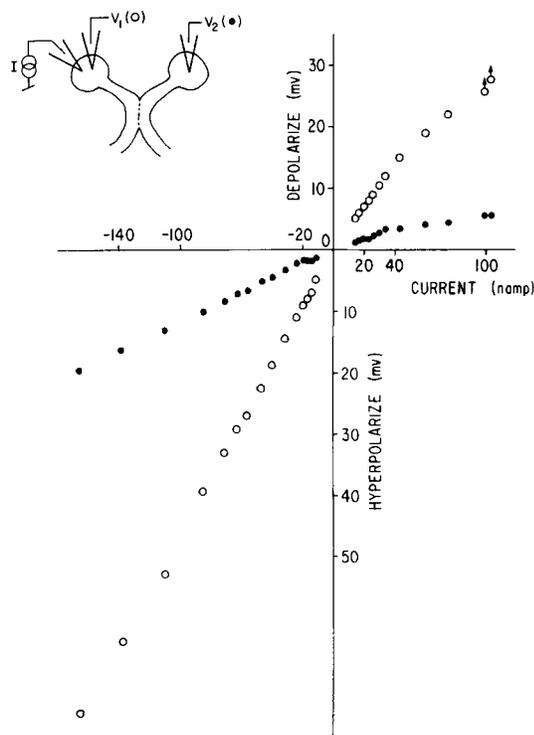


FIGURE 3. Steady-state polarization change (ordinate) in G-L (V_1) and in G-R (V_2) in response to hyperpolarizing or depolarizing current steps (abscissa) applied to G-L. Upward arrows over certain V_1 data points indicate that spikes were superimposed upon the step depolarization responses.

rectification (Hodgkin et al., 1949) appears in the directly polarized giant cell at depolarization levels near to, but slightly more negative than the spike threshold, and this is reflected indirectly in the coupled cell. The anomalous rectification (Kandel and Tauc, 1966) that characterizes many molluscan neurons either was absent or represented less than a 20% increase in the conductance between the resting level and -100 mv.

The coupling coefficient of two cells has been defined as the ratio, $V_2:V_1$ between the secondary and primary steady polarizations (Bennett, 1966). Table I lists coupling coefficients of several identified cells, with each vertical

TABLE I
COUPLING COEFFICIENT FOR DIRECT CURRENT

	G-R	M-R	C-RV	C-RD	G-L	M-LV	M-LD	C-LV	C-LD	C-LM
G-R	X	0.17	0.01	0.03	0.25		0.1		0.02	0.02
M-R	0.5	X		0.02	0.10	0.01	0.03		0.02	
C-RV	0.03		X							
C-RD	0.1	0.04		X						
G-L	0.25	0.05	0.01		X	0.1	0.2		0.05	0.03
M-LV	0.02	0.04			0.5	X	0.06		0.01	0.01
M-LD	0.2	0.03			0.5	0.02	X		0.03	0.01
C-LV							0.02	X		
C-LD	0.14	0.04			0.25	0.04	0.08		X	0.01
C-LM	0.1	0.01			0.12		0.02		0.01	X

The coupling coefficient of two cells is the ratio between the secondary and primary steady polarizations. Cells are labeled G-R, M-R, etc. according to Fig. 1. Directly polarized cells are listed at the top of each vertical column. The coupling coefficients were in general higher and varied less from ganglion to ganglion when the connective sheath was not opened. Because of the possibility of injury during dissection the largest coefficient from 2 to 12 observations was entered in the tables. Those among M and G cells are probably more indicative of the coupling which actually exists in the intact animal since a greater number of observations were made on these large cells and they could be studied without dissection of the sheath. Since relatively fewer observations were made on C cells and these smaller cells were more susceptible to damage during dissection, coefficients of 0.01 may not be representative of the strength of coupling which these cells exhibit in the intact ganglion.

