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FURTHER STUDIES ON THE ELECTROPHYSIOLOGICAL ANATOMY OF THE LEFT AND RIGHT GIANT CELLS IN *APLYSIA*

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Electrophysiological studies on cells of *Aplysia* central ganglia have become of general interest in recent years and have developed from work on the nature of integrative mechanisms (Arvanitaki & Chalazonitis, 1959; Tauc, 1955; Tauc & Hughes, 1963) to investigations of plastic change at a cellular level (Bruner & Tauc, 1966; Kandel & Tauc, 1965). Earlier work showed the range of phenomena found in a variety of cells, particularly in the abdominal ganglia, but research has now become more concentrated on certain cells which are readily identifiable in all preparations (Strumwasser, 1965; Hughes & Tauc, 1961; Kandel & Tauc, 1965). Of these cells, the large cell in the right upper quadrant, which is often called the giant cell, or right giant cell (RGC), has been used a great deal in studies on the basic phenomena of spike initiation in molluscan cells (Tauc, 1962). The anatomy of this cell was shown to be quite complex as a result of electrophysiological studies (Hughes & Tauc, 1963) which also revealed the presence of a comparable cell (LGC) on the left side of the C.N.S. Because of similarities in the general pattern of branching of these cells, they were considered homologous.

During recent years, more extensive studies on these two cells have revealed an even more complex anatomical arrangement (Hughes, 1965) and a direct synaptic connexion between them (Hughes & Tauc, 1965). The present paper describes recent electrophysiological work on the anatomy of the two cells, their synaptic inputs and certain connexions between them.

MATERIALS AND METHODS

The animal used in this investigation was the large *Aplysia* which is found at Arcachon in September and October. This is usually called the Black Aplysia (*l'Aplysie noire*) and was earlier thought to be *A. depilans**; this is the only large species recorded for the Atlantic coast in the Faune de France (Pruvot-Fol, 1954). More recently the possibility that this was wrongly named has been investigated and there is now no doubt that the Black Aplysia at Arcachon is, in fact, *A. fasciata*. *A. depilans* does occur, however, and has been used in several experiments. This species is smaller and more brown in colour; the two parapodia are joined high up behind the mantle cavity and

* I am indebted to Dr Nellie B. Eales for confirming my suspicion that this might not be so and for providing me with a list of distinguishing features.

the animals very rarely swim, whereas swimming is a very characteristic feature of *A. fasciata.*

The animals were taken from the aquarium and dissected as described previously (Hughes & Tauc, 1963). The whole of the C.N.S. was isolated and pinned out with the main nerves across pairs of chlorided silver electrodes. Glass capillary microelectrodes were inserted into the two giant cells, if possible two electrodes into at least one of them. In the early experiments the sheaths surrounding the ganglia were dissected off but both giant cells survived in very few preparations. Later, electrodes made in a Nastuk-type puller were inserted through the sheaths with greater success than those made in a de Fonbrune microforge, but because of their construction, these electrodes were noisier. The glass capillary microelectrodes were usually of 20–50 MΩ impedance, but during their insertion through the sheath, the tip often broke, reducing their impedance to less than 10 MΩ when measured *in situ.* The intracellular electrodes fed into cathode followers which were either of a modified Bak negative capacitance type or of a design with an electrometer valve input. Other stimulation and recording methods were the same as those used in previous investigations. The results described below are based upon work with about fifty preparations.

RESULTS

The technique used for recognizing the presence of a branch of the giant cell in one of the nerves was generally the same as used previously (Hughes & Tauc, 1963). This consisted in: (1) the recognition of the antidromic spike intrasomatically following stimulation of a nerve, (2) the extracellular recording of an action potential in a nerve following intracellular stimulation of the giant cell soma. In addition, the technique has been used of showing the possibility of collision between impulses when stimuli were applied to two nerves thought to contain a branch of a given axon (cf. Tauc & Hughes, 1963).

The path of the giant cell axons

In previous work it was shown that the RGC axon extended into the main parapodial and pedal nerves on the right side and that the axon continued through the right cerebro-pleural connective and descended into the left cerebro-pleural connective. Its path from this point was not traced further. In a few experiments, however, it was noted that firing of a large unit in the left cerebro-pleural connective which may have been the RGC axon, was followed by an action potential in the left parapodial nerves (Fig. 13, h.i.; Hughes & Tauc, 1963). The axon of the LGC was found to branch into the parapodial and pedal nerves on the left side of the body. No branch of this axon was found in the left connective. Transmission across the branching from the parapodial nerves to the cerebro-pleural connective was also found to be possible, usually in both directions.

In previous work no unequivocal evidence was found for the presence of an antidromic spike in one of the giant cells when stimuli were applied to nerves on the contralateral side. Such experiments were not performed on many occasions, however, and there were no experiments in which simultaneous recordings were possible from both giant cells. In more recent work evidence was obtained for the recording of an antidromic spike in a giant cell when contralateral parapodial nerves were stimulated.

There is some variability in the ease of eliciting this response which explains why it was overlooked previously. In some preparations it is readily observed, as shown in Fig. 1, where an antidromic spike is recorded in both giant cells after stimulating right parapodial nerves (see also Fig. 5). In most cases where an antidromic spike was recorded in both cells the threshold for the ipsilateral spike was lower than that of the contralateral response.

The left giant cell. Experiments with the left giant cell have been carried out with far more preparations than previously, owing to the improved technique for inserting microelectrodes. The effect of hyperpolarizing currents on the form of the antidromic potentials is similar to that known for the RGC (Fig. 2). At the same polarization or degree of fatigue, stimulation of the contralateral nerves often did not lead to invasion

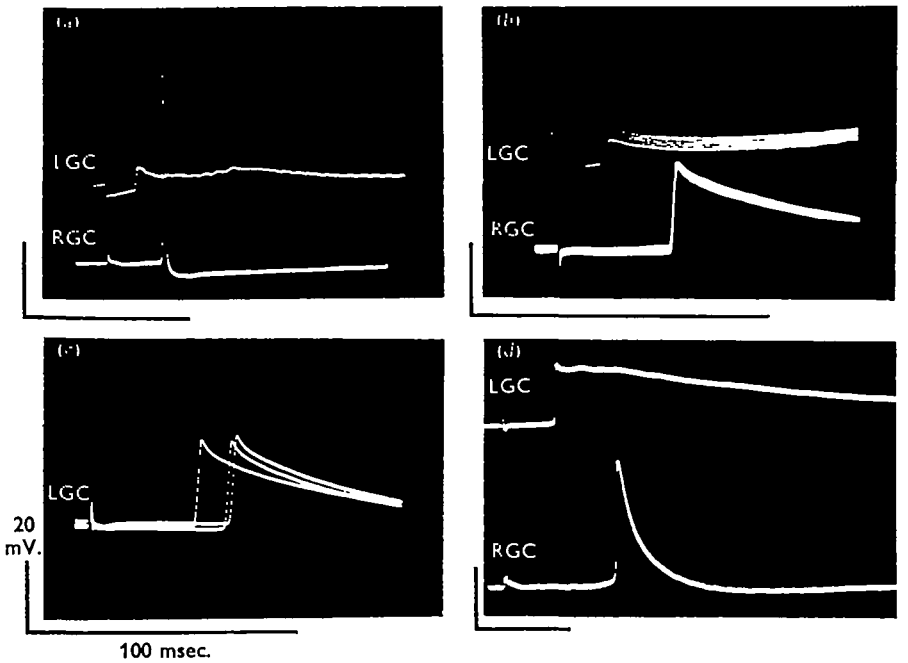

Fig. 1 *a*, *b*, *d*. Simultaneous recordings from the right and left giant cells following stimulation of the pedal (*a*), middle (*b*) and posterior (*d*) parapodial nerves on the right side. In these recordings an A spike is recorded in both cells and indicates the presence of a branch of the axon of both giant cells in these nerves. In (*b*) many sweeps are superimposed and show the constant delay. (*c*) Identical A spikes in the LGC following stimulation of the posterior and middle parapodial nerves and the pedal nerve of the left side. Note different delay times.

of the soma. In addition, there is also a difference in the size of the A potential recorded before invasion of the soma occurred. That resulting from ipsilateral stimulation is often larger than that evoked when nerves on the right side are stimulated (Fig. 1). The difference in size of the A potentials may be due to several causes such as the relative size of the branches where they join the main axon or differences in the distance of this junction from the soma, or a combination of these and other features of the neurone. The importance of the size difference and/or local variations in excitability is supported by experiments involving stimulation of a nerve on each side of the body at different intervals of time after one another (Fig. 3). In this case, stimulation of the

right nerve produces only a small A potential, whereas that of the corresponding left nerve produces an AS spike. This was true for all the parapodial and pedal nerves stimulated in this preparation. Varying the interval between ipsi- and contralateral stimulation showed that if the antidromic impulse from the left side preceded that on the right side in its arrival at the cell, then it could block the contralateral spike, thus giving definite evidence that the impulse can be transmitted across the branching from left to right. On the other hand, an impulse from the right side which arrived before that from the left did not block it, suggesting that transmission was not possible in this direction.

