THE INPUT-OUTPUT ORGANIZATION
OF A PAIR OF GIANT NEURONES IN THE MOLLUSC,
ANISODORIS NOBILIS (MACFARLAND)

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INTRODUCTION

One of the ultimate aims of neurophysiological research is the description of well-defined behavioural acts in terms of the input-output organization of single cells within a neural aggregate. As the basic tool of neurophysiology is the recording of bioelectric potentials, the ideal preparation for study should contain a manageable number of cells, organized in a small number of functional classes with representative members of each class susceptible to intracellular penetration. The central ganglia of the gastropod molluscs come close to satisfying these basic requirements (Tauc, 1966). They contain several thousand neurones, whose somata are densely packed around an inner core of axons—the neuropile. The recent studies of the abdominal ganglia of Aplysia (Frazier et al. 1967; Kandel & Wachtel, 1968) and the cerebral ganglia of Tritonia (Willows, 1968) have shown that an understanding of the functional organization of a large but well-defined group of nerve cells is possible. In the central ganglia it is often difficult to examine the complete output organization of cells because of the large number of neurones involved and the complex arrangement of their axons in the neuropile. Even simpler types of neuronal organization are found in the peripheral nervous system of molluscs which may be better suited for the investigation of elementary neural populations and the transmission of information in a neural aggregate. In the ‘sympathetic nervous system’ of the nudibranch molluscs a series of small ganglia and isolated nerve cells are linearly and symmetrically arranged along two nerves which supply the oesophagus (Alder & Hancock, 1845; Bergh, 1879). In Anisodoris nobilis (MacFarland) most of the cells are large enough to be investigated with one or more intracellular microelectrodes, and the linear organization of the peripheral nervous system makes practicable an investigation of the interrelationship between different cells. In this paper we describe the input-output relationship of two symmetrical giant cells which receive synaptic information from more central ganglia, and whose output participates in the control of an electrically interconnected population of peripheral neurones.

ANATOMY

The cells investigated are found in a chain of ganglia and nerves situated on the oesophagus of the nudibranch mollusc, Anisodoris nobilis (MacFarland) (Fig. 1). The most rostral members of this chain, the buccal ganglia, are attached by two long con-
nectives to the cerebral ganglia. The buccal ganglia give off nerves to the buccal bulb (pharyngeal nerves) and to the odontophore. The buccal ganglia are joined by a short connective to the smaller gastro-oesophageal ganglia (GOG) (Pl. 1 a) which contain the somata (200–400 μ in diameter) of a giant neurone (G cell) and of three medium-sized cells ($M_1$, $M_2$, and $S$ cells, 100–200 μ in diameter). Two nerves leave the GOG: the salivary nerve and the gastro-oesophageal nerve. The latter is divided into two major branches: the lateral branch (lateral nerve), which innervates the most rostral
part of the oesophagus, and the medial branch (medial nerve), which innervates the remaining part of this organ. Numerous large neurone somata (100–300 μ in diameter) are found scattered along the length of the medial nerve (P cells, Pl. 1b). In addition, several ganglia, each containing from 2 to 5 neurone somata, are also found along this nerve. Smaller nerves (oesophageal nerves) leave the medial nerve at irregular intervals and join with nerves from the opposite side to form a reticular network which covers the whole surface of the oesophagus (Pl. 1c). At the nodes of this reticulum another class of nerve cells (O cells) is found.

At the boundary of the oesophagus with the stomach the two medial nerves join together, thus effectively closing a ring. From the most caudal portion of this ring several large nerves are given off to the stomach and to the liver.

All the large neurones of the oesophageal ring contain a bright orange pigment and their dimensions, even of the smaller ones, are such that they can always be seen under a dissecting microscope. The axons of the major cells tend to have a characteristic irregular shape and thus can be easily followed in cross sections of the nerve. Serial reconstructions of sections revealed that the axon of the S cell runs in the salivary nerve, while those of the M cells and G cell run in the medial nerve. In addition, a rather large process of the G, S and M cells runs in the bucco-gastro-oesophageal connective to join the neuropile of the buccal ganglion.

Much smaller neurones, 10–20 μ in diameter, are found in the buccal and gastro-oesophageal ganglia. These neurones are always found in well-defined clusters and appear to constitute a distinct population. In the GOG the number of these small cells varies from 20 to 50 without any obvious correlation with the size of the ganglion. Small cells (30–50 μ in diameter) are sometimes found in the peripheral ganglia and often in the neighbourhood of the O cells. The scarcity of these elements and the fact that their presence is not constant indicates that these are smaller P or O cells instead of a separate population of cells.

