INPUT ORGANIZATION OF TWO SYMMETRICAL GIANT CELLS IN THE SNAIL BRAIN

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SUMMARY

1. The ventral surface of the snail brain contains two symmetrical giant cells which are readily identifiable in each preparation. These cells lie in the left and right metacerebrum, a cerebral integrative structure which makes afferent and efferent connexions with the periphery and with the infraoesophageal ganglia on each side by means of four ipsi- and four contralateral peripheral nerves and three ipsi- and three contralateral connectives.

2. In order to examine the functional consequences of this anatomical symmetry single or double micro-electrodes, for intracellular recording and for direct stimulation, were placed in one or both of the ventral metacerebral giant cells.

3. Responses from 6 ipsi- and 6 contralateral inputs were examined for orthodromic and antidromic components. The results of these experiments revealed that the output organization (the pathways of the three axonal branches of the giant cells) as well as the input organization were completely symmetrical in the two cells. The anatomical symmetry therefore seems to have been functionally preserved. Simultaneous recordings from both cells failed to reveal direct interconnexions, but did show that the cells do share in common the output of at least two interneurones.

4. By recording from both cells it was also possible to demonstrate, for each of the symmetrical nerves and connectives, that laterality of an input was signalled by latency and by synaptic efficacy; the ipsilateral input produced EPSPs that were consistently more effective and of shorter latency than the contralateral ones.

5. Pharmacological studies revealed that both cells fall into the category called D cells by Tauc & Gerschenfeld (1961); they responded

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with depolarization to iontophoretic injection of ACh and all excitatory synaptic inputs were blocked by d-tubocurarine.

6. With none of the twelve afferent inputs was synaptic inhibition observed. The results support the notion that D cells do not receive inhibitory input.

INTRODUCTION

The ventral surface of the brain of the snail contains two symmetrical giant cells. These two cells are among the five giant neuronal elements in the nervous system of the snail which are readily identifiable in each preparation by their characteristic position and appearance (Kunze, 1918). The cells lie in the left and right metacerebrum, an integrative structure which makes afferent and efferent connexions both with the periphery and with the lower order infraoesophageal ganglionic masses. The distribution of the nerves and connectives to the brain is also symmetrical; there are four major peripheral nerves and three connectives on each side. In addition, the two cerebral ganglia of the brain are interconnected through a commissural pathway (Kunze, 1919; Schmaltz, 1914).

In the present study, single and double micro-electrodes have been placed in each and in both metacerebral giant cells in order to investigate the functional consequences of this anatomical symmetry. The first of this series of papers is concerned with the physiological and pharmacological aspects of the input organization of these two cells. The second paper (Kandel & Tauc, 1966) is concerned with an anomalous biophysical property of these cells and its functional consequence for synaptic transmission.

METHODS

Preparation. Helix aspersa and *H. pomatia* were used exclusively. The *H. aspersa* obtained from Greece were particularly suitable because their connective tissue was easier to dissect, but the common French edible snail was also used. Similar results were obtained with hibernating and non-hibernating individuals. The snails were put under running tap-water in order to make them emerge from their shell. With the animal fully extended, the shell and visceral hump were stretched and cut off from the 'Kopffuss', the head and foot region. The whole circumoesophageal ring of ganglia was removed and immersed in physiological snail solution. During the dissection of the nervous system great care was taken in identifying and in tracing long lengths of the various cerebral nerves. The cerebral ganglia were pinned, by the edges of the connective tissue, to the paraffin base of a chamber, ventral side upwards. The chamber contained ten pairs of electrodes which were used for stimulating the three connectives on each side and two of the four readily available peripheral nerves.

The snail brain is covered with several layers of connective tissue which proved a formidable obstacle to electrode impalement. All these layers had to be removed, since even the final layer could not be penetrated without breaking the pipette and/or destroying the cell. The connective tissue could not be sufficiently softened by connective tissue enzymes to facilitate penetration and was difficult to dissect by hand. The technique we finally used was that of mechanical microdissection with a DeFonbrune micromanipulator. This gave satisfactory results in most preparations.

A second technical difficulty was posed by contraction of the nerves following stimulation. In early experiments, the resulting movement of the ganglion frequently dislodged the micro-electrode. This movement, presumably due to epidural cells of the connective tissue (Schlote, 1957) was eliminated by carefully stripping the nerves and the connectives of all covering connective tissues.

Recording technique. Intracellular impalement was obtained under direct vision with micropipettes filled with 3 M-KCl or 0·6 M-K₂SO₄. In all experiments the recording electrode could be switched into one arm of a Wheatstone bridge so that current could be injected through the micropipette. The bridge employed (Kandel, Spencer & Brinley, 1961) had the advantage of placing the preparation on earth and thereby permitting the use of two symmetrical circuits with a common reference earth for recording from, and injecting current into, two independent cells.

In some experiments another independent micro-electrode was used for stimulating the impaled cell or for iontophoretically injecting ACh on the somatic membrane.

Pharmacological studies. A perfusion system, similar to that used in previous investigations of molluscan neurone pharmacology (Tauc & Gerschenfeld, 1961), permitted changing of solutions and/or perfusion with snail physiological solution without dislodging the recording micropipette. ACh was applied by perfusion to the whole ganglion (5×10^{-5} M) or by iontophoretic injection to a single cell by means of an extracellular placed micropipette. *d*-tubocuranine was applied by perfusion.