column corresponding to a particular polarized cell. Thus, for example, a direct polarization of G-R producing a primary change of 20 mv was seen as secondary changes of 10 and 5 mv in M-R and G-L, respectively. Hence the coupling coefficients between cell couplets G-R:M-R and G-R:G-L are approximately 0.5 and 0.25, respectively. When on the other hand M-R is polarized directly, and the response in G-R is observed, the coupling coefficient is close to 0.2; i.e., the response is attenuated by a factor of 5. The coefficient between the two giant cells is 0.25. The coupling coefficient between two cells depends therefore upon the relative sizes of the directly and indirectly polarized cells and is greater when the directly polarized cell is the larger of

the couplet (Hagiwara and Morita, 1962). This follows from an examination of the equivalent circuit (Bennett, 1966).

B. Attenuation of Sinusoidal Voltages Subthreshold sinusoidally varying voltages of different frequencies were applied to one giant cell and the response was recorded in the other. The Bode plot of attenuation as a function of frequency, shown in Fig. 4, indicates that between the giant cells signals up to about 1 Hz are attenuated about four times, and that above this frequency, the attenuation rises in a nonlinear manner. This relation between attenuation and frequency is characteristic of a transmission cable with resistance and

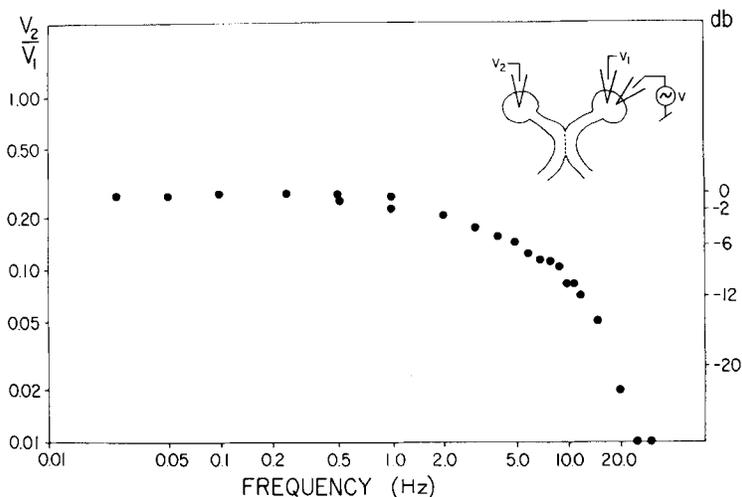


FIGURE 4. Bode plot of the attenuation (V_2/V_1) of a sinusoidal signal as a function of frequency. V_1 and V_2 are peak-to-peak voltages recorded in G-L and G-R, respectively. Ordinate at left is ratio of peak-to-peak voltage in indirectly (V_2) and directly (V_1) polarized cells. The same ratio is expressed in db at right ordinate, with 0 db corresponding to the asymptotic value approached at low frequencies.

capacitance distributed along its length, and is to be expected when the neuron behaves as a passive conductor. The phase difference between the primary and secondary sinusoidal voltages recorded in the two somas had the characteristics of a lag that increased with frequency, having values of about 18° at 1 Hz and about 70° at 20 Hz.

C. Transmission of Action Potentials When an action potential was recorded in the soma of one of the 10 identifiable cells, a fast, positive voltage deflection could often be detected in the other cell. When large enough to be seen clearly, the deflection was biphasic with an initial depolarization followed by hyperpolarization. Each phase had a time course somewhat more prolonged than that of the corresponding sign deflection of the soma spike in the

primary cell (Fig. 5). The hyperpolarizing phase of the secondary response may in part reflect the primary spike undershoot during the afterpotential.

Frequently a delay of several milliseconds was observed between the rising phase of the spike and the voltage deflection in the coupled cell (Fig. 5). If one assumes that the major contributor to the secondary response is the spike which occurs where the axons of the two cells are most closely associated, then a substantial part of the delay time can be attributed to the conduction time for the spike between the soma where it is recorded and the junction point.