**METHODS**

The buccal and gastro-oesophageal ganglia are located on the dorsal surface of the oesophagus at the line of its attachment to the buccal bulb. This region was exposed by opening the anterior third of the animal with a longitudinal incision along the midline of the dorsal wall. After isolating the oesophagus with the attached ganglia from the surrounding organs it was excised, its dorsal wall slit, and the whole oesophagus pinned flat on a paraffin dish for further dissection and isolation of the ganglia and nerves.

For the purposes of the present research, several types of preparations were used (see Fig. 1). In the simplest type one gastro-oesophageal ganglion was completely isolated with only the more proximal portion of the medial nerve left attached; alternatively, a longer part of the medial nerve containing one or more peripheral ganglia or cells plus the lateral and salivary nerves were left. In more complex experiments either one or both buccal ganglia with or without the contralateral gastro-oesophageal ganglion were left. Finally, the whole gastro-oesophageal ring as shown in Fig. 1 was studied intact.

The fine dissection of the ganglia and nerves was carried out under a high-power binocular microscope. As the distal portions of both the medial and lateral nerves are partially embedded in the oesophageal wall, particular attention had to be paid to this
part of the dissection, as it was easy to inadvertently damage the axons of the $G$ cell or peripheral cells. When isolated, both the oesophagus and nerve sheaths contract continuously, making fine dissection and subsequent long-term recording from some types of preparations difficult. In these cases the whole preparation was pre-exposed to a weak solution of chilled glutaraldehyde ($0.5\%$ in sea water) for a brief period (less than 45 sec.) to abolish contractions of the sheath and oesophagus. At this concentration and exposure time glutaraldehyde does not affect the membrane properties of nerve cells (Mirolli & Gorman, 1968).

Because of the small dimensions of the isolated GOG ($300-500\mu$) it was impossible to hold it for stable intracellular recording. A simple technique was used employing a mild negative hydrostatic pressure to hold the ganglion (Tyler et al. 1956). With this method the ganglion could be held firmly for indefinite periods with one or more microelectrodes inserted into cells.

Artificial sea water of known ionic composition (Aquarium System Inc., Wickliffe, Ohio, U.S.A.) was continuously circulated through the recording chamber by a battery-driven peristaltic pump. The temperature in the chamber was controlled by pre-circulating the artificial sea water at various speeds through an ice bath. As the animals (kindly furnished by Dr Rimmon Fay, Pacific Bio-Marine Co., Venice, California, U.S.A.) normally live throughout the year at a temperature between 10-13°C, the experiments were performed at temperatures between 10-11°C.

Visualization of the cells of the ganglia in the recording chamber was achieved by partial dark-field illumination. One or more $3\text{M}$-KCl-filled micropipettes ($8-10\,\text{M\Omega}$ resistance) were inserted through the intact sheath of the ganglion into the somata of cells under visual control. Intracellular signals were recorded with a negative capacitance amplifier, displayed on a 4-channel oscilloscope for photographic analysis, and simultaneously recorded with a rectilinear D.C. pen recorder. Nerve discharges were picked up with a fine insulated silver wire and amplified by a high-gain differential R.C.-coupled amplifier.

Intracellular stimulation was accomplished either through the same electrode used for recording with a modified Wheatstone bridge circuit (Araki & Otani, 1955) containing a $10^8$ or $10^9\,\Omega$ resistor; or, alternatively, in larger cells where two micropipettes could be inserted, through separate microelectrodes. Peripheral nerve stimulation was achieved by drawing the cut end of the nerve into a fluid-filled pipette, and applying short duration (1-2 msec.) stimuli between the holding pipette and adjacent pipette in the pool. The polarity of the stimulus was arranged so that the pool electrode was made the cathode and the holding pipette the anode (Gorman & Mirolli, 1968). Both intracellular and peripheral nerve stimulation were applied through battery-powered stimulus-isolation units and controlled with a digital timing device.

RESULTS

The axonal pathways of the GOG cells

The axonal pathways of the larger cells in the GOG were identified by recording the presence or absence of antidromic invasion following stimulation of various nerves (Tauc, 1957). In agreement with our anatomical findings (see Fig. 1), the results showed that the axonal paths of the two giant cells ($G$ cells) are symmetrical. The axon
of each G cell runs in the medial nerve (Fig. 2 A) for the full length of the oesophagus, passing through the several peripheral ganglia encapsulated in the sheath of the nerve. At the boundary of the oesophagus and stomach the axons bifurcate and run in each of the branches of the gastric nerve. The axonal pathways of the other larger cells in the GOG are also symmetrical. The M cells send their axons into the medial nerve (Fig. 2 B), while the S cell sends its axon into the salivary nerve (Fig. 2 C). None of the larger cells in the ganglion sends axons into the lateral nerve.