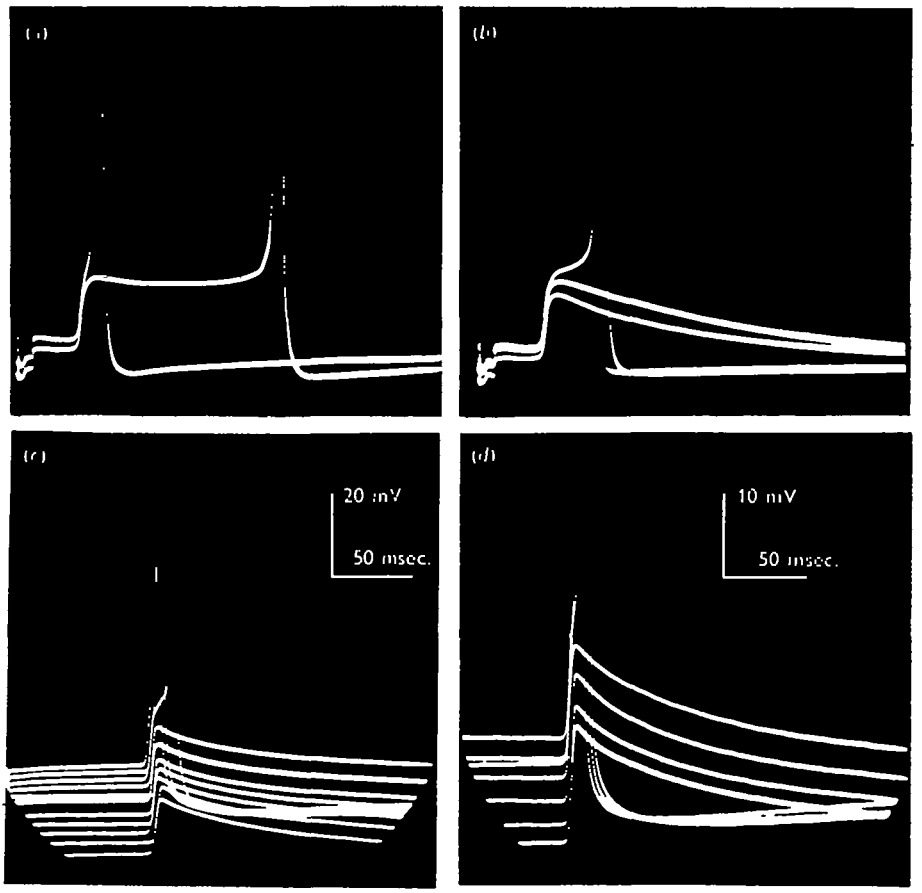

Fig. 2. The left giant cell. (*a*) and (*b*) superimposed traces of different forms of A and AS spike resulting from single shocks to the right middle parapodial nerve, (*c*) and (*d*) show the effect on the antidromic spike of changing the membrane potential by passing currents through a second electrode inserted into the cell. The antidromic potentials result from single shocks to the right posterior parapodial nerve in a different preparation from (*a*) and (*b*). Calibration for (*a*) and (*b*) is same as in (*c*).

Corresponding experiments, in which stimuli were applied to two nerves on the same side, show that it was nearly always possible for stimulation of any parapodial or pedal nerve to block an impulse initiated in another nerve on the same side, thus showing not only that each nerve contained a branch of the giant cell axon but also that transmission was possible in both directions at their branching.

Further evidence for branches of the LGC being present in the right parapodial nerves has been obtained from extracellular recordings following firing of the LGC, either as a result of synaptic stimulation (Fig. 4*a*) or depolarization of the LGC soma (Fig. 4*d*).

The right giant cell. In earlier work branching of this cell seemed to be confined to the right side, but further experimentation has shown that the main branch of the axon, which was previously traced into the left cerebro-pleural connective, descends farther and sends branches into the main left parapodial and pedal nerves (Fig. 6). As mentioned below, it also has a synaptic connexion with the LGC in the left pleural ganglion. Whichever of these nerves is stimulated, on either the left or right side, the antidromic impulse is propagated down the main axon in the right connective and

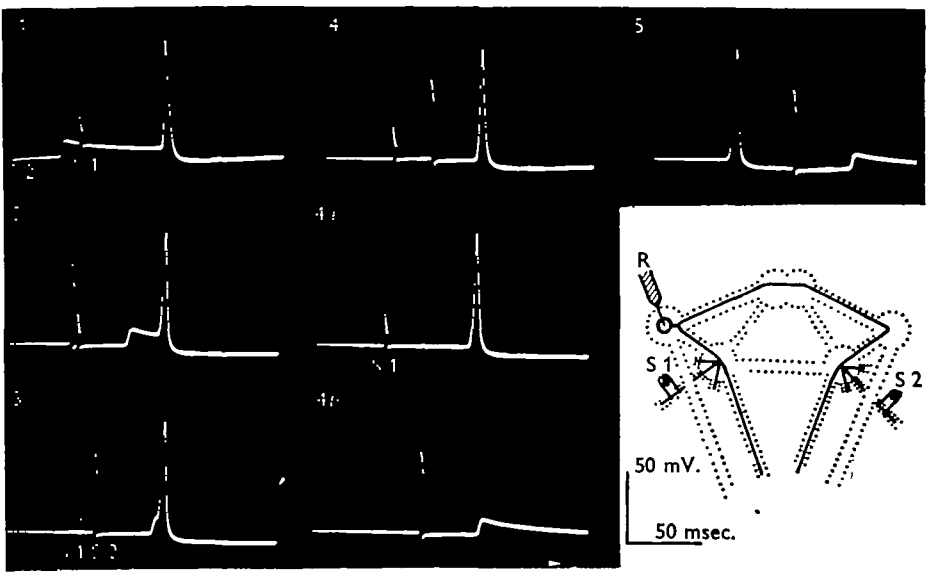

Fig. 3. The left giant cell. Antidromic potentials recorded following stimulation of the middle parapodial nerves on both sides. The A spike from the left side is followed by invasion of the soma whereas stimulation on the right side (S2) is not. Successive recordings are shown in which the interval between these two shocks is varied. In 1–3 the S2 spike precedes S1 and there is no blocking action. But (4) when the S1 spike precedes the S2 spike, the A spike resulting from S2 is absent. 4*a* and 4*b* show the effect of stimulating S1 and S2 alone at the same delay as in 4. In 5, S2 is sufficiently delayed after S1 that the A spike it produces is not blocked by S1.

gives rise to an antidromic potential in the RGC soma. Collision experiments have also been performed between the different branches of this axon, either on the same side or on opposite sides. Conduction of the impulse appears to take place equally in all directions, though in some preparations there seems to be some sort of block at the branching (Hughes & Tauc, 1961) which, in some instances, can be overcome at higher frequencies. A new and rather unexpected finding in later work is definite evidence for a branch of the RGC axon entering the branchial nerve. This was suggested not only by recording an A spike in the soma but also by recording a small potential in the branchial nerve following intrasomatic stimulation of the RGC. Collision experiments further showed that transmission occurs in both directions

between this branch and the main axon in the right connective. It appears, then, that these two branches divide off from the main axon leaving the RGC.

In a unique specimen (Fig. 7), it was found that the two giant cells were placed symmetrically on both sides of the body in the pleural ganglia. In this preparation studies were made of the branching which showed that it was fundamentally the same as would be expected from work on preparations having the normal orientation of the two giant cells. Thus an antidromic spike was recorded in both cells after stimulating one of the parapodial or pedal nerves (Fig. 5). In these records it can be seen that stimulation of the left posterior parapodial nerves leads to an antidromic spike in both

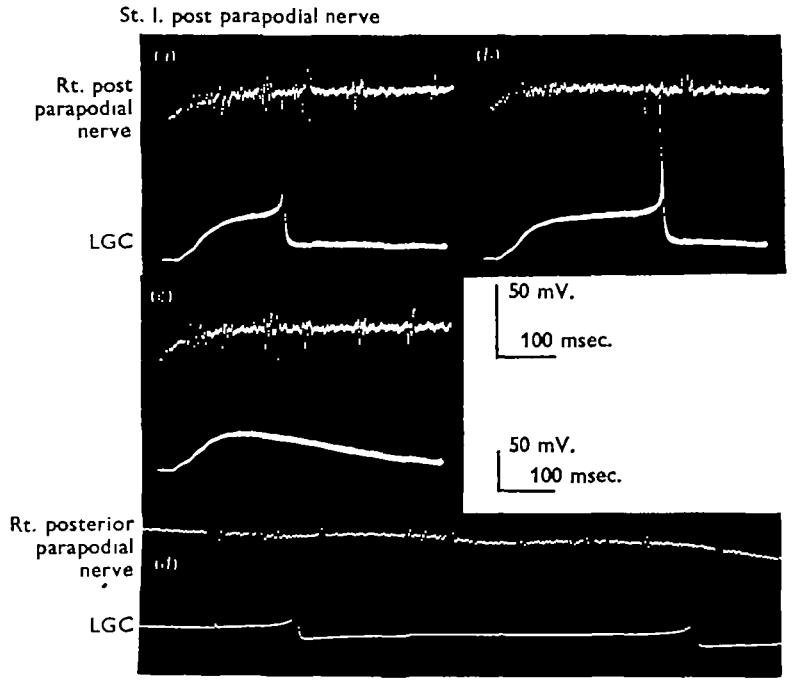

Fig. 4. Extracellular recordings from the right posterior parapodial nerve and intracellular recordings from the LGC following stimulation of the left posterior parapodial nerve. (*a*–*c*). Three frames showing slight variations in the response to single shocks of the same intensity. In two, (*a*) and (*b*), the synaptic input to the LGC is sufficient to evoke a somatic discharge and this is followed after a constant delay by the extracellularly recorded spike in the right posterior parapodial nerve. In (*d*) the LGC is firing as a result of depolarization of the cell soma and is followed after the same delay by a similar spike in the right posterior parapodial nerve. These recordings clearly demonstrate the existence of a branch of the LGC in the right posterior parapodial nerve. Low calibration applies to (*d*) only.