<div align="center">RESULTS</div>

The brain of the snail is made up of two symmetrical (supraoesophageal) cerebral ganglia which interconnect by means of a thick commissure. The cerebral ganglia are connected to the buccal ganglion on each side by the cerebro-buccal commissure, and to the seven intraoesophageal ganglia by the left and right cerebro-pedal and cerebro-pleural commissures. In
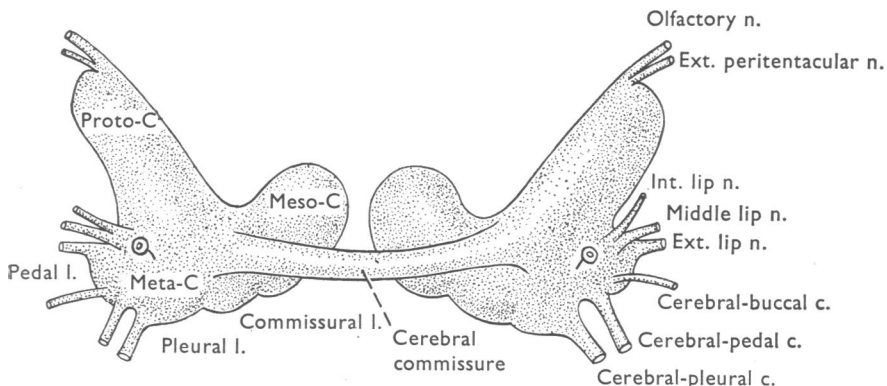

Fig. 1. Schematic drawing of the ventral surface of the cerebral ganglia of the snail indicating position of two metacerebral giant cells and of the main nerves and commissures.

addition to these commissural connexions with other ganglionic regions, the cerebral ganglia give rise on each side to three lip nerves, to a large sensory nerve (the olfactory nerve), as well as to several smaller nerves (Fig. 1). The lip nerves supply the oral lobes and smaller tentacles (the

middle lip nerve), the skin between the tentacles (the internal lip nerve) and the musculature of the buccal cavity (the internal and the external lip nerves).

The cerebral ganglia can be divided into three regions, the meso-, the proto-, and the metacerebrum. The metacerebrum is further subdivided into the pedal, pleural, and commissure lobes. The metacerebrum receives the major portions of the sensory and associative inflow into the brain and is, on these anatomical ground, considered the highest integrative region of the snail nervous system (Kunze, 1919). The most prominent cells of the metacerebrum are the symmetrical giant cells that lie on the dorsal and ventral surface.

The two symmetrical cells on the ventral surface of *Helix aspersa* are about 140 μ in diameter. They lie on the periphery of the metacerebrum near the origin of the internal lip nerve.

When these cells were satisfactorily impaled with a micro-electrode, a resting potential of 50 mV was usually recorded. In most experiments, the cells were silent in the absence of stimulation and did not generate action potentials. However, in some experiments, even without apparent injury, the cells tended to fire in repetitive bursts for relatively long periods of time (30–60 min). Figure 2 illustrates data from an experiment in which simultaneous recordings were obtained from the two cells when they fired in this bursty manner. In this and all subsequent records, the upper channel record is from the right cell and the lower channel record from the left one. Parts $2A_1$ and $2A_2$ are a low and high gain recording respectively showing two different bursts in the right cell. Parts $2B_1$ and $2B_2$ show an orthodromically triggered (B_1) and spontaneously occurring (B_2) burst in the left cell. The transients in the high-gain records are artifacts due to the capacitative coupling between the two recording micropipettes. As these figures illustrate, spike generation in one cell did not produce a detectable alteration in the transmembrane potential of the other cell. This finding indicates that the cells do not make direct synaptic connexions with each other.

Repetitive firing similar to that occurring spontaneously or in response to orthodromic volleys could also be produced by passing sufficient depolarizing current through the micropipette (Fig. 3, parts A_1–A_4). As was the case for spontaneously occurring or synaptically evoked bursts, the spikes were followed by an additive hyperpolarizing after-potential which was 5 mV in amplitude and up to 1 min in duration following a train of action potentials (Fig. 3, Parts A_3 and A_4).

The action potential of these cells could be triggered antidromically (Fig. $3C_1$) as well as orthodromically (Fig. $3C_2$) by stimulation of the appropriate connective or nerve. The antidromic action potential occurred

in two steps indicating sequential invasion with an axonal region (A spike) firing before the cell body (S spike). As can be seen in parts C_1 and C_2, the firing level for the S spike was approximately the same with both anti- and orthodromic invasion. In these standard properties, the metacerebral cells resemble other molluscan neurones (Tauc, 1962).
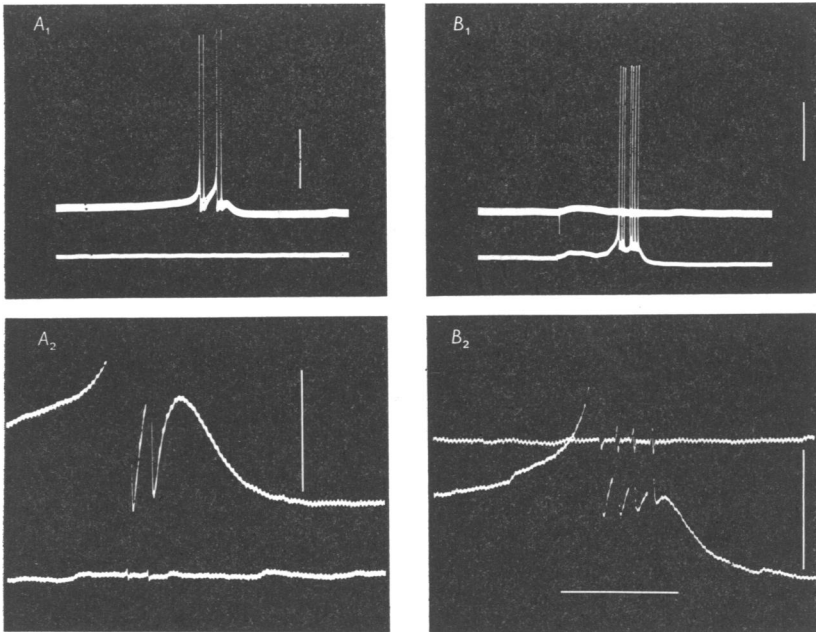

Fig. 2. Simultaneous recordings from both giant cells. In this and all subsequent recordings the right giant cell is illustrated in the upper trace and the left giant cell in the lower trace. A_1 and B_1 are low gain, slow sweep records (voltage cali- bration is 20 mV, time calibration 1 sec); A_2 and B_2 are high gain records (voltage calibration is 2 mV, time calibration is 0·5 sec). Repetitive firing in one cell failed to produce a detectable potential change in the other cell. The transients in the quiescent cell, noticeable in the high gain records, are artifacts due to the capacitative coupling between the two recording pipettes. The action potentials in A_1 and B_1 have been retouched. Traces of 50 cycles a.c. current appear on A_2 and B_2 and on some of the subsequent figures.