One difficulty in the identification of axonal pathways by antidromic stimulation should be mentioned. For some undetermined reason, perhaps related to their size, the axons of large cells appear to be more subject to trauma than the small axons in the nerve. Therefore, a small body of negative evidence can not be used to exclude a possible anatomical pathway, particularly when a difficult nerve dissection is involved.

**The Input to the G Cell**

*Input pathways through the Buccal Ganglion.* The G cells receive symmetrical inputs from several sources. Stimulation of either the ipsilateral or contralateral cerebral connectives was followed by a long-duration (> 1 min.), complex synaptic potential...
consisting of both hyperpolarizing and depolarizing components (Fig. 3A). In the early phase of the response the inhibitory phase dominated (Fig. 3A). This component was often succeeded by a depolarizing phase and cell discharge. Small fluctuations of potential often occurred during the response. Their origin and relationship to the long-lasting biphasic response has not been studied. Save for differences in latency the response evoked by both pathways on either cell was similar (Fig. 3B).

Often, after several stimuli, spontaneous inhibitory potentials occurred nearly synchronously in both cells at irregular intervals. The response evoked by pharyngeal nerve stimulation was also biphasic. However, in this case a small depolarizing potential preceded a long-duration hyperpolarization potential. Although the input from the cerebral connectives and pharyngeal nerves passes through the buccal ganglia

![Figure 3](image-url)
to reach the G cells, direct stimulation of many of the larger cells in the buccal ganglia failed to reveal a direct or synaptic connexion between these cells and the G cell.

The peripheral input to the G cells. The two main branches of the ipsilateral GOG nerve provide major synaptic inputs to the G cell. Stimulation of the medial nerve even at low intensities evoked strong synaptic excitation, often resulting in cell discharge in addition to the response due to antidromic invasion (Fig. 4A and C). This synaptic potential could be distinguished from the antidromic potential evoked by

![Image](image_url)

**Fig. 4.** The response of the gastro-oesophageal giant cell to medial nerve stimulation. In A, antidromic and synaptic responses. In B, the effects of intracellular hyperpolarization on the antidromic and post-synaptic response. The medial nerve response is shown between the current (top) and soma potential (bottom) response. In C, the prolonged synaptic response of the G cell which follows antidromic invasion.

stimulation of the same nerve by several methods. First, destruction of the G cell axon abolished antidromic invasion without affecting the occurrence of the PSP. Second, strong hyperpolarizing currents which blocked the S spike and reduced the amplitude of the A spike (Bennett, Crain & Grundfest, 1959; Tauc & Hughes, 1963) increased the amplitude of the excitatory post-synaptic potential (PSP) (Fig. 4B). Last, reversal of the polarity of the stimulus to the medial branch was usually followed by the PSP without evoking antidromic invasion (A. L. F. Gorman and M. Mirolli, in preparation). At high stimulus intensities the PSP was both more complex and very long in duration (Fig. 4C). Isolation of the GOG from the buccal ganglia did not affect the amplitude of the peripherally evoked PSP (Fig. 4C). This finding indicates that the pathway for the response does not depend on the presence of the buccal ganglion. Stimulation of the lateral branch of the GOG nerve also resulted in the appearance of a synaptic response in the G cell (Fig. 5A, B). At low stimulating intensities this response was complex, consisting of an initial excitatory PSP followed by a long-duration inhibitory potential (Fig. 5C, D). At higher stimulus intensities the response was dominated by strong excitatory synaptic drive (Fig. 5A), which often resulted in single or repetitive cell discharge.
Interaction between GOG cells. The synaptic input to the $G$ cells could be mediated by the $M$ or $S$ cells in the same ganglion. However, the results of transmembrane stimulation showed that there were no direct electrical or synaptic connexions between

![Fig. 5. The response of the gastro-oesophageal G cell to lateral nerve stimulation. In A, synaptically evoked discharge of the $G$ cell. In B, graded PSP's evoked by different intensities of stimulation. In C and D, simultaneous oscillographic (C) and pen-recorded responses (D) to show the long-duration hyperpolarizing which follows the depolarizing phase of the lateral response.](image)