giant cells which is followed by EPSPs. Stimulation of the right posterior parapodial nerve produced an antidromic spike in the RGC, but it was not possible to record one in the left cell. The other two recordings (*c*, *d*) are at higher sweep speed and show the delay in the A spike recordings following stimulation of the left posterior parapodial nerve and of the left cerebro-pleural connective. As expected, the interval (25 msec.) between the appearance of an antidromic spike in the LGC and in the RGC following parapodial nerve stimulation is equal to the sum of the delays

(9 + 16 msec.) following stimulation of the cerebro-pleural connective. In the latter case, of course, the two antidromic impulses were conducted in opposite directions from the point of stimulation. These measurements suggest conduction velocities of 60–80 cm./sec. for the giant cell axons in these connectives. As a result of the finding of a branch of the axon entering the branchial nerve in normal preparations, it was expected that in this specimen a branch of the RGC might descend in the right connective and enter the branchial nerve. Fig. 5*e* shows that this was in fact the case, for intracellular stimulation of the RGC was followed by a potential recorded extracellularly in the

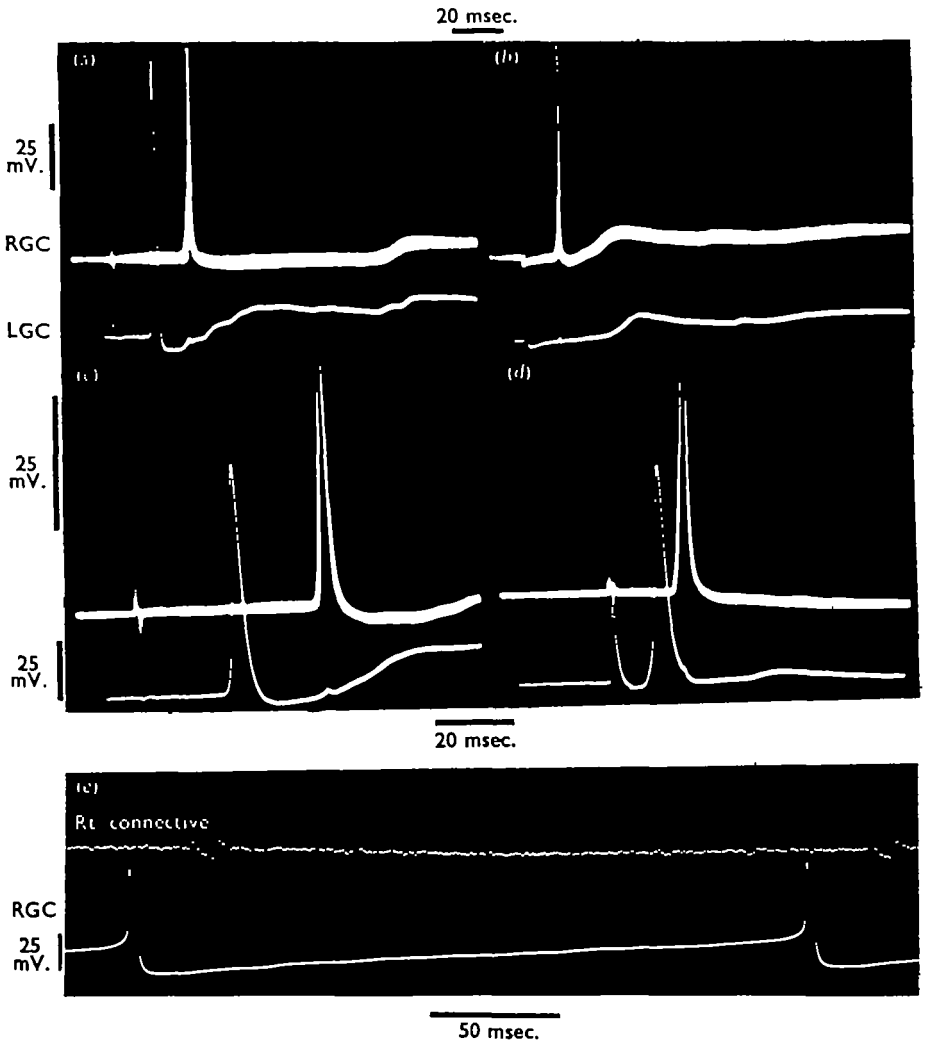

Fig. 5. Recordings from a unique preparation in which both giant cells were in the pleural ganglia (Fig. 7). Stimulation of the left posterior parapodial nerve is followed by an AS spike in both giant cells (*a* and *c*). Stimulation of the right posterior parapodial nerve (*b*) gives rise to an AS spike in the RGC as well as an EPSP, but in the LGC there is no A spike. Notice the similarity in general form of the compound EPSPs. In (*d*) stimulation of the left cerebro-pleural connective is followed by A spikes in both giant cells. In (*e*) firing of the RGC gives rise to an action potential in the right connective. Calibrations for all intracellular recordings are 25 mV. Sweep speeds are the same for (*a*) and (*b*) and for (*c*) and (*d*).

right connective. In figure 15 are shown recordings which demonstrated the bilateral symmetry of the direct synaptic connexion between these two cells. This is the only instance in which intracellular stimulation of the LGC was followed by the recording of a biphasic postsynaptic potential in the RGC. The similarity in the shape of these two synaptic potentials is quite notable. It is particularly striking that a BPSP has never been recorded from the RGC when in its normal position.

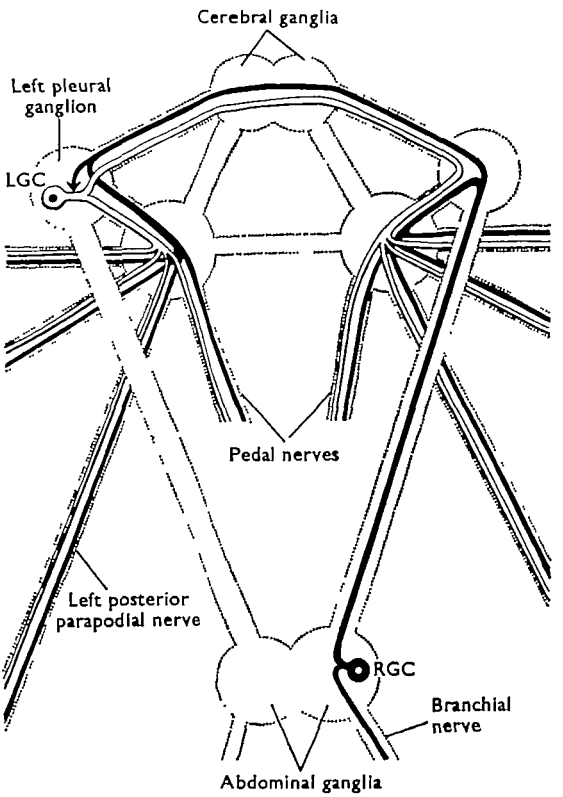

Fig. 6. Diagram of the main central nervous ganglia and connectives in *Aplysia fasciata* showing the typical branching pattern of the two giant cells as indicated by electrophysiological evidence discussed in this paper.

As a result of the experiments summarized here, the present evidence indicates the branching of the axons of the two giant cells shown diagrammatically in Figs. 6 and 7. As yet there is little detailed information about the precise nature of the branching in the pleural/pedal ganglia. In the case of the LGC it seems probable that all the branches from the left side come off the same main axon as shown in the diagram because they produce identical A spikes in the soma (Fig. 1*c*).

Synaptic connexions of the giant cells

Sensory inputs. In previous work recordings from the RGC in whole animal preparations (Hughes & Tauc, 1963) showed that this cell could be excited by mechanical stimulation almost anywhere on the body surface. Similarly, electrical stimuli applied to any of the nerves led to recording of a synaptic potential even if it did not

fire. 'Habituation' of the responses to both mechanical and electrical stimulation was a marked feature of these preparations. In the present work, with microelectrodes inserted into both giant cells, many inputs were stimulated in order to compare their effect on the two cells. Because of their different positions in the C.N.S., a marked difference in response to a given input seemed probable. Stimuli anteriorly or on the left side were expected to be more effective on the LGC; those more posteriorly applied and to the right side being more effective on the RGC.

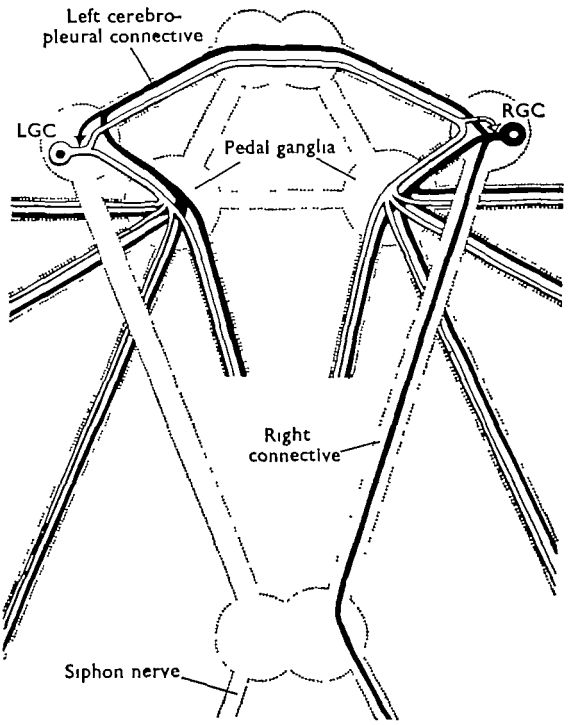

Fig. 7. *Aplysia fasciata*. Diagram to show the position and branching of the two giant cells in a unique preparation in which the RGC soma was situated in the right pleural ganglion.