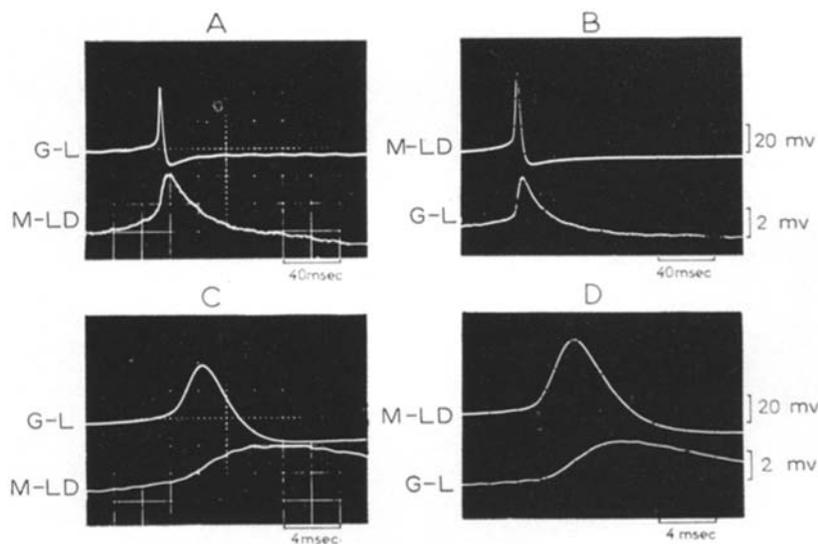


FIGURE 5. Spike transmission between electrically coupled cells, G-L and M-LD, bathed in high magnesium seawater. In A depolarization of G-L initiated a spike, which was seen as an attenuated voltage deflection in M-LD. In C the same potentials are shown at a 10 times faster sweep rate. A spike in M-LD produced a similar voltage deflection in G-L (B and D).

Although the time to onset of the passive voltage deflection is about the same whether the spike is produced in G-L or M-LD, the delay between the maxima of the spike and the passive voltage deflection is longer when the spike appears in cell G-L (Fig. 5 C) than when it appears in M-LD (Fig. 5 D). This difference might be because the two cells have different time constants, and/or the junction between the two axons is closer to the soma of cell G-L than to that of cell M-LD, since passive propagation of the maximum is slower than active propagation.

Cells G-L, G-R, and M-LV could be excited antidromically by stimulation of both the ipsilateral and contralateral nerve to the dorsolateral pharynx. However, antidromic spikes could be evoked in M-LD and M-R only by ipsilateral stimulation of this nerve. The cells, M-LD and M-R, located in

different halves of the ganglion, may therefore be coupled by virtue of their coupling with those cells which send axonal processes through the contralateral half of the ganglion. One cannot exclude, however, the possibility that cells, M-LD and M-R, send processes across the midline which do not emerge in the contralateral nerves.

The coupling coefficient for spike activity was considerably lower than for DC (Table II). This is due to the greater attenuation of high frequencies. However, the spike attenuation was less than that which one would have predicted from the plot of attenuation vs. frequency, if one assumed that a spike width of 5 msec is equivalent to a 100 Hz sinusoid. This inconsistency can be explained by the fact that subthreshold oscillations used to determine

TABLE II
COUPLING COEFFICIENTS FOR SPIKE ACTIVITY

	G-R	M-R	C-RV	C-RD	G-L	M-LV	M-DL	C-LV	G-LD	C-LM
G-R	X	0.05			0.08		0.01		0.01	
M-R	0.02	X			0.01					
C-RV	0.01		X							
C-RD				X						
G-L	0.08	0.01			X	0.02	0.07		0.01	0.01
M-LV					0.02	X				
M-LD	0.05				0.07		X		0.01	0.01
C-LV								X		
G-LD	0.03				0.05	0.01	0.01		X	
C-LM	0.01				0.02		0.01			X

The coupling coefficient for spike activity is the ratio of the peak of the positive voltage deflection in the secondary cell to the spike amplitude in the primary cell. Spikes appear in cells listed at top of each vertical column.

the Bode plot are electrotonically conducted along the axons of both cells to the other soma of the couplet, whereas a spike is conducted without attenuation along one axon, attenuation of the electrotonic effect occurring only between the axonal junction and the soma of the secondary cell. A similar conclusion was reached concerning the attenuation of high frequency components among puffer supramedullary neurons (Bennett et al., 1967 a).