![Fig. 6. Non-interaction between the major cells of the gastro-oesophageal ganglion. In A, stimulation of $M$ cell; current shown on top, $M$ cell response in middle and $G$ cell potential on bottom. In B, stimulation of the $G$ cell; $M$ cell potential shown on top, current in the middle and $G$ cell response on bottom. A and B are from the same experiment. In C, stimulation of the $S$ cell; $G$ cell potential shown above the $S$ cell response (no current trace shown). In D, stimulation of the $G$ cell; $S$ cell potential shown above the $G$ cell response. C and D are from the same experiment, time and voltage calibrations for A and B are shown in B; for C and D are shown in D.](image)

the large cells of the GOG. Stimulation of the $M$ cell while recording from the $G$ cell (Fig. 6A) showed the complete lack of direct or synaptic coupling between the two cells. Identical results were obtained when the stimulating-recording conditions were
reversed (Fig. 6B). Stimulation of the S cell while recording from the G cell (Fig. 6C) and vice versa (Fig. 6D) showed a similar lack of interaction.

**Connexions between G cells.** Stimulation of the contralateral medial and lateral nerves had no effect on the behaviour of the G cells. The results suggest that there are no direct or indirect synaptic connexions between the two cells. Direct confirmation of this supposition was obtained from experiments in which electrodes were placed in both G cells. Antidromic invasion of either cell, evoked by stimulation of the medial nerve, occurred without affecting its symmetrical partner (Fig. 7A, B). Similarly, repetitive discharge of a G cell evoked by transmembrane stimulation was ineffective in evoking PSP's in the contralateral G cell (Fig. 7C).

![Graph showing non-interaction of giant cells](image)

Fig. 7. Non-interaction of giant cells. In A, antidromic invasion of left giant cell (top) produced by stimulation of the ipsilateral medial nerve. Note lack of response in right giant cell (bottom). In B, from the same experiment, antidromic invasion of right giant cell (bottom) without a response in left giant cell (top) following stimulation of the left medial nerve. In C, alternating intracellular stimulation of right and left giant cells to show lack of direct interaction between the symmetrical cells. The fluctuations of potential shown in the bottom trace were spontaneous and not linked to the discharge of the other G cell.

**THE PERIPHERAL AND OESOPHAGEAL CELLS**

Experiments were performed on preparations in which regions of the medial nerve containing P cells or regions of the nerve and oesophagus containing O cells could be isolated. For purposes of identification, the portion of the medial nerve which is directed toward the GOG ganglion will be referred to as the central side and the opposite end of the nerve the peripheral side.

**The axonal pathway of the P and O cells.** The axons of the P cells are contained within the medial nerve; and, like the axon of the G cells, are directed toward the periphery. Stimulation of the peripheral side of the nerve elicited antidromic invasion of both G and P cells (Fig. 8). Antidromic invasion of P cells was never observed following
stimulation of the central side of the nerve. Occasionally $P$ cells were not invaded following stimulation of either side of the nerve. However, in these cases the possibility that some axonal elements were damaged during the dissection of the nerve or that the axons of the uninvaded cells exited in smaller branches of the nerve between the recording and stimulating points must be considered.

![Diagram](image)

*Fig. 8. Pathways of the peripheral cell axons. Antidromic invasion of two peripheral cells (top and middle traces) in different portions of the nerve (see insert) and the gastro-oesophageal giant cell (bottom trace) following stimulation of the peripheral end of the medial nerve.*

The axonal pathway of the $O$ cells has been incompletely explored. However, in the cells that have been examined, stimulation of the peripheral side of the medial nerve elicited an antidromic response suggesting that an axon (or an axonal branch) of the $O$ cell entered the medial nerve through one of the oesophageal nerve filaments and travelled away from the GOG and the more central ganglia.

*The excitatory response of the $P$ and $O$ cells.* The $P$ and $O$ cells receive an excitatory input from both the central and peripheral sides of the medial nerve. Stimulation of the central side of the nerve with single shocks elicited discharge of $P$ cells (Fig. 9A). Stimulation of the peripheral side of the GOG nerve also elicited PSP’s in the $P$ cells (Fig. 9B). Usually this PSP was difficult to examine in detail because of the complications introduced by the antidromic response. Following a train of stimuli the postsynaptic response appeared as a progressive and prolonged depolarization underlying successive antidromic invasions. In those $P$ cells that were not invaded antidromically the peripherally evoked PSP was clearly visible (Fig. 9C, bottom trace). The results shown in this figure also illustrate that at least two types of excitatory potentials occur following nerve stimulation. A larger PSP which could lead to cell discharge was preceded by a series of smaller potentials.