However, although this was sometimes the case, it was by no means as marked as expected. Instead, the most characteristic feature was the remarkable similarity in the synaptic responses of these two cells, and in some preparations the LGC showed larger EPSPs to both ipsilateral and contralateral inputs. Low intensities of stimulation often evoked an apparently unitary synaptic potential in the two cells, which suggested the response of both cells to a common interneurone. With higher intensities of stimulation the cells showed larger compound EPSPs which sometimes reached the firing level. Further increases in intensity decreased the delay before the origin of a spike from the EPSP (Fig. 8). The LGC had the lower threshold of nerve stimulation required to fire the cell in most preparations, but this was not invariable. Fig. 9 shows the response of the two cells to stimulation of a variety of nerves; in some cases the synaptic potentials are preceded by the antidromic spike but this is not present in all instances. The delay between the potentials recorded in the two cells depended upon

the point of application of the stimulus; but, as can be seen, differences in the absolute size of the synaptic potentials were small. Stimulation of the lateral tentacular nerve on the right or left side produces very similar effects on the two cells. The synaptic potential recorded following stimulation of the posterior parapodial nerves is larger in the RGC than in the LGC, though the latter fired a spike following a shock to the right nerve, whereas this was not so for the RGC (Fig. 9*c*). Furthermore, stimulation of nerves leaving the abdominal ganglia which might have been expected to have a much greater input to the RGC than the LGC, affected both cells similarly. Thus stimulation of the branchial nerve, although slightly more effective on the RGC,

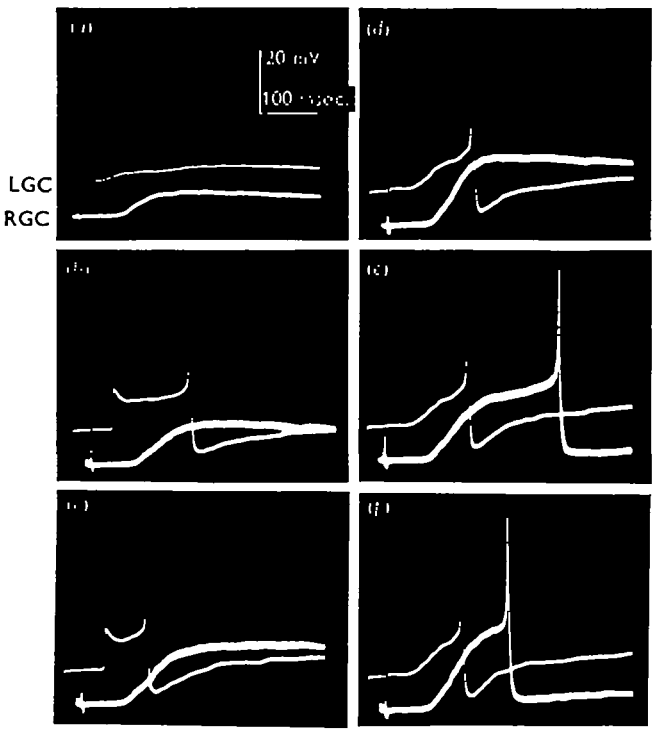

Fig 8. Recordings from the LGC and RGC resulting from single shocks of gradually increasing (*a–f*) intensity applied to the right middle parapodial nerve. Notice the gradual increase in size of the EPSP which leads to firing of the cell soma, and a decrease in the delay for spike initiation at higher intensities. In (*b*) and (*c*) the LGC axon in this nerve is stimulated and gives rise to an A spike in the cell soma.

produced a good-sized EPSP in the LGC. In this particular preparation, it is apparent that all these inputs elicited a spike in the LGC more readily than in the RGC.

More detailed recordings from another preparation are shown in Figs. 10–12. Stimulation of the lateral tentacular nerves (Fig. 10*c*) at low intensity evoked a unitary postsynaptic potential. With an increase in intensity this led to a double response in which, on the left side, the LGC response preceded that in the RGC. With stimulation on the right side, the reverse was the case (Fig. 10*d*), but at higher intensities this brought in another unitary EPSP in which the LGC preceded the RGC. This latter response was obtained alone with stimulation of low intensity (Fig. 10*c*). At higher

intensities the LGC showed a much more marked EPSP which reached the firing level (Fig. 10*b*). Unitary responses following stimulation of these nerves could often be obtained quite pure and were of interest from the point of view of habituation as they usually showed a marked reduction to the second shock (Hughes & J. Bruner, unpublished).

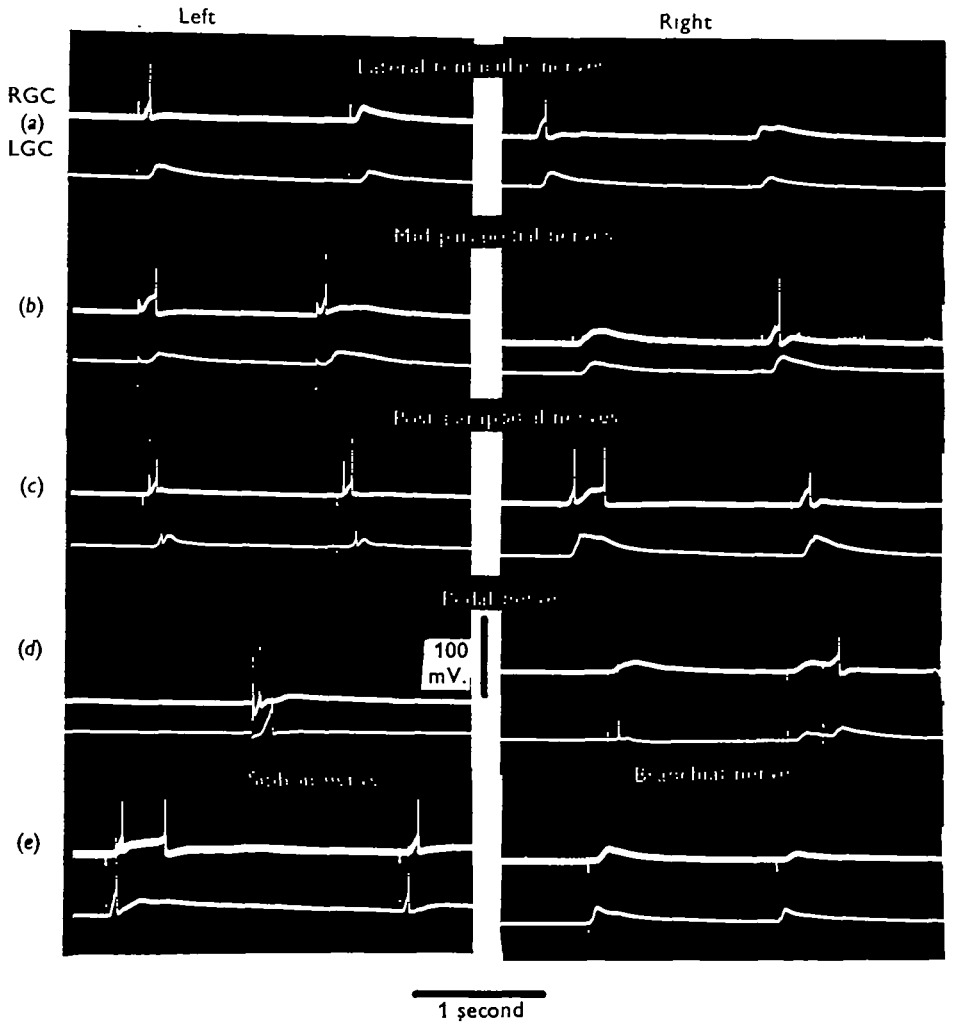

Fig. 9. Responses of the right and left giant cells to stimulation of pairs of nerves from the left and right sides of the body, and of two nerves which enter the abdominal ganglia. Notice the general similarity in the form of the synaptic potential recorded in the two cells following a given synaptic input.

The response of the two giant cells to ipsilateral and contralateral stimulation of the middle and posterior parapodial nerves and the main pedal nerves are shown in Fig. 11. In this preparation the antidromic spike was only obtained on the ipsilateral side; and, again, the LGC appears to receive a greater synaptic input than the RGC even following stimulation of nerves on the right side.