As in other molluscan neurones, orthodromic EPSPs could not always be clearly distinguished from antidromically produced axonal (A) spikes on the basis of configuration alone. In other molluscan cells (Tauc & Hughes, 1963) this distinction can be facilitated by the differential response of EPSPs and A spikes to hyperpolarizing currents; progressive hyper- polarization decreases and ultimately blocks the A spike while it pro- gressively increases the amplitude of the EPSP. As will be described in the next paper, this criterion could not be strictly employed in these cells

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(Kandel & Tauc, 1966). EPSPs and A spikes could, nonetheless, be easily distinguished by tests for occlusion (Fig. 3 *B*). EPSPs were not occluded by directly initiated spikes and the conductance change of the synaptic potential altered the decay of the hyperpolarizing after-potential (Fig. 3 B_2). By contrast the A spike was readily occluded by an appropriately timed direct spike (Fig. 3 B_1). This distinction permitted us to map the output and input relations of the two cells. In the sections that follow, the path of the giant cell axons will be defined by means of antidromic activation similar to that shown in Fig. 3 C_1. Subsequently, the synaptic input organization will be examined.
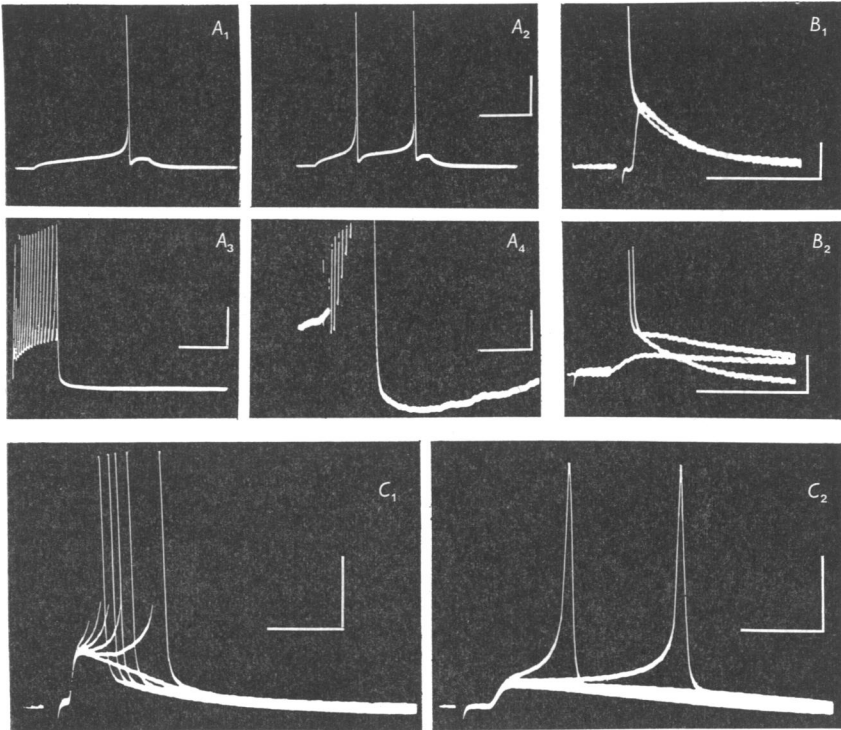

Fig. 3. Direct, orthodromic and antidromic action potentials. A_1–A_4 directly initiated action potentials and after-potentials. The voltage calibration is 20 mV in A_1–A_3 and 2·5 mV in A_4. The time calibration is 500 msec for A_1 and A_2 and 1 sec for A_3 and A_4. The high gain slow sweep record (A_4) shows the magnitude of the after-potential following a train of spikes. C_1 and C_2 illustrate antidromic (C_1) and orthodromic (C_2) initiated action potential and the underlying A spike (C_1) and EPSP (C_2). The firing level for the S spike is approximately the same with both modes of activation (voltage calibration is 20 mV, time calibration is 100 msec). B_1 and B_2 show the effects of A spike (B_1) and EPSP (B_2) on the hyperpolarizing after-potential (voltage calibration is 10 mV, time calibration is 250 msec).

Path of the giant cell axons

By means of antidromic activation it was possible to demonstrate that the paths of the axon branches of these two giant cells are symmetrical. Each cell sends one axonal branch into the ipsilateral external lip nerve and into the ipsilateral cerebro-buccal connective as well as another branch into the contralateral cerebro-buccal connective. The top two parts of Fig. 4 show the action potential in the left and right cell produced by stimulating the left and right lower lip nerves, respectively. The action potentials are quite similar in appearance. In each case, the action potential has three components, one S spike and two A spikes of differing amplitude and safety factor. These three components could be separated in two ways: (1) by use of repetitive stimulation to bring out the differential refractoriness and safety factor in each of the three components (Fig. 4, upper row right); and (2) by hyperpolarizing the cell and therefore first blocking elements that are closer to the current passing micro-electrode in the soma (Fig. 4, upper row left). Antidromic action potentials were also produced by stimulating the ipsilateral and contralateral buccal connectives (Fig. 4, middle and bottom row). The bottom section of Fig. 4 illustrates a simultaneously obtained record from both cells in response to stimulating the left buccal connective. Note that the left cell fired before the right and that the A spike component of the homolateral spike was larger than that of the contralateral spike. The difference in configuration of the A spikes from homolateral and contralateral branches of the same neurone suggests that the two branches differ in diameter, the contralateral one being narrower, so that the antidromic axonal action potential blocks further away from the cell body in contralateral branch than in the homolateral one (Hughes & Tauc, 1963). The latency difference presumably reflects not only this difference in size but also the greater conduction distance for the contralateral branch.