D. Synaptic Input to Electrically Connected Cells In the isolated ganglion preparation, very complex spontaneous synaptic activity is recorded in the identified cells. At the normal resting levels of approximately -60 mv all such activity is depolarizing (i.e. positive-going). The inversion potential for much of this activity is about 5 mv more positive than the resting potential (approximately -55 mv) whether intracellular microelectrodes filled with KCl or K_2SO_4 are used. We call these IPSP's since the inversion potential is more negative than the threshold for initiating spikes.

There is considerable similarity in the spontaneous synaptic activity of the G and M cells (Fig. 6 A). The question arises whether the nearly identical potentials seen simultaneously in these cells are due to the low resistance electrical communications which enables most of them to reflect the synaptic activity received by any one, or results from the fact that all these cells receive

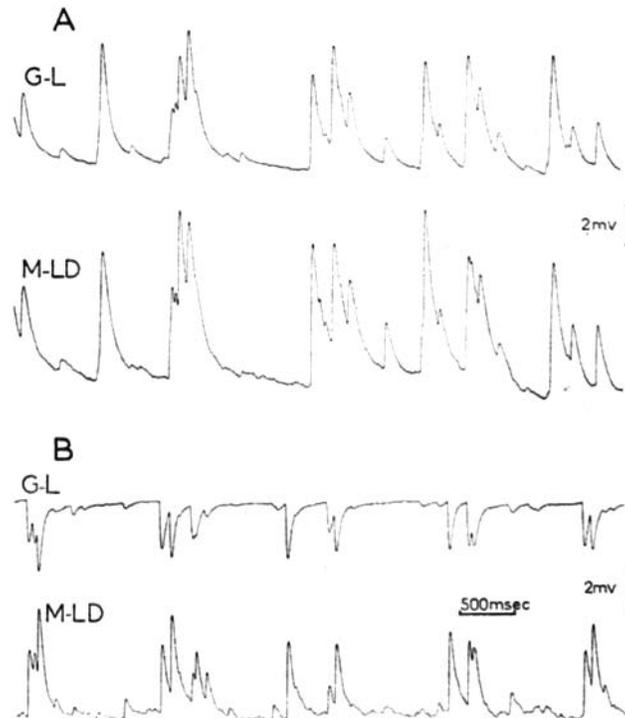


FIGURE 6. Spontaneous synaptic activity recorded simultaneously from cells G-L and M-LD. In A the membrane potential means are artificially maintained at average levels of about -86 mv in the somas of G-L and M-LD, respectively. In B the membrane potential of cell G-L is depolarized to -43 mv, beyond the inversion potential for IPSP's, while that of M-LD is maintained artificially at about -80 mv. Opposite sign of synaptic potentials indicates that these electrically coupled cells individually receive input from a common source.

separate synaptic input from a common source. The question was answered by depolarizing artificially one of the cells beyond the inversion potential for most of the observed spontaneous synaptic activity. Under these conditions the synaptic potentials, while still synchronous, are of opposite sign (Fig. 6 B). Therefore, these cells individually receive input from a common source in addition to being electrically connected.