A detailed comparison of the response of the two giant cells to stimulation of the two main nerves leaving the abdominal ganglia is shown in Fig. 12. Both cells are affected at low intensities by stimulation of the siphon nerve and the response appears to be unitary and probably monosynaptic. The EPSP recorded in the LGC is about twice the amplitude of that in the RGC. A gradual increase in intensity brings in other steps in the compound EPSP and eventually leads to firing of the LGC and, at higher intensities, the RGC. The delay before the EPSP is shorter for the RGC than for the LGC, as expected. The shortness of these delays (20 and 50 msec.) suggests that a relatively rapidly conducting pathway (more than 100 cm./sec.) is involved, the difference in the delays being about 30 msec. in this case. A similar situation is found following stimulation of the branchial nerve; and, again, the difference in the delay

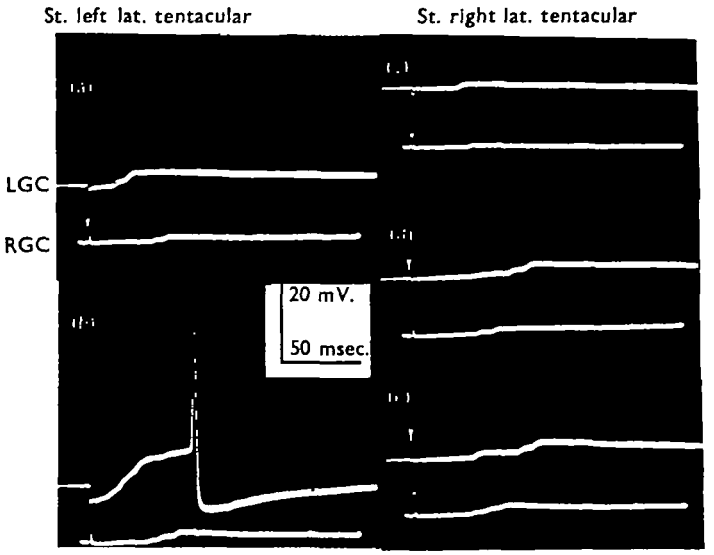

Fig. 10. Responses of the LGC and RGC to stimulation of the left (*a*, *b*) and right (*c*, *d*, *e*) lateral tentacular nerves. (*a*) Low intensity, (*b*) high intensity. Notice the much greater size of the compound EPSP in the LGC. (*c*)–(*e*) stimulation of the right lateral tentacular nerve. At low intensities (*c*) the unitary EPSP in the LGC precedes that in the RGC, but with reversal in the polarity of the stimulating electrodes (*d*), the EPSP in the RGC precedes that in the LGC but with further increase in the intensity (*e*) the unit evoked in (*c*) is also brought in.

times between synaptic potentials is about 25 msec. Clearly a more direct and probably more rapidly conducting pathway is involved between the abdominal ganglia and the LGC than that followed by the RGC axon because the latency for the BPSP is much longer (0·15 sec.).

In most recordings a similarity in the general form of the two compound EPSPs suggests that there are interneurones which are common to both cells. Further evidence for common interneurones has been obtained on many occasions where unitary postsynaptic potentials having a 1:1 relationship were recorded in the two cells. This can often be observed following a single shock to one of the nerves which results in the repetitive firing of such an interneurone, and sometimes it occurs in bursts. Fig. 13*d* shows some recordings of this type, following stimulation of the right connective. In this case the repetitively firing interneurone produced an EPSP in the RGC

which preceded that in the LGC by about 0·2 sec. At lower gain these EPSPs only appear as very small undulations of the base line (Fig. 13*d*). Burst-firing of this interneurone leads to stepwise summation of the unitary EPSPs in the giant cells (Fig. 13*e*). The relatively long delay between the RGC and LGC responses suggests that this interneurone is situated in the abdominal ganglia. These recordings also show that the EPSP produced by this particular interneurone is larger in the LGC than in the

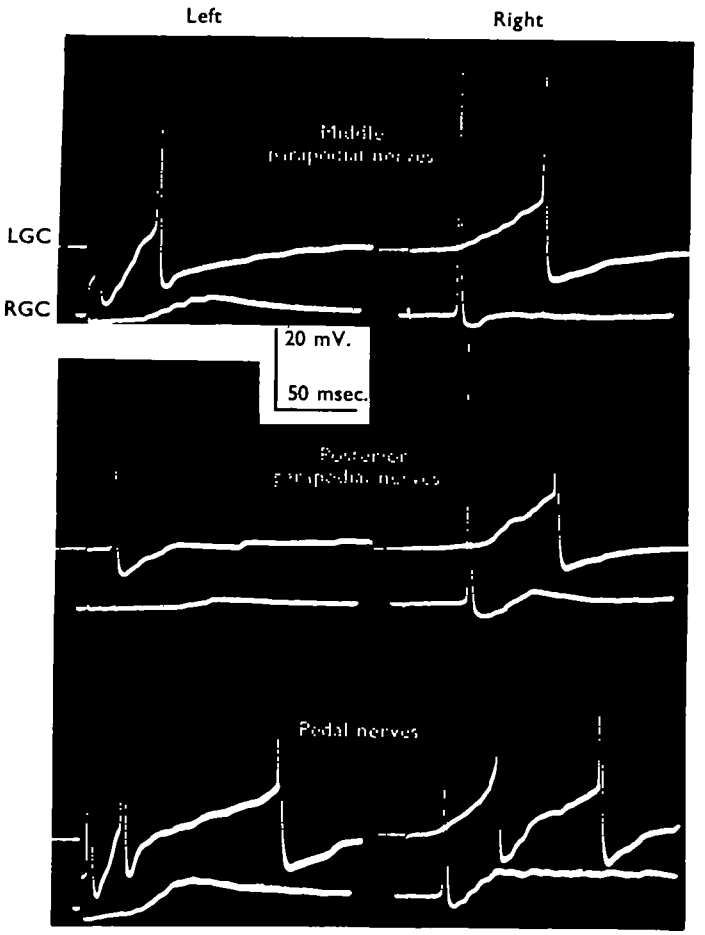

Fig. 11. Responses of the two giant cells to stimulation of parapodial and pedal nerves of the left and right side. In this preparation recordings from the LGC following stimulation of nerves on the left side always show an antidromic (AS) spike which precedes the synaptic response. Stimulation of nerves on the right side correspondingly produce an AS spike only in the RGC. Notice that the LGC fires more readily than the RGC, regardless of whether stimulation is applied to the right or left nerves.

RGC. In other cases the reverse is true, and the EPSP in the LGC may precede or be simultaneous with that in the RGC (Fig. 13*b*). This strongly suggests differences in the location of these interneurones or at least in their regions of spike initiation. Searches for such interneurones in different ganglia have so far been unsuccessful, but if it were possible to find such a cell it would be extremely valuable for further investigation of the integrative action of the giant cells.

In some preparations the output pattern of the LGC was analysed in more detail because it was found that the soma was not always invaded, so that the response consisted not only of synaptic potentials and somatic (AS) spikes but also of A spikes. When the delay before the appearance of an A spike was short and constant it was clear that it had been conducted directly from the stimulated nerve, but in other cases it had a synaptic origin. Presumably the latter had been propagated 'antidromically' from a site of spike initiation, which may have been associated with one of the branches

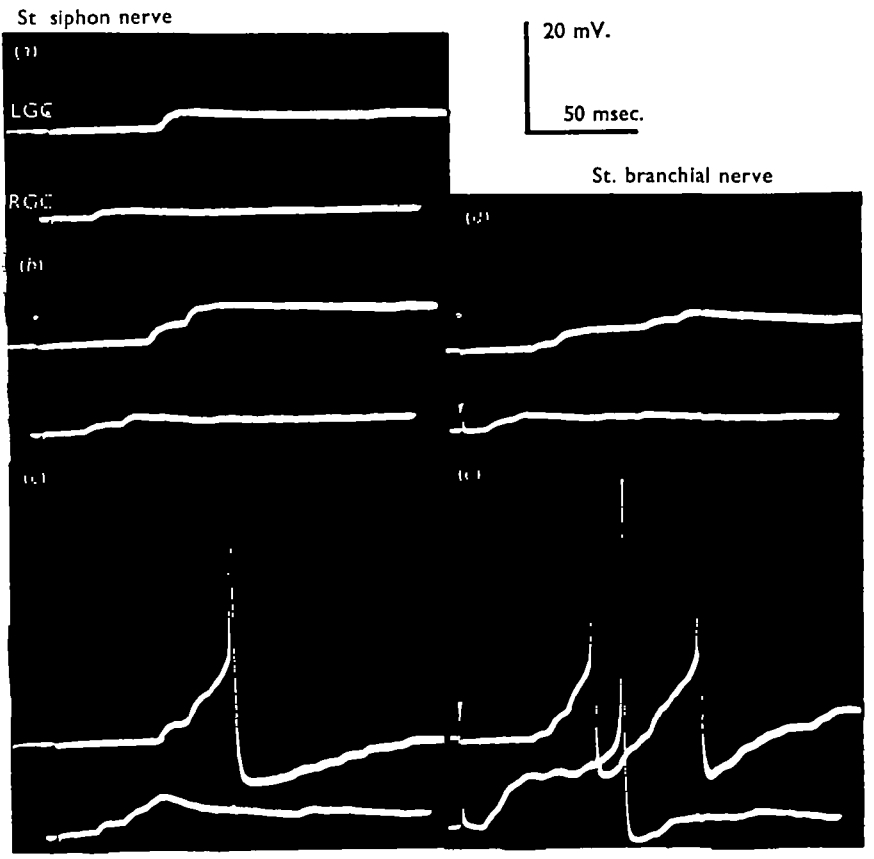

Fig. 12. Synaptic responses of the LGC and RGC to stimulation of the siphon (*a*, *b*, *c*) and branchial (*d*, *e*) nerves. The intensity of the single shocks is increased from (*a*) to (*c*) and (*e*) is at a higher intensity than (*d*). For description see text.

of the LGC axon. Because of differences in the size of the A spike recorded in the soma, depending upon which of the two main axons carries the antidromic impulse, these recordings suggest that in some cases one type of synaptic input tends to excite synapses near the branch to the left side, whereas in other cases those on the right seem to be more excited. Thus in Fig. 14 stimulation of the left parapodial nerves tends to elicit either full-fledged somatic spikes or A spikes of the larger type, and hence these are most probably due to synaptic inputs which affect LGC branches to the left side of the animal. During these investigations, as with other studies of the synaptic responses, it was observed that the output pattern of the LGC often remained

surprisingly constant when a given stimulus was repeated. The only difference was the substitution of an A spike for an AS spike (Fig. 14*c*); and in some instances the large A spike was replaced by the smaller type, occurrences of the latter type indicating that this particular synaptic input was affecting regions of spike initiation associated with both main branches. This sort of evidence indicates a complex integrative action of the LGC, depending upon the number and nature of the converging pathways on to it and their relationship to the branching of the cell, in addition to the summation and other properties of the synaptic membrane.