Input organization

Ipsilateral inputs. The evoked ipsilateral orthodromic inputs are also symmetrical. Figure 5 illustrates the effects of stimulating ipsilateral nerves and connectives for both the left and the right cells. These data were obtained in the same preparation and have been arranged in focus columns, each column illustrating the respective response of the cell (upper row) and of the right cell (lower row) to the indicated ipsilateral nerves and connectives. As is evident from the figure, the ipsilateral inputs produced large excitatory synaptic potentials and their configuration, for a given ipsilateral input, was comparable in both cells. No inhibitory PSP were observed.
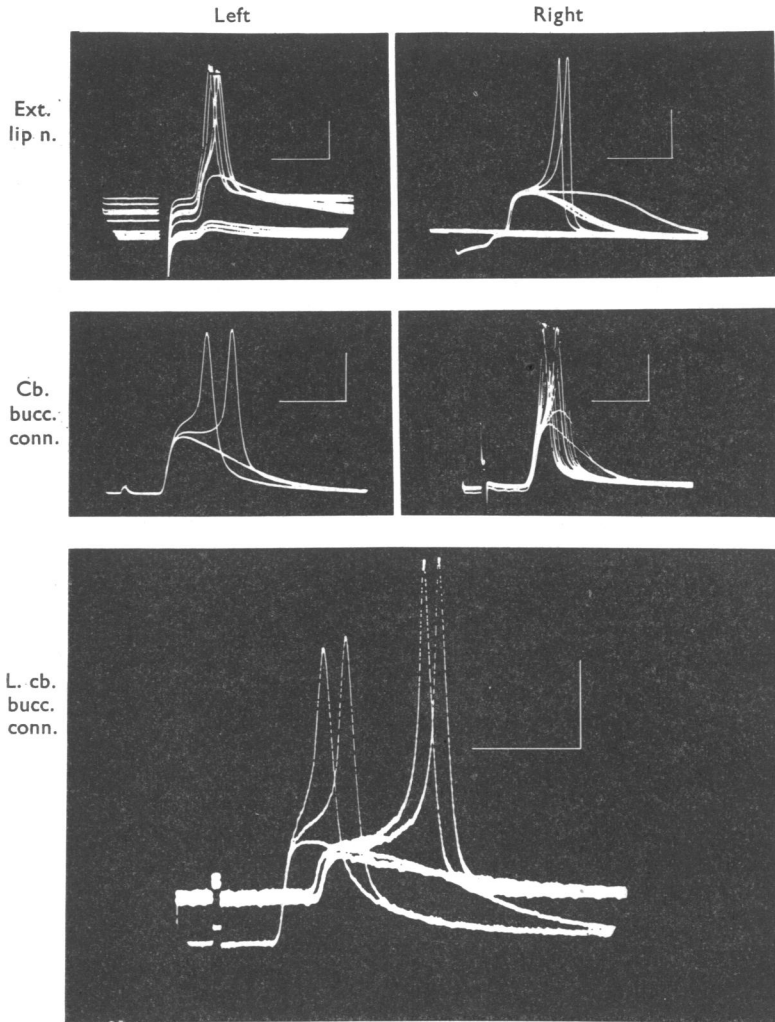
Fig. 4. Pathways of the giant cell axons. The left cell (left column) and the right cell (right column) are shown responding to antidromic activation following stimulation of their ipsilateral external lip nerve (upper figure) and buccal connective (middle figure). The lower figure is a simultaneous recording of the two giant cells showing antidromic activation in both cells with different latency and action potential configuration in each, following stimulation of the left cerebro-buccal connective. In all records, the voltage calibration is 20 mV and the time calibration is 50 msec. Superimposed records to illustrate several components in antidromic action potential. These could also be separated by differential hyperpolarization (upper left).
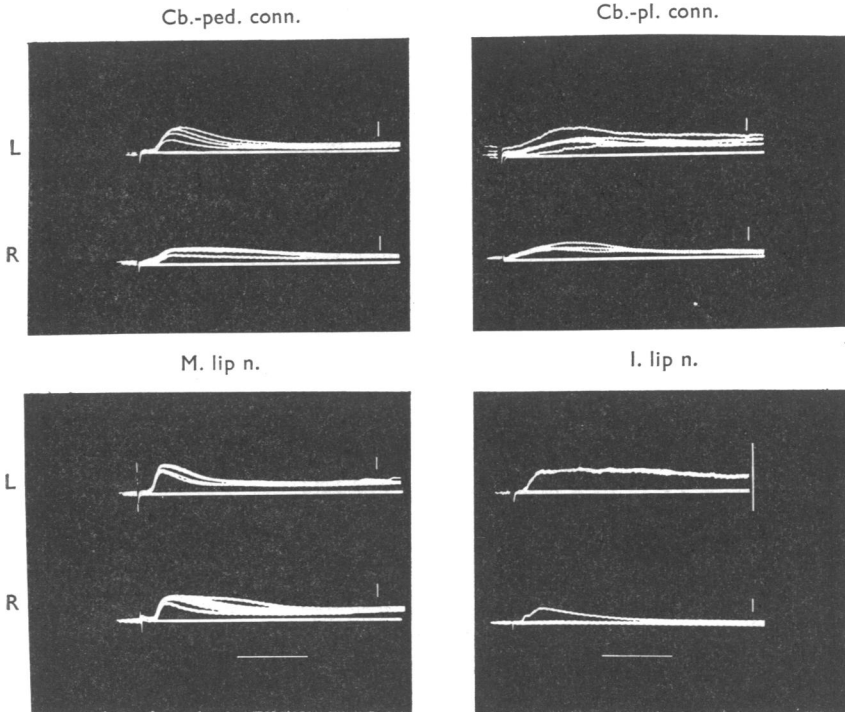
Fig. 5. Organization of ipsilateral inputs. Synaptic potential produced in the left (L) and right (R) giant cell by stimulation of the two main afferent lip nerves and the afferent two connectives. Note the general similarity in the latency and configuration of the ipsilateral PSPs in the two cells. The calibrations in all records are 5 mV and 250 msec.