*Electrotonic connexions between $P$ cells.* While the larger PSP’s shown in Fig. 10C may represent a chemically mediated response, our evidence suggests that the smaller PSP results from electrotonic coupling between $P$ cells. Electrotonic spread between two adjacent $P$ cells is illustrated in Fig. 10A, B. Both cells were penetrated with single
microelectrodes connected to bridge circuits. Stimulation of one cell evoked a potential change in the membrane of the adjacent cell. The time course of the potential was considerably more rapid in the polarized cell than in the non-polarized one and the amplitude of the electrotonically coupled spikes much smaller (Fig. 10 A, E). While these two observations may be explained by a large membrane capacitance and a small coupling coefficient between the two cells (Bennett, 1966), there is good reason to assume that the electrotonic coupling between P cells is located far from the soma. Approximately the same degree of electrotonic couplings was observed between cells with distant as well as adjacent soma locations (Fig. 10 C, D). In every case the electrotonic spread of maintained polarization, as recorded in the cell soma, was much greater than the transmission of spike potentials.

![Fig. 9. The synaptic response of peripheral cells. In A, synaptically evoked discharge of two adjacent peripheral cells produced by stimulation of the central side of the medial nerve. In B, synaptically evoked discharge of two peripheral cells produced by stimulation of the peripheral side of the medial nerve. At the stimulus intensity used the cell shown in the bottom trace was antidromically invaded. In C, same experiment as shown in B: high gain recording to show depolarizing potentials evoked in a peripheral cell (bottom) by stimulation of the peripheral side of the medial nerve.]

**Fig. 9. The synaptic response of peripheral cells.** In A, synaptically evoked discharge of two adjacent peripheral cells produced by stimulation of the central side of the medial nerve. In B, synaptically evoked discharge of two peripheral cells produced by stimulation of the peripheral side of the medial nerve. At the stimulus intensity used the cell shown in the bottom trace was antidromically invaded. In C, same experiment as shown in B: high gain recording to show depolarizing potentials evoked in a peripheral cell (bottom) by stimulation of the peripheral side of the medial nerve.

**THE OUTPUT OF THE G CELLS**

*The synaptic connexion between G and P cells.* The PSP recorded from P cells following stimulation of the central side of the medial nerve can be partially accounted for by a monosynaptic connexion between the G and P cells. Discharge of the G cells was associated with the appearance of a post-synaptic response of the P cells (Fig. 11). This PSP was constant in amplitude and latency, unitary in character, and dependent on the frequency of G cell discharge (Fig. 11 A). Repetitive stimulation of the G cell evoked a progressive depolarization of P cells which resulted from the summation of individual PSPs. The amplitude of each PSP was not constant, but increased during the progression of the train. However, the total P cell response to single or repetitive discharge of the G cell differed from the response evoked by nerve stimulation in that the former seldom attained sufficient amplitude to elicit P cell discharge. One limiting
factor in the amplitude of the depolarization attained by the P cells following repetitive stimulation of the G cells was that the latter could not be driven at frequencies greater than 20 impulses/sec. In all cases studied, synaptic transmission between the G cell and P cells was unidirectional; stimulation of P cells had no effect on G cell behaviour (see Fig. 11 B, C, E, F).

![Diagram](image)

Fig. 10. Electrotonic connection between adjacent and distant peripheral cells. In A and B, depolarizing and hyperpolarizing potentials elicited by intracellular stimulation of a peripheral cell (bottom trace) and their effect on an adjacent peripheral cell (top trace). In C and D, depolarizing and hyperpolarizing potentials evoked by intracellular stimulation of a peripheral cell (bottom trace) and their effect on a peripheral cell 2-8 mm. distant (top trace). In E, same cells as shown in C and D: effect of intracellular evoked cell discharge on distant peripheral cell.

In some P cells the G cell discharge was associated with a double rather than a single PSP (Fig. 12). This could be due to electrotonic coupling between P cells or to the branching of the pre-synaptic axon. Moreover, in these cells a single G cell spike could produce either type of response. While the electrotonic coupling between P cells was seldom of sufficient amplitude to account for the double PSP of the P cell, it may represent a mechanism by which the total potential of the P cell is increased. In occasional cells the effect of G cell discharge was minimal (Fig. 12 D, E). In this case the small progressive build-up of potential following repetitive discharge of the G cell was similar to the electrotonic coupling observed between P cells (Fig. 12 D). In addition, under these conditions electrotonically transmitted spikes were observed superimposed on the membrane depolarization (Fig. 12 D).