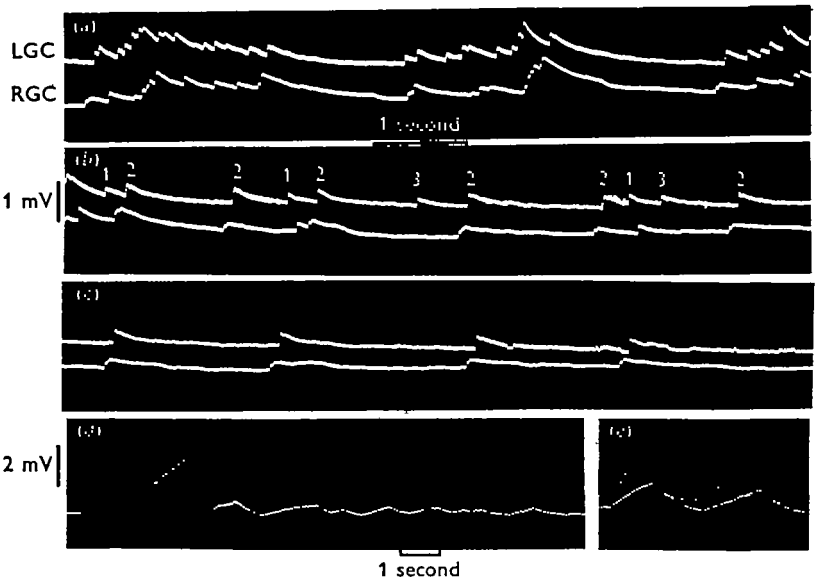

Fig. 13. Recordings from the two giant cells showing unitary EPSPs resulting from the firing of common interneurones. Later in the response (*b*) three of these can be distinguished, as indicated. For the most active interneurone (2) the EPSP in the RGC precedes that in the LGC but in another interneurone (1) the reverse is true. A third interneurone (3) has input to the LGC but is not associated with an EPSP in the RGC, and hence is not a common interneurone.

Direct connexion between the two giant cells. During the first attempts to establish whether or not the RGC had any synaptic connexions with cells in other ganglia, a large sample of cells was impaled in the right pleural and pedal ganglia to see whether any synaptic potential was recorded following mechanical or electrical stimulation of the RGC soma. In no case was any suggestion found of a direct synaptic connexion (Hughes & Tauc, 1963). In those studies it had not been possible to record from both giant cells simultaneously. In the very first experiment in which both giant cells were dissected out from the surrounding membranes and microelectrodes were inserted into them, it was discovered that a shock applied to the RGC was followed after a delay of about 0·15 sec. by a small synaptic potential in the LGC. No evidence was found for the reverse effect, i.e. stimulation of LGC eliciting a synaptic potential in the RGC. Later investigations of this preparation revealed that the synaptic potential recorded in the LGC has a characteristic biphasic shape, which give it some interesting summation properties (Hughes & Tauc, 1965, 1967). Only in one preparation was it possible to record a direct synaptic connexion from the left to the right giant cell.

This was the specimen in which the giant cells were symmetrically placed, one in each pleural ganglion (Fig. 7). Stimulation of either giant cell led to the recording of an almost identical biphasic postsynaptic potential (BPSP) in the other cell (Fig. 15). Such a finding is further evidence supporting the view that these two cells are homologous.

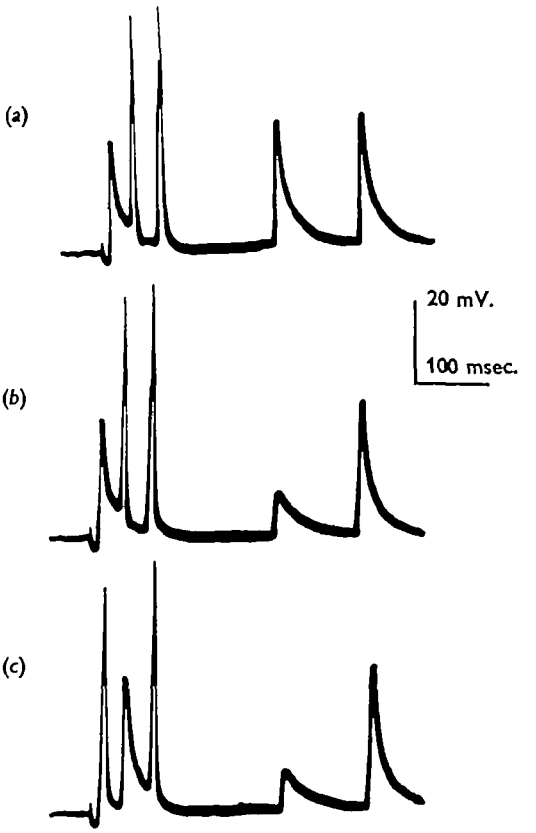

Fig. 14. Recordings from the LGC following single shocks to the left posterior parapodial nerve. Notice the presence of a directly-conducted A spike but also other A spikes with longer and variable delays indicating their synaptic origin. There are also differences in the sizes of the A spikes which may indicate fractionation in the output of this neurone. Notice also the similarity of the discharge pattern in these three sweeps.

The symmetrical arrangement of the two giant cells in this preparation suggests that the normal position of the RGC soma in the abdominal ganglia results from a lengthening of the main axon before its division into branches to the left and right side. This suggests the possibility that the BPSP synapse from the LGC may normally remain in the right pleural ganglion. Experiments designed to test this possibility have so far been unsuccessful (Hughes & Tauc, 1967).

Analysis of the results obtained with different species of *Aplysia* has not so far shown the existence of any direct synaptic connexion between the two giant cells in *A. depilans*. The preparation is more difficult in this species because of the thicker sheaths, but evidence for the BPSP has been obtained in nearly all preparations of *A. fasciata*.

Electrical properties of the giant cell somatic membranes

The large size of these two cells makes them very convenient for the study of cell membrane properties by experiments in which two microelectrodes are inserted into the cell soma. One electrode with a 100 MΩ series resistance was used for passing current and the other for recording changes in the membrane potential. Experiments were performed routinely in which the responses to rectangular pulses of different strength were tested as shown in Fig. 16. The slow rate of change in membrane potential to such currents immediately emphasizes the long time-constant of these cells (100–200 msec.). Using equal steps of ingoing current it was apparent that the successive

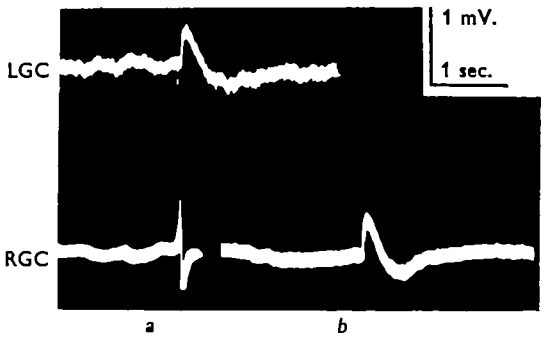

Fig. 15. Synaptic responses recorded in the left (*a*) and right (*b*) giant cells following stimulation of the other giant cell intrasomatically. In this preparation a BPSP is recorded following stimulation of either giant cell as the two cells were symmetrically placed in this unique preparation (see Fig. 7) (after Hughes & Tauc, 1967).

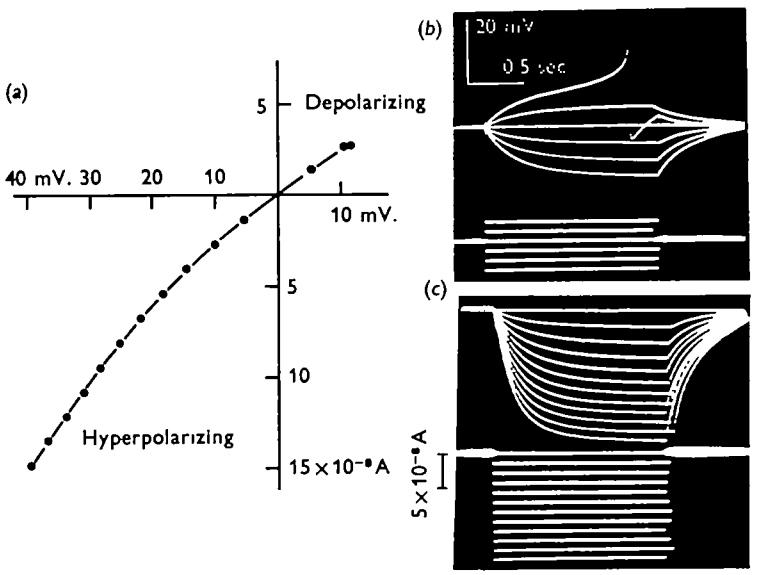

Fig. 16, (*b*) and (*c*) responses of the LGC (19. x. 64) soma to rectangular pulses of 1·5 sec. duration, passed through a second micro-electrode. Eleven equal steps of current were used and gave rise to smaller increments of potential change at higher strengths. The relationship between current and membrane potential is plotted in (*a*) where a change in slope resistance from $3{\cdot}55 \times 10^6\,\Omega$ to $2{\cdot}33 \times 10^6\,\Omega$ takes place between 10 and 20 mV. hyperpolarization relative to the normal resting potential.