Contralateral inputs. In this series of experiments simultaneous recordings from both cells were obtained and their responses to orthodromic inputs were tested in order to determine how the two cells distinguished between ipsi- and contralateral inputs. Each portion of Fig. 6 contains the simultaneously recorded responses from the right (upper trace) and left (lower trace) cell. Responses produced by stimulation of the left cerebral ganglion nerves are arranged in the left column and responses from the right nerves in the right column. Each row therefore represents the response of both cells to the left and right member of a given pair of nerves.

In all instances examined the distinction between ipsi- and contralateral input was the same. The ipsilateral input was considerably more effective and of slightly shorter latency than the contralateral one. The contralateral PSP had much the same time course as the ipsilateral but it was about five times smaller. The latency differential between ipsi- and contralateral inputs varied; in some cases the differences were small; in others great.

Left nerve   Right nerve

Cb.-ped. conn.

Cb.-pl. conn.
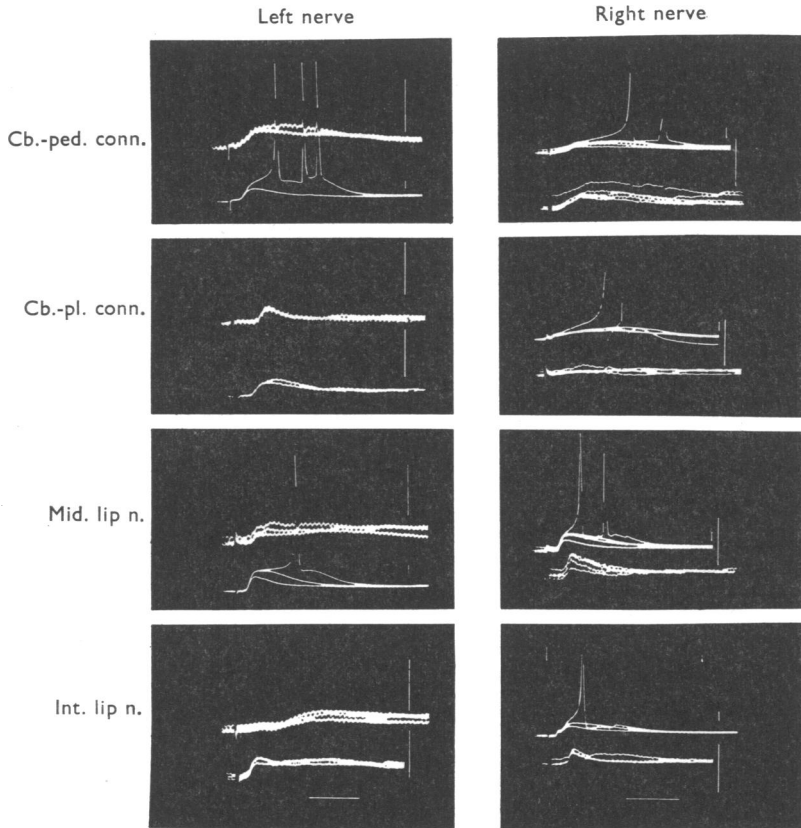
Mid. lip n.

Int. lip n.

Fig. 6. Organization of contralateral inputs. Simultaneous recording from both cells to permit comparison of ipsilateral and contralateral responses to a given input. Responses to stimulation of the left nerve and connectives in the left column and responses to stimulation of right nerves and connectives in the right column. All calibration signals are 5 mV and 250 msec.

Interneurones

In addition to those large, evoked EPSPs, the giant cells also showed two types of spontaneously occurring elementary EPSPs. On the basis of their relative constancy of configuration and firing pattern, it was possible to attribute these PSPs to the activity of two different interneurones.

One (the 'phasic' EPSP) tended to fire in a phasic manner, and frequently gave rise to high-frequency bursts. This EPSP had a relatively slow rise time and a long decay; it therefore had a considerable tendency for temporal summation. The other (the 'tonic' EPSP) tended to fire in a more regular discharge; this EPSP had a faster rate of rise and of decay. Figure 7 illustrates the pattern of firing of the PSPs of these two inter-

neurones as recorded in the left cell. Part *A* shows the phasic PSP, part *B*, the tonic PSP and part *C* the concomitant occurrence of both PSPs.

The elementary PSP produced by the tonic interneurone lasted 70–100 msec, that of the phasic interneurone lasted 200–300 msec. The time constant of decay for the former was about 50 msec, which is similar to the input time constant of the membrane as measured, at the resting level of membrane potential, by the decay of a comparably sized depolarizing electrotonic potential. The decay of the phasic PSP and that of the synchronous PSPs produced by ipsilateral nerve stimulation was longer, but even these were in part dependent on the passive membrane properties, as was evident in experiments in which the latter were systematically changed (Kandel & Tauc, 1965; Figs. 2 and 7). The longer duration of the phasic and of the evoked PSPs may be due to a peristent transmitter action (Fessard & Tauc, 1958; Tauc, 1958).

In general, the duration of PSPs in the metacerebral cells and in other molluscan neurones is considerably longer than that encountered in the mammalian motoneurone. This is due to longer action of the transmitter (Tauc, 1958) and to different membrane properties. For example, in the metacerebral cells, the specific capacity is 5 μF/cm^2 and the specific resistance, at the resting level, is 10^4 Ω cm^2 (Kandel & Tauc, 1965). In some molluscan cells the specific capacity may reach 10 μF/cm^2 (Fessard & Tauc, 1958). These values are 5 to 10 times higher than those of cat motoneurones (Eccles, 1964). The durations of the metacerebral PSPs are, however, quite similar to the PSPs of mammalian supraspinal neurones (see, for example, Kandel *et al.* 1961; Anderssen & Eccles, 1962; Purpura & Cohen, 1962).

The interneurones generating the PSPs tended to be under the control of different nerves. Although the separation was frequently not complete there was a tendency for the 'tonic' interneurone to be driven more effectively by the left peripheral nerves and by the right connectives while the 'phasic' interneurone was driven more effectively by the right peripheral nerves and by the left connectives (Figs. 7 and 8).