**Effects of current on the PSP.** The finding that the P cell discharge does not influence the G cell’s response, as recorded from its soma, does not exclude the possibility that the P cell PSP is mediated by an electrical synapse. Because of the uncertainty of the locus of the synaptic contact to the recording site in the P cell soma, the comparison between the delay of the G cell axonal spike and the P cell, PSP can not be used to differentiate between an electrical or chemical response. To provide an electro-
physiological identification the $P$ cell membrane was polarized to different levels to determine if the PSP amplitude was changed as a function of membrane potential. In most instances, the PSP amplitude remained unchanged or decreased (Fig. 13 A) during hyperpolarization and increased during depolarization. This finding may be related to the properties of the $P$ cell post-synaptic membrane. The $P$ cell current-voltage relation is not linear and the membrane rectifies when it is hyperpolarized below the resting potential (A. L. F. Gorman and M. Mirolli, in preparation). For comparison, the effects of membrane polarization on a depolarizing potential elicited in the same cell by intracellular stimulation is illustrated in Fig. 13 B to show that similar changes occurred in the amplitude of both the transmembrane evoked depolarization and the PSP.

Fig. 11. The pathway from giant cell to peripheral cells. In A, a single discharge of the giant cell (middle) evoked by an intracellular stimulus (top) produced a single PSP in a peripheral cell (bottom). In B, double discharge of the giant cell, evoked by a longer-duration stimulus, produced by a double PSP in the peripheral cell. The unidirectional nature of the synaptic pathway between the giant and the peripheral cell is shown in C, where intracellularly evoked discharge of the peripheral cell (middle) had no effect on the giant cell (bottom). In D, antidromic invasion of both cells following stimulation of the peripheral side of the medial nerve to show the axonal pathway of the two cells ($P$ cell, top; $G$ cell, bottom). In E, hyperpolarization of the giant cell membrane to show lack of effect on the peripheral cell. In F, hyperpolarization of the peripheral cell membrane to show lack of effect on the giant cell membrane. Calibrations for A–C, E and F are shown in F. Calibration for D is shown in D. All records taken from the same experiment.

**DISCUSSION**

Despite their connexion to a highly rhythmic structure, neither the gastro-oesophageal giant cells nor the peripheral cells are autorhythmic, or subject to a rhythmic synaptic bombardment. Quite the contrary, under the several experimental conditions
used to investigate their behaviour (including recording from the gastro-oesophageal ganglia with more central ganglia and portions of the oesophagus attached) the membrane of both the $G$ and $P$ cells exhibited an extraordinary stability. However, the cells are excitable and receive powerful synaptic drives both from the periphery and from more central ganglia.

![Graphs of neuron activity](image)

**Fig. 12.** Characteristics of the giant cell's synaptic effect on the peripheral cells. In A and B, repetitive discharge of the giant cell (middle) to show presence of a single or dual PSP in the peripheral cell (bottom). In C, repetitive discharge of the same giant cell shown in A and B to show facilitation of the PSP in the peripheral cell. In D and E, the effect of repetitive discharge of the giant cell (second trace) on two peripheral cells (third and fourth traces). In E, the arrow points to an electrotonic spike-like response in the fourth trace in a cell with a very weak response to giant cell discharge (see text for further discussion). Calibrations for A and B are shown in C; those for D are shown in E.

The organization of the gastro-oesophageal chain is shown in schematic form in Fig. 14. The output relation of the gastro-oesophageal giant cells indicates that they act as *command interneurones* participating in the control of the behaviour of a group of more peripherally located cells. The axon of the $G$ cell makes an excitatory synaptic contact with most of the peripheral neurones encapsulated in the medial nerve. This type of side branch synaptic junction allows a single giant interneurone to influence the behaviour of a topographically dispersed population of cells. Further, the absence of a synaptic contact from the peripheral cells to the $G$ cell indicates that the transmission of information through this population is one-way—without feedback to its command interneurone. However, the presence of electrical junctions between the peripheral cells provides a mechanism for positive feedback within this neural population. The system thus represents one of the most elementary of the functional neural populations defined by Kandel & Wachtel (1968), i.e. their 'elementary divergent aggregate'. While the ultimate function of the $P$ cells has not been investigated, the direction of their axonal pathway toward the periphery is clear and suggests that they may serve as motoneurones for the oesophagus.
The control function of the giant cells over the peripheral cells is partial rather than complete. This is in contrast to what is known about the function of more centrally located giant neurones in other molluscs. Thus, while direct stimulation of some molluscan giant cells can evoke gross behavioural responses (Willows, 1968) or simple reflexive movement of muscles (Kupfermann & Kandel, 1968), the giant cell’s excitatory input to the peripheral cells was seldom capable of eliciting cell discharge. It is possible that other neurones either in the GOG or in other ganglia may participate in the control of the peripheral cell’s behaviour. This lack of complete control may also reflect the more restricted function of giant interneuronal cells located in peripheral ganglia versus the widespread control of more central commanding interneurones.