increments of potential change tended to decrease (Fig. 16*c*). From plots of voltage change against current, in such experiments, the slope resistance of the cell was determined (Fig. 16*a*). It is clear that the slope resistance changes with an increase in current strength. In the particular experiment plotted in Fig. 16 the change is from a resistance of $3{\cdot}55 \times 10^5$ ohms to one of $2{\cdot}33 \times 10^5$ ohms when the cell is hyperpolarized by more than 20 mV. Over the normal physiological range ($\pm$ 10 mV with respect to the resting level) the slope is constant. Very similar results are obtained with the RGC, and slope resistance changes have been observed which were of the same order as that from 10×10^5 ohms to $2{\cdot}2 \times 10^5$ ohms described in the 10–20 mV. hyperpolarizing range by Kandel & Tauc (1966). This rectifying effect of hyperpolarizing

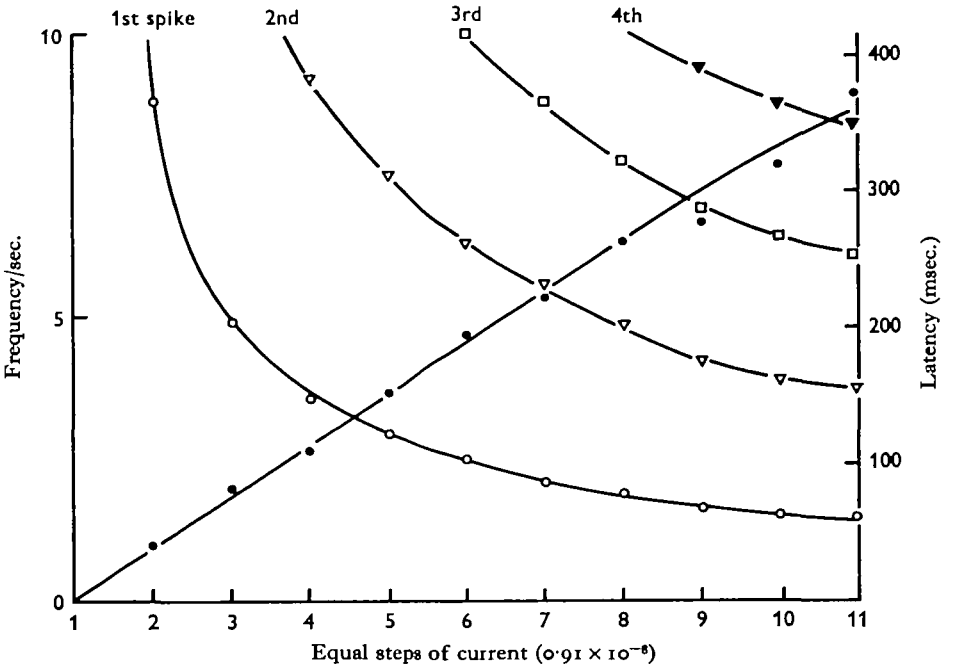

Fig. 17. The left giant cell (30. x. 64). Plot of the mean (over 3 sec.) frequency (●) of firing of the cell in response to depolarizing currents of increasing strength. The latency of the first (○) and subsequent spikes (▽, □, ▼) is also plotted for responses to the same current steps.

currents in molluscan nerve cells has been called 'anomalous rectification' (Tauc & Kandel, 1964) or 'ingoing rectification' (Hughes & Tauc, 1965), and is not uncommon in ganglion cells of *Aplysia*. Thus in a sample of twenty-five abdominal ganglion cells chosen at random this phenomenon was shown by eight of them. There is, however, some variation in the extent of the rectification for corresponding cells in different preparations as has been observed for both the LGC and RGC. The rectification is usually less for the left cell than for the right cell; that plotted in Fig. 16 has been chosen as an example of a less well-marked case. Evidence for anomalous rectification is readily obtained by recording the dynamic current/voltage curve by using the oscilloscope as an XY recorder, a technique used by Furshpan & Potter (1959) and Tauc & Kandel (1964). For the LGC this technique (Hughes & Tauc, 1965) showed an increase in conductance by a factor of about two for a hyperpolariza-

tion of about 20 mV. Thus in this detailed feature of their current/voltage curve the giant cells are very similar.

When currents are passed in the depolarizing direction there is a local response of the somatic membrane which gives rise to a spike at a critical firing level. With increasing current strengths the latency before the origin of the spike decreases, as plotted in Fig. 17. This decrease in latency may be due to properties of the somatic membrane, but there is also the possibility that the region of spike initiation comes closer to the soma and this would have the same effect. The firing frequency increases in an approximately linear way with increasing strength (Figs. 17, 18), but generally it gradually flattens out at higher current strength. In some cases (as shown for the LGC in Fig. 18) there is a suggestion that the curve is made up of two components and this may be due to a change in the region where spikes are being initiated at greater current strength, the increased slope indicating a more sensitive locus. Once again, some variation was found in the shape of this curve, particularly for the LGC. Fig. 18 shows the relationship between firing frequency and current strength for the two cells from the same preparation. The LGC is usually more sensitive than the RGC; the ratio of increase in frequency for a given current increment for the LGC is about twice that for the RGC (Table 1). In both cases this figure is low relative to other nerve cells (3·0 for lobster cardiac ganglion cell, Otani & Bullock (1959), and 4·0–13·5 for cat spinal motor neurones (Frank, see Strumwasser & Rosenthal, 1960), presumably because of the large size of the cell and its large capacitance effect.

Table 1. *Membrane properties of somata of three* Aplysia *neurones*

	(1)	(2)	(3)
Position in C.N.S.	Abdominal ganglia	Abdominal ganglia	Left pleural ganglion
Diameter (μ)	178	500–700 (RGC)	600 (LGC)
Resistance	2·2 MΩ *	Slope $10 \times 10^6\Omega$† and $2{\cdot}2 \times 10^6\Omega$	$3{\cdot}55 \times 10^6\Omega$ and $2{\cdot}33 \times 10^6\Omega$
Specific membrane resistance (Kohm/cm.^{2})	2·2*	11·3–2·5†	4·01–2·63
Time constant (msec.)	50*	100–200	160
Specific membrane capacity (μF/cm.^{2})	23*	12·4–56†	40–60
Pacemaker sensitivity $\left(\frac{\text{impulses/sec.}}{A \times 10^{-6}}\right)$	0·7–4·5*	0·3–0·4	0·7–0·9
Rheobase ($A \times 10^{-6}$/cm.^{2})	50–200*	—	177
Acetylcholine	Hyperpolarize? or depolarize?	Hyperpolarizes	Hyperpolarizes

* Fessard & Tauc (1956) and Tauc (1955).
† Kandel & Tauc (1965).

Table 1 summarizes some of the membrane constants determined for the two giant cells in these experiments and in those of previous authors. Some data for a smaller cell from the abdominal ganglia is also included for comparison. It is apparent from this table that these two cells are extremely similar in many respects, and this adds further support for the view that they are homologous cells. Calculations in this table assume that the surface of the cell soma is uniform. In fact the actual surface is much larger because of the many infoldings of the membrane in large cells of *Aplysia* (Schlote, 1957; Bullock, 1961).

A detailed comparison has not been made of their pharmacological properties, but when acetylcholine is applied to the cell soma it results in a marked hyperpolarization in both the LGC and RGC. These cells are therefore H cells according to the classification of Tauc & Gerschenfeld (see Gerschenfeld, 1966). As in all experiments of this type with molluscan ganglion cells, it is difficult to know their exact significance for studies on the nature of the synaptic processes. However, most of the evidence seems to suggest that they give a good indication of the local membrane properties in the synaptic regions. The recording of a unitary biphasic postsynaptic potential

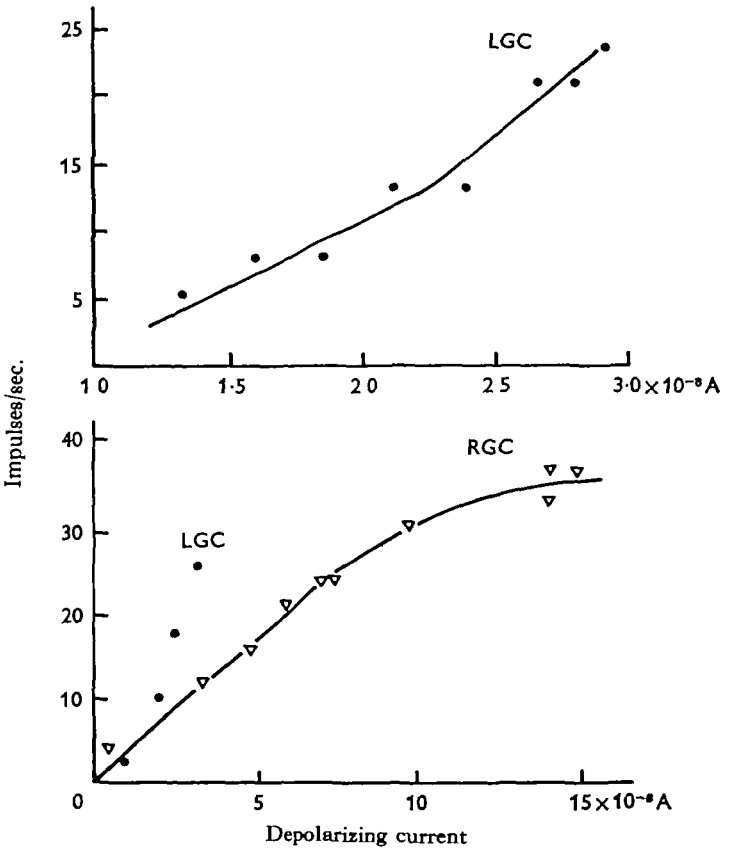

Fig. 18. Plot of the mean firing frequency in response to different intensities of rectangular pulses of 0·37 and 0·75 sec. duration applied intrasomatically to the left and right giant cells respectively of the same preparation. The left cell is more sensitive to current as shown by the position of the points for this cell (●) on the same scale as the RGC plot. The form of the curve for the left giant cell is suggestive of two components but is not present in all cases, the general form of the curve for the RGC being more typical for both cells.

in the LGC which appears to be chemically mediated (Hughes & Tauc, 1965) suggests the possibility that a given transmitter can have hyperpolarizing or depolarizing effects on different regions of the postsynaptic membranes. This would not be surprising as it is already known that the same transmitter (ACh) liberated from a given cell may have a hyperpolarizing effect on one *Aplysia* ganglion cell and a depolarizing effect on another. Alternatively, the presynaptic neurone may release different transmitters and this would contravene Dales's principle (1935). However, in view of the demon-

stration of more than one type of synaptic vesicle at a given synaptic region, as shown by recent electron microscope studies on molluscan ganglia (Gerschenfeld, 1963; Rosenblueth, 1963; Amoroso, Baxter, Chiquoine & Nisbet, 1964), it seems quite possible that a given cell may in fact be able to release different transmitters even at endings quite close to one another and on the same postsynaptic cell. Which of these alternatives applies in the case of the BPSP is an important subject for further investigation.