When simultaneous recordings were obtained from the left and right cells, the EPSPs produced by the two interneurones could be noted to occur simultaneously in both cells indicating that the interneurones were common to both giant cells. Therefore, each interneurone presumably sends one branch to each of the two giant cells. As these data indicate, the two branches always fired together. The fact that the PSPs occurring in the two cells were in almost perfect synchrony also indicates that the conduction distance for the branches must be comparable. This suggests that the two interneurones are probably centrally located.

A distinctive feature of the 'phasic' PSP was its tendency to fire in bursts (Fig. 7*C* and Fig. 8). In many preparations, these bursts would persist for a half hour or longer in a fairly regular pattern but, after a while, particularly after repeated stimulation of nerves, the bursts would turn off and not return for the remainder of the experiment.

Figure 8 illustrates these bursts as they occurred in both cells. Upon stimulation of the left middle lip nerve firing rate of the 'tonic' PSP was increased, but its change of pattern did not interfere with the generation of the burst discharges by the 'phasic' PSP.
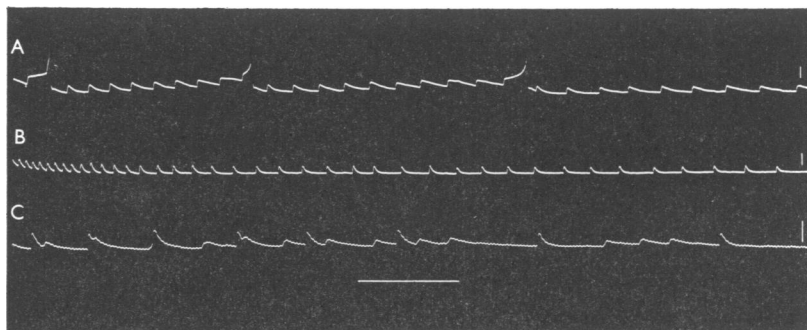
Fig. 7. Two types of interneurones. Parts A–C are from the same experiment on the left cell and show the distinctive PSPs produced by the repetitive firing of the 'phasic' (A) and the 'tonic' (B) interneurone following stimulation of the left (A) and right (B) connectives. In C, a higher gain, faster sweep record, the two types of PSP can be seen occurring together. Note bursty firing of phasic PSP in part C and its capacity for temporal summation in part A. The voltage calibration in all records is 1 mV; the time calibration is 3 sec for A and B and 1·5 sec for C.
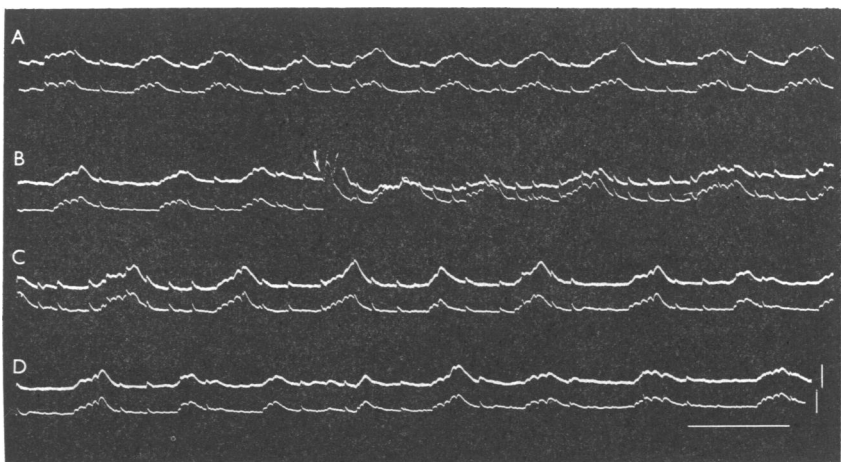
Fig. 8. Independent activation of interneurones. A–D is a continuous record obtained simultaneously from both cells. Note that both PSP have same configuration and occur simultaneously in each cell. At the arrow (B) the left-middle lip nerve was stimulated and the tonic interneurone was activated. The consequent repetitive activity of the tonic interneurone did not alter the tendency for recurrent repetitive bursts by the phasic interneurone. Voltage and time calibration is 2·5 mV and 6 sec, respectively.

Pharmacological properties of the synaptic input

In a previous investigation of molluscan neurones, Tauc & Gerschenfeld (1961) found that all the neurones in the abdominal ganglion of *Aplysia* and in the visceral mass of the snail responded to acetylcholine (ACh) with
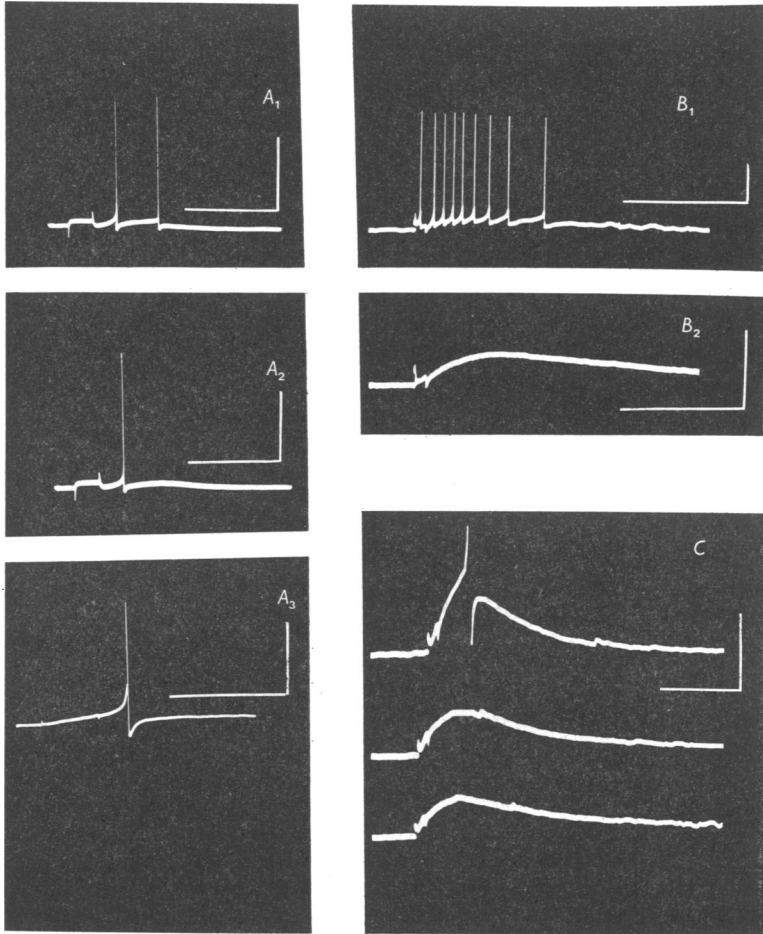