The 10 electrically connected cells listed in Table I were not interconnected directly via chemical synapses. The frequency of the observed spontaneous

synaptic potentials often increased, however, when the large cells were subjected to a subthreshold depolarization. This suggests that the large cells are electrically connected with one or several unidentified interneurons which in turn produced synaptic potentials. We call such a neuron an interneuron because it makes synaptic contact with the identified neurons in the ganglion. In Fig. 7 simultaneous recordings are shown from an M and a G cell in opposite halves of the buccal ganglion. Subthreshold depolarization of the G cell not only depolarized the M cell indirectly but also activated a previously quiescent interneuron which provided synaptic input to the M and G cells. This newly aroused synaptic input is hyperpolarizing in the giant cell and

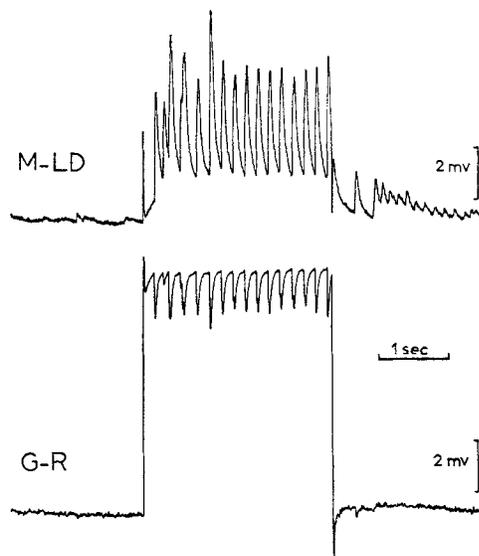


FIGURE 7. Simultaneous recordings from M-LD (top) and G-R cells (bottom). Direct depolarization of G-R produces attenuated depolarization in M-LD, and also excites an interneuron which feeds back hyperpolarizing PSP's to the former and depolarizing PSP's to the latter. Resting potential is about -60 mv in both cells prior to polarization of G-R.

depolarizing in the M cell. The opposite sign was due to the different levels of polarization of the two cells and not to differences in input function since the synaptic activity could be made hyperpolarizing in the M cell also if the steady-state level was less negative. Polarization changes in the M cell modulated the activity of the interneuron only if the latter was already active or had been brought close to activity by G cell depolarization. If the interneuron was silent and if the G cell was near its normal, -60 mv, resting level, an M cell depolarization of about 30 mv (which caused it to fire rapidly) did not excite the interneuron. It would seem therefore that the interneuron has better electrical coupling with the G cell than with the M cell and that the modulatory effect which the latter exerts on interneuronal activity is by way of the M cell: G cell coupling.

On one occasion an electrically coupled unidentified interneuron, located on the dorsolateral side of the ganglion, was penetrated together with an

M cell on the same side (Fig. 8). The interneuron was firing spontaneously and each spike was associated with a positive-going PSP in the M cell. Sub-threshold depolarization of the M cell increased the firing rate of the interneuron and correspondingly accelerated the synaptic input to the M cell (Fig. 8 A). The secondary polarization change in the interneuron was slight. More marked depolarization of the M cell (Fig. 8 B) made the latter fire, reversed the sign of the PSP's evoked by the interneuron, and increased their rate.