DISCUSSION

The results of this further analysis of the giant cell system in *Aplysia* have emphasized the complexity of its organization. Although these cells are situated asymmetrically in the C.N.S., nevertheless they are almost symmetrical in their branching pattern, responses to synaptic input and membrane properties. It is apparent that not only

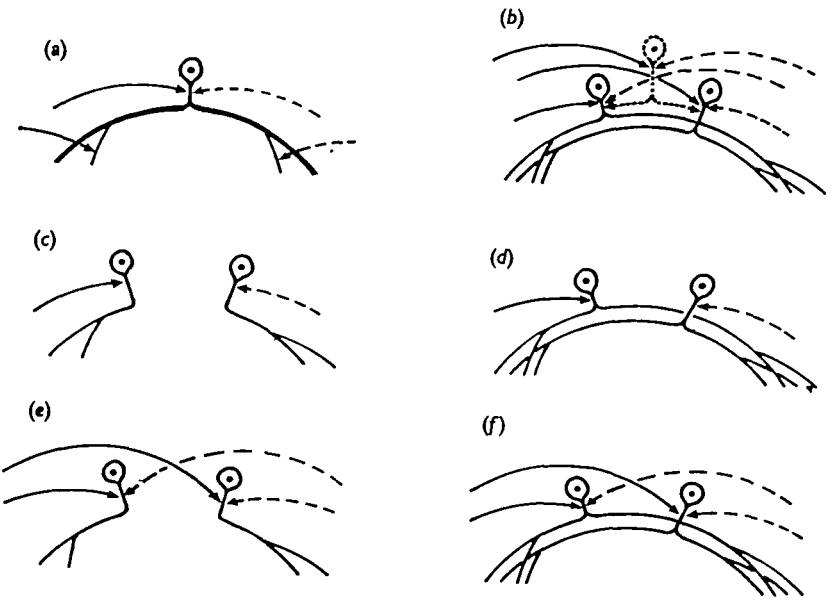

Fig. 19. Diagrams showing some different types of neuronal connexions involved in the integration of inputs from both sides of the body. Inputs from many sensory neurones are indicated by arrows, full lines from the left and dashed lines from the right side. These inputs are shown synapsing with motor neurones (full lines) and with a common interneurone (dashed lines) in (*b*). For detailed description see text.

does the output of these cells diverge so that it can affect all regions of at least the foot and parapodia, but also that they are subjected to convergent synaptic input from all parts of the body. Such an arrangement is presumably ideal where it is necessary for sensory input anywhere on the animal to influence all parts of these organs. The system utilizes cells which are probably homologous, and perhaps this can be regarded as an advanced neuroanatomical arrangement for such a function (Hughes & Chapple, 1967).

In Fig. 19 are illustrated other possible ways in which input from both sides of the body can be integrated. In (*c*) and (*e*) each cell only innervates ipsilateral structures but in (*e*), because of converging input from both sides of the body, stimuli on either side may elicit a response on both sides of the effector system. Such a neuronal

arrangement is probably involved in the symmetrical excitation of the siphons, etc., of *Ensis* as described by Drew (1908). In *Mya* a postsynaptic response recorded extracellularly in nerves and connectives occurs on both sides in response to a unilateral stimulus (Horridge, 1958). It was not possible to decide whether this was true for single neurones in these experiments because of the complexity of the recordings in multifibre preparations.

Recently Mellon (1966) has used intracellular recording techniques to establish for single cells in the ganglia of *Spisula* that axonal branches are present only in ipsilateral nerves and that these cells are subject to convergent input from both sides of the body. Another theoretical possibility is shown in Fig. 19*d*, where neurones send axons to nerves on both sides but the input to each neurone is solely ipsilateral. In this case also, stimulation on either side of the body would result in a symmetrical response. Built in to such a system is the possibility of having two different types of symmetrical response depending on which side of the body is stimulated, but little possibility of producing alternation of the two sides. Such systems do not seem to have been described, however, but clearly they must play a part in local ganglionic mechanisms. A common situation is one in which paired cells are affected from both sides but the influence of the ipsilateral input is greater and the two cells innervate structures on both sides of the body (e.g. snail giant cells, Kandel & Tauc, 1966). The organization of the two giant cells in *Aplysia* appears to be of this general type, but synaptic input from a given side affects both cells almost equally. In *Tritonia* it has also been shown D. A. Dorsett (1967) that some giant neurones send branches to several nerves on both sides of the body.

The present investigations have shown the existence of a number of interneurones which are common to the giant cells, and it has been suggested that such pathways are involved in the response of the two cells to both ipsilateral and contralateral inputs. The system clearly becomes very complex when a given input can affect not only the ipsilateral and contralateral giant cell but also a number of interneurones which are common to them. The resulting convergence must lead to a close similarity in the size and time-course of the compound synaptic potential. Such neuronal connexions are shown diagrammatically in Fig. 19*b*. To this arrangement must also be added the direct connexion between the two giant cells.

Further degrees of freedom exist in the giant cell system because of the possibility that different output pathways of the LGC may be brought into action independently of one another. This is indicated diagrammatically in Fig. 19*a* where a single cell is shown sending axonal branches of different sizes to the two sides of the body. Such a neurone may function so that a given input only affects ipsilateral output; or, where the input influences a more central region of the axonal branching it will affect all outputs equally, regardless of whether they are ipsilateral or contralateral. For both giant cells the experimental evidence suggests that the branches in the ipsilateral nerves are larger than those on the contralateral side. In the case of the LGC, there is also evidence for the synaptic input being more effective on the ipsilateral side when fractionation of the total output occurs. These observations add support to the model suggested by Tauc & Hughes (1963) for the independent functioning of parts of molluscan neurones.

Such considerations indicate some integrative mechanisms which might be investi-

gated further in the *Aplysia* C.N.S. Clearly it would be invaluable if the position of some of the common interneurones could be found. This would provide a preparation with three interconnected neurones in which responses to different pattens of input might be studied. Such investigations might show the importance of the two giant cells in some function in which a bilaterally symmetrical output plays a fundamental role. Several possible functions suggest themselves, but as yet there is no definite evidence for any of them. The existence of a direct connexion between the giant cells of *A. fasciata* and not *A. depilans* may indicate some role in swimming but there is no direct evidence for this function.

SUMMARY

1. Electrophysiological investigations have shown that the giant cell in the right upper quadrant of the abdominal ganglia (RGC) and the giant cell of the left pleural ganglion (LGC) have axonal branches in the main parapodial and pedal nerves on both sides of the body. There is also a branch of the RGC in the branchial nerve.

2. The A spike recorded in the LGC following antidromic stimulation of nerves on the left side is usually larger than that resulting from stimulation of nerves on the right side. Collision experiments suggest that transmission can always occur at the main branching from left to right, but that it is not always possible in the opposite direction.

3. The effectiveness of synaptic inputs to the LGC and RGC from the main nerves has been compared. The synaptic potentials recorded simultaneously in the two cells were very similar in their general form following stimulation of a given nerve, but there were differences in latency. Inputs from the left side tend to be more effective on the LGC and from the right side on the RGC, but only slightly and not in all preparations. Inputs from nerves which enter the abdominal ganglia were generally more effective on the RGC than the LGC, but again not in all cases. The threshold for spike initiation is usually lower for the LGC than the RGC.

4. The presence of a number of interneurones common to these two cells was shown by simultaneous recording. The precise location of such interneurones was not established, but the order of appearance of unitary EPSPs, and the interval between them, showed that some interneurones were in the abdominal ganglia and others in ganglia of the circumoesophageal ring. It is suggested that the presence of these interneurones accounts for the general similarity in the compound EPSPs recorded in the two cells in response to synaptic inputs.

5. There is a direct synaptic pathway between the cells from right to left, but evidence for a connexion in the reverse direction was only found in a single specimen of *Aplysia fasciata*. The unitary postsynaptic potential recorded for these direct connexions is biphasic in form. This direct connexion does not seem to be present in all species of *Aplysia*.

6. Comparison of some of the membrane properties of the LGC and RGC is made. Both cells are similar with respect to their resistance and capacitance and both show anomalous rectification in their current voltage curves. They are both H cells. The excitability of the LGC is usually greater than that of the RGC as indicated by the responses to intrasomatic stimulation.

7. It is concluded that the two giant cells are homologous cells which innervate

many parts of the foot and parapodia on both sides and receive convergent input from all over the body. Possible ways in which they might integrate such inputs are discussed.

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