Fig. 9. ACh response in the giant cell. A_1 and A_2 illustrate the generation of spikes after iontophoretic application of ACh. A_3 is from another experiment and shows, on a fast sweep, the short latency of onset of the ACh response when the injecting micro-electrode is well positioned. B_1 repetitive discharge to strong injection of ACh. In B_2 the membrane was hyperpolarized and the ACh potential underlying the burst was shown at higher gain. In C the ACh response is shown at constant gain but at different levels of hyperpolarization. The voltage calibration is 40 mV in A_1–A_3, 20 mV in B_1 and 10 mV in B_2 and C. The time calibration is 2 sec in A_1, A_2 and C, 1 sec in A_3, 5 sec in B_1 and B_2. The action potentials in parts A and B have been retouched.

either a depolarization (D cells) or a hyperpolarization (H cells) of their membrane potential.

In the current series of experiments, when ACh was applied by perfusion to the metacerebral giant cells, both cells showed a similar depolarizing response. These could also be demonstrated when ACh was injected iontophoretically on the synapse-free cell body (Fig. 9) indicating that here as in other molluscan neurones the chemosensitive area was not restricted to the synaptic region. Repeated iontophoretic injections caused desensitization (Fig. 10) similar to that which occurs at the muscle end plate of the frog (Katz & Thesleff, 1957) and in molluscan D and H cells (Tauc & Bruner, 1963).
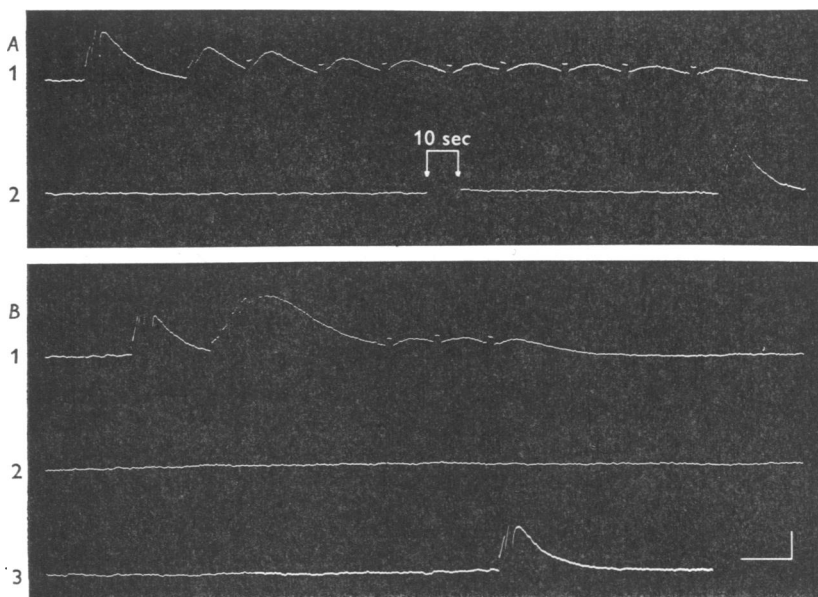

Fig. 10. Desensitization of ACh response. A_1 and A_2 and B_1–B_3 represent continuous strips with the exception of the temporal deletion indicated. A and B show the decline of ACh response during and after two different rates of repetitive injection and the recovery of the response after 0·5–1 min. Time, 5 sec; voltage, 15 mV.

The finding that ACh simulated the action of the excitatory transmitter substance was further strengthened by experiments with curare. Curare in concentrations of 5×10^{-5} M blocked the action of the ACh injection (Fig. 11 A) as well as the synaptic potential produced by the natural transmitter (Fig. 11 B).
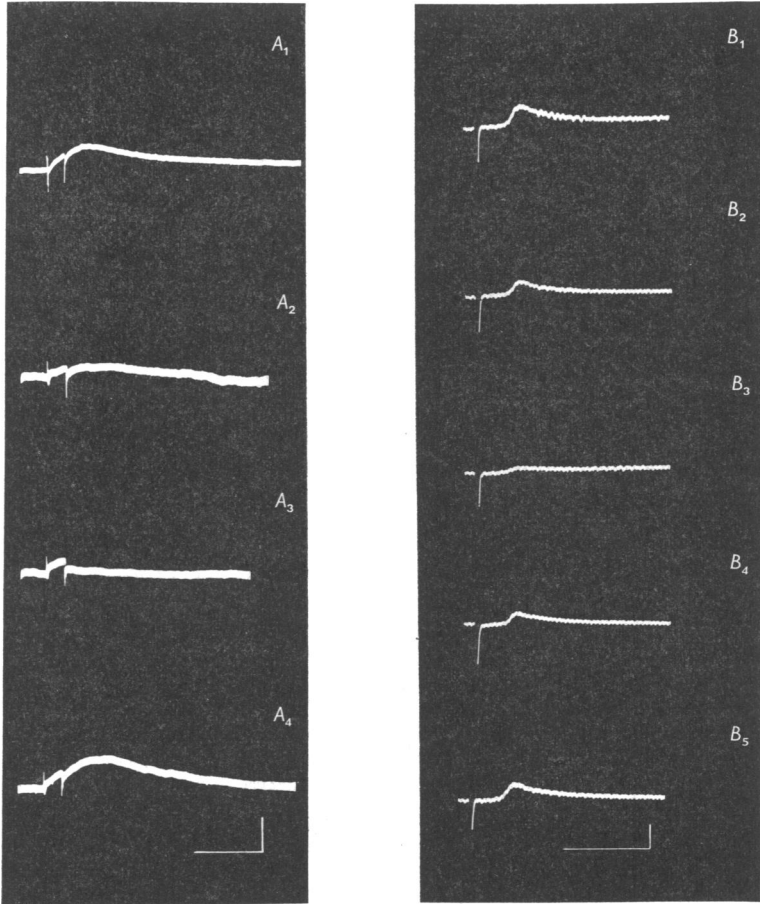
Fig. 11. Curare blockade of ACh response and EPSP. A_1–A_4 ACh response. B_1–B_5 EPSP. A_1 control; A_2 and A_3 2 min and 3·5 min after application of 10^{-4} curare; A_4 after 10 min washing. B_1 control; B_2 and B_3 1·5 and 3 min after application of 10^{-4} curare; B_4 and B_5 after 6 and 9 min washing (EPSP produced by ipsilateral mid-lip nerve). Voltage and time calibrations are 5 mV in A and B and 2·5 sec in A and 500 sec in B.