Fig. 13. Effects of changes of membrane potential on the synaptic potential of the peripheral cells. In A, the bottom trace shows the triple PSP of the peripheral cell evoked by a triple discharge of the G cell (third trace from the top) during different levels of membrane polarization. The top trace shows the current trace for the G cell and the second trace current for the P cell. In B, the effect of the same changes in membrane potential level as shown in A on an intracellularly applied depolarization. In A and B, the top current and voltage calibration refers to the G cell current and voltage traces; the bottom calibrations to the P cell current and voltage traces.

As illustrated in Fig. 14, the G cells display a functional as well as an anatomical symmetry in their input-output relation. The functional symmetry of anatomically symmetrical pairs of cells in the molluscan nervous system has been recognized (Hughes & Chapple, 1967; Hughes & Tauc, 1968; Kandel & Tauc, 1966a; Strum-
Despite the symmetry of the $G$ cells, simultaneous recording from both neurones shows that there are no direct connexions between them. Kandel & Tauc (1966a) have reported a similar lack of direct connexions between two giant symmetrical cells in the metacerebral ganglia of Helix (but see Hughes & Tauc (1968) for a recently demonstrated case of direct connexions). The results of stimulation of the cerebro-buccal connectives reveals the presence of powerful symmetrical excitatory and inhibitory connexions from the central ganglia to the $G$ cells. The long delay

Fig. 14. Schematic diagram of the input-output relationship of the two giant cells. The soma and axon of the $G$ cells are shown in black. See text for further discussion.
observed between the stimulus and the appearance of this synaptic potential suggests that the connexion from the cerebral ganglia is not direct but may involve inter-neurones in the buccal ganglia. Equally powerful synaptic drives reach the G cells from the periphery via the lateral and medial nerves.

However, an important distinction must be drawn between inputs from the buccal ganglia (central inputs) and those from the branches of the gastro-oesophageal nerve (peripheral inputs); as in the former case the inputs are bilateral, while in the latter case the inputs are restricted to the ipsilateral side. Further, the output from each G cell is restricted to the ipsilateral side of the oesophagus. Therefore, given the absence of direct connexion between G cells, the only manner by which a symmetrical and simultaneous input from the G cells can reach the periphery is by way of a synchronized input from the central ganglia.

The data obtained about the synaptic response produced by stimulation of peripheral inputs to the G cell could be taken to indicate that a powerful excitatory feedback system exists in the oesophagus. However, the peripheral neurones responsible for this effect have not been identified. While this type of negative evidence is not sufficient to exclude a peripheral origin for the PSP evoked by stimulation of the medial and lateral nerves, an alternative possibility must be considered. The presence of the pre-synaptic cell soma is not necessary for the transmission of synaptic activity. A long axon with a side branch synaptic contact, such as the contact the G cell must make with peripheral cells, can be activated by antidromically as well as orthodromically travelling impulses. It follows that if peripherally directed axons of cells in more central ganglia make synaptic contacts with the giant cell our present technique could not distinguish between the two cases where synaptic discharge was evoked by axons with peripherally located somata or by axons with their somata situated in central ganglia.

Discharge of the G cell was generally associated with the appearance of a postsynaptic response in P cells. As our anatomical results show that there are no unidentified cells interposed between the G cell axon and the P cells, the pathway involved must be monosynaptic. The constant amplitude and latency of the response, in addition to its one-to-one relationship with discharge of the G cell, indicates that the response is also unitary in character. Whether this response is electrically or chemically mediated, or involves some combination of both types of synaptic action, remains to be determined. The usual electrophysiological criteria for determining whether a synapse depends on electrical or chemical transmission (Grundfest, 1961, 1966) are unfortunately of doubtful value, as the conductance of the peripheral cell membrane is voltage-dependent. As a consequence of this hyperpolarizing conductance increase (Grundfest, 1961, 1966; Kandel & Tauc, 1966b) the amplitude of an excitatory PSP decreases when the membrane is hyperpolarized and increases when it is depolarized. This sequence of change is opposite to that expected from a chemically mediated excitatory PSP with an equilibrium potential which is closer to the zero potential than to the resting potential of the cell membrane. Under these conditions it is impossible to differentiate between a chemically mediated synaptic action occurring at a distant location and an electrically mediated one, as both would tend to act as constant-current sources for the potential developed across the soma membrane and to be dominated by the voltage-dependent conductance changes occurring there.
The finding that peripheral cell stimulation does not have an observable effect on the giant cell membrane excludes the possibility of a direct feedback path, but cannot be used to exclude the possibility that there is an electrical junction between the two cells. In either case the effects of peripheral cell stimulation probably would not be directly observable at the soma membrane of the giant cell unless the potential evoked in the giant cell axon was non-decrementally conducted back to its soma.