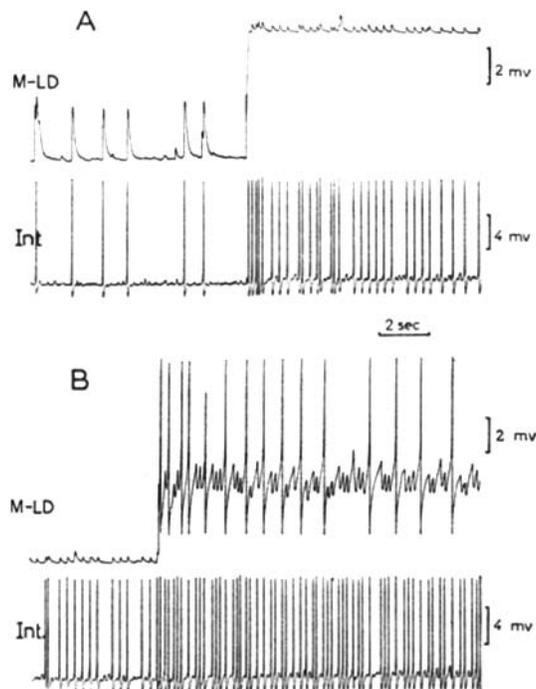


FIGURE 8. Simultaneous recordings from M-LD cell (top) and unidentified interneuron (bottom). The two cells are coupled both electrically and chemically. Each interneuronal spike produces a chemically mediated PSP in the M cell. Direct depolarization of M cell (A) from a resting potential of -65 mv accelerated interneuronal firing and thus the frequency of the PSP's. The resting potential of the interneuron is about -40 mv. Additional polarization of M cell beyond inversion potential for the PSP's (B) further accelerated the interneuron and therefore the PSP's. Spike amplitudes are attenuated because of high frequency limitations of ink recorder.

DISCUSSION

The role played by electrical coupling in the normal functioning of the organism has been frequently speculated about and rapid transmission favoring synchronous mass activity has been stressed (cf. Bennett et al., 1967 *b*). A relatively fast, synchronous contraction of the pharyngeal musculature is an

important component of the behavior of a *Navanax* capturing its prey. It is tempting to suppose therefore that electrical communication in the buccal ganglion plays an important role in this activity.

This does not seem to be tenable in the present case, however, and even if these cells are motoneurons, the syncytial role of the electrical coupling will be minimal. This conclusion is derived from the fact that most if not all these neurons receive considerable synaptic input from common interneuronal sources, and synaptic activity is most alike in neurons which are also most strongly connected electrically. Thus these junctions are not necessary for the transmission of the PSP's which they are best suited to pass. Action potentials are passed relatively poorly by the electrical junctions and, in the isolated preparation at least, rarely provoke secondary spiking. Moreover the giant cells, (G-L and G-R), which have the strongest coupling to other cells, have high thresholds and generate spikes rarely. In short, it seems that the electrical coupling between these neurons does not play a major role in the high degree of synchrony required for swallowing.

One consequence of the coupling is that synaptic input to *one* cell will be less effective in causing a polarization change than it would be if the same neuron were electrically isolated. This is because a larger membrane surface must be charged. In this sense the electrical coupling among the 10 largest cells does insure that the cells involved act in concert primarily when they receive input simultaneously.

The electrical coupling which may have the greatest interest from the integrative viewpoint is that between the large cells that have been identified and several unidentified interneurons which feed back synaptically upon the large cells. In the lobster cardiac ganglion (Watanabe and Bullock, 1960) the small pacemaker cells provide excitatory synaptic input to the large follower cells which in turn are electrotonically connected to the pacemakers. The feedback is thus positive. In the *Navanax* buccal ganglion the situation is somewhat more complex. Since the normal resting level of the large neurons is several millivolts more negative than the IPSP inversion potential, positive feedback is perhaps the most usual effect. If on the other hand, a giant cell polarization level is less negative than the inversion value, the IPSP's fed back will be hyperpolarizing and the feedback will be negative.

With such a coupling and feedback arrangement, only a rapid and strong depolarization, determined for example by a potential prey within striking distance, could effectively bring the large cells to spike threshold and initiate a discharge. The rapid and strong depolarization produced by an excitatory input should be common to the set of large cells, since they share PSP's and are electrically coupled. Only at this moment can the electrical coupling among the large cells contribute to their synchronization. The shared depolarization and especially that of the largest cells, would be transmitted electrically

to the interneurons. The discharge of these interneurons would elicit a negative feedback of inhibitory PSP's in the large cells. The IPSP's would reduce the depolarization of the large cells and abbreviate their firing. In this manner the proximity of a palatable prey could produce a brief burst of synchronous activity in the M cells and perhaps a sudden pharyngeal expansion. Slowly rising depolarization on the other hand would be prevented from exciting the M cells because of the earlier mobilization of the negative feedback.

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