DISCUSSION

The data presented about the metacerebral giant cells indicate that the anatomical symmetry has been functionally preserved. Not only are the input and the output organizations symmetrical but the two cells also share at least some common pharmacological properties. Finally, as will be illustrated in the following paper, both cells have identical anomalous biophysical properties.

Simultaneous recording from both metacerebral cells indicate that they

lack direct interconnexions but share the output from at least two distinctively different interneurones. The difference in the rates of rise and decay of the two types of EPSPs generated by these interneurones may be due to differential electrotonic effects. This suggests that PSPs are generated on spatially separated portions of the synaptic region of the giant neurone, with the 'tonic' PSP being electrically 'closer' to the intrasomatic pipette than the 'phasic' one.

In studies with both cells, it has been possible to demonstrate that laterality of the input is determined by two factors: latency and efficacy. The ipsilateral inputs are more effective and of shorter latency than the contralateral ones. The schematic diagram of Fig. 12 summarizes the data obtained on the pathways of the giant cell axons and on the organization of the inputs.

The input organization depicted in the diagram of Fig. 12 is incomplete as far as two sets of data are concerned. First, we failed to observe effects following stimulation of the olfactory and external peritentacular nerves. These nerves were particularly difficult to dissect and failure to obtain responses following their stimulation may simply reflect damage due to dissection. Secondly, although, as indicated on the diagram, the nerves carrying ipsilateral branches of the axons of the giant neurones (ext. lip nerve and cb. buccal connexion) also conveyed orthodromic EPSPs it was difficult to be certain if the nerve carrying the contralateral axonal branch (the contralateral cerebral buccal nerve) also conveyed afferent information. Our experiments suggest that this was not the case but there are not enough data on this point to be certain.

In a study of the pharmacological properties of cells in the visceral ganglion Tauc & Gerschenfeld (1961) divided cells into two classes, D and H, depending on whether they responded with depolarization or hyperpolarization to ACh and had a corresponding excitatory and inhibitory cholinergic input. The H cells also had a non-cholinergic excitatory input, while the D cells appeared to have no inhibitory input (Tauc & Gerschenfeld, 1961). A limitation of these earlier experiments was that only two peripheral nerves were usually stimulated and therefore only a limited number of all possible inputs were tested. The metacerebral giant cells have, therefore, permitted a more rigorous testing of the D cell hypothesis. It proved possible to specify the properties of twelve different peripheral inputs and of two interneurones on each of these cells and in no case was inhibition observed. Finally, it was also possible to demonstrate a transmitter uniformity for all inputs since all of them were blocked by *d*-tubocurarine and are therefore presumably cholinergic.

Some of the techniques utilized here have previously been employed to illustrate functional symmetry in other molluscan giant cell pairs. In a study of afferent inputs and common interneurones Strumwasser (1962) documented the functional symmetry of the cells in the left and right buccal ganglion of *Aplysia*. On the basis of symmetrical axonal path-

ways, as demonstrated by antidromic volleys, Hughes & Tauc (1961, 1963) suggested functional symmetry for two anatomically asymmetrical giant cells in *Aplysia*, one located in the left pleural ganglion, and the other in the right upper quadrant of the abdominal ganglion. Paired giant neurones
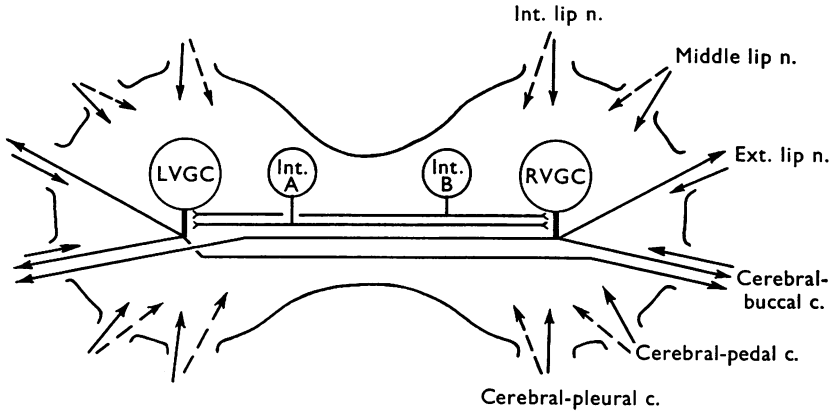

Fig. 12. Schematic outline for input and output organization of the metacerebral giant cells.

such as those described by Mauthner (Bodian, 1937), also occur in the medulla of lower vertebrates and these cells, in addition to their other interesting and distinctive electrophysiological properties, seem to show functional symmetry (Furshpan & Farukawa, 1962).

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