In rare instances discharge of the $G$ cell axon had no effect, or only a very weak effect, on a peripheral cell. However, in these cases the possibility of some damage to the $G$ cell axon or to the synaptic connexion between the two cells could not be excluded. In other peripheral cells discharge of the $G$ cell was associated with a double rather than a single PSP. Whether the appearance of a double PSP represents the activation of: (a) two different synaptic mechanisms operating at the same synapse (as occurs in the chick ciliary ganglion (Martin & Pilar, 1963)), (b) two separate synapses from the $G$ cell occurring at different locations, or (c) a second peripheral cell acting as an interneurone, remains to be determined.

The peripheral neurones are joined by a series of resistive junctions into an electrical network. In this respect a clear differentiation can be made between the cells in the gastro-oesophageal ganglion and those in the periphery. In the former case electrical connexions are absent, while in the latter case they are widespread. This type of non-specific electrical contact between cells may represent one aspect of the more primitive organization of the molluscan peripheral nervous system. If this is true, then the paucity of information about electrical connexions between molluscan neurones (Tauc, 1959, 1966) may simply reflect the amount of attention devoted to central ganglion cells rather than a genuine lack of electrical synapses. The finding that an electrical interaction occurs between distant as well as adjacent somata, and the large attenuation of potential observed in both cases suggests that the electrical junction occurs at some distance from the soma. Perhaps the most striking feature of the peripheral cell electrical junctions is their poor performance in transmitting high-frequency signals (spikes). They effectively act as low-pass band filters; and for this reason are poorly suited for the type of fast synchronization of cell discharge which occurs in many electrically coupled systems (Bennett, 1966). However, the system is well suited to spread a state of general excitation throughout a large population of cells; and may provide the slow integrative amplification and synchronization which probably underlies repetitive synaptic activation of the peripheral cell population by the cells which control their behaviour.

**SUMMARY**

1. Each of the two gastro-oesophageal ganglia of the nudibranch mollusc, *Anisodoris nobilis*, contains one giant neurone ($G$ cell) whose axon is directed toward the oesophagus in the gastro-oesophageal nerve.

2. In the absence of stimulation the $G$ cells are normally silent. However, they receive inhibitory and excitatory synaptic inputs from more central ganglia and a predominantly excitatory input from the periphery. The inputs from the central ganglia are bilaterally distributed to both $G$ cells, whereas the inputs from the periphery are limited to the ipsilateral $G$ cell.
3. Intracellular stimulation shows that there is no interaction between the G cells, nor between the G cell and other cells in the same or contralateral gastro-oesophageal ganglia.

4. The axon of the G cell makes synaptic contact with a series of peripheral cells (P cells). In most P cells the post-synaptic potential elicited by intracellular stimulation of the G cell is constant in amplitude and latency and probably results from a unitary monosynaptic contact. Intracellular stimulation shows that the P cells are not connected to the G cell.

5. The P cells are inter-connected by low-resistance electrotonic junctions which allow slow potentials of either polarity to spread between cells. These junctions exist between distant as well as adjacent peripheral neurones.

6. Our results show that the G cell functions as a command interneurone for an aggregate of electrically interconnected peripheral neurones.

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EXPLANATION OF PLATE

In a, unstained whole mount of the gastro-oesophageal ganglion from a small animal (45 mm. long). The giant cell (G cell) practically fills the whole capsule of the ganglion. The bucco-gastro-oesophageal connective is directed toward the top and the gastro-oesophageal nerve toward the bottom of the photograph. In b, unstained whole mount of a cluster of four peripheral cells from a larger animal (78 mm. long) in the medial branch of the gastro-oesophageal nerve. The musculature of the oesophageal wall is also visible in the background. In c, glytaraldehyde-osmium stained whole mount of the oesophagus (same animal as shown in b) showing the medial branches of the gastro-oesophageal nerves and the peripheral nerve network.