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PROPERTIES OF A SYMMETRIC PAIR OF SEROTONIN-CONTAINING NEURONES IN THE CEREBRAL GANGLIA OF *PLANORBIS*

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SUMMARY

1. There is a bilaterally symmetric pair of large serotonin-containing neurones in the cerebral ganglia of *Planorbis corneus*.

2. In some animals these neurones are connected by a non-rectifying electrotonic synapse, and fire in synchrony even at prolonged high frequency. In other animals the neurones are not coupled, and fire independently except when driven by common input. Occasionally the coupling is weak.

3. Both coupled and non-coupled serotonin neurones have processes in the major nerve trunks of both buccal ganglia.

4. Synapses are made with many neurones in the buccal ganglia. The serotonin neurones can initiate firing in several motoneurones and thus produce movements of the buccal mass.

5. During spontaneous feeding cycles the input and firing pattern of the serotonin neurones do not bear any obvious relation to the movements of the buccal mass.

6. The data suggest that the serotonin neurones are modulatory cells, altering the level of excitability of buccal ganglion neurones.

INTRODUCTION

In the cerebral ganglia of the pulmonate molluscs *Helix*, *Limax* and *Planorbis*, and the opisthobranch molluscs *Aplysia* and *Tritonia* there is a bilaterally symmetric pair of giant serotonin-containing neurones (Dorsett, 1967; Cottrell & Osborne, 1970; Marsden & Kerkut, 1970; Pentreath, Osborne & Cottrell, 1973; Weinreich, McCaman, McCaman & Vaughn, 1973; Cottrell & Macon, 1974; Gerschenfeld & Paupardin-Tritsch, 1974). These neurones, which appear to be homologous in the various genera (see Osborne & Cottrell, 1971; Weiss & Kupfermann, 1974) have provided valuable information on the transmitter role of serotonin and the behavioural role of single identified neurones. In *Helix* and *Aplysia* each serotonin neurone has been shown to exhibit extensive axonal branching and to make excitatory and inhibitory synaptic connexions with numerous other neurones in the buccal ganglia. Serotonin appears to be the transmitter at each synapse (Cottrell, Berry & Macon, 1974; Cottrell & Macon, 1974; Gerschenfeld & Paupardin-Tritsch, 1974). The neurones may serve

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a modulatory role in feeding behaviour, which is governed principally by the rhythmic pattern of motor output from the buccal ganglia (Pentreath, 1973; Weiss, Cohen & Kupfermann, 1975; Weiss & Kupfermann, 1974).

The present paper describes the synaptic connexions made by the serotonin neurones in *Planorbis*, and their influence on the feeding movements of the buccal mass. The neurones show an extensive and overlapping distribution of peripheral axons, and appear to play a modulatory role in feeding via synaptic connexions with neurones in the buccal ganglia. In some animals the serotonin neurones were strongly coupled electrically, but in others no connexion between them could be found. In general, the properties of the neurones suggest that they are structurally and functionally homologous to the cerebral giant neurones of *Helix*, *Limax*, *Aplysia*, and *Tritonia*.

METHODS

Specimens of *Planorbis corneus*, obtained from Gerrard & Haig Ltd, East Preston, Sussex, were maintained in aquaria at room temperature (16–20 °C). Experiments were performed on the isolated circumoesophageal and buccal ganglia which were pinned to the plastic base of a 5 ml Perspex chamber and bathed with a continuous flow of physiological solution (Berry, 1972*a*) at room temperature. For behavioural experiments semi-intact preparations were used, in which the buccal mass and associated muscles were left *in situ* with their nerve supply intact. The cerebral ganglia and sometimes the buccal ganglia were then immobilized by pinning them to a plastic platform which allowed almost unrestricted movement of the buccal mass.

Neurones were impaled with double barrelled microelectrodes containing 0·6 M-K_2SO_4 (2–25 MΩ resistance); one barrel was used to pass current and the other for recording potential. Part of the connective tissue above the neurones to be impaled was removed with forceps. Records were made on a Brush 220 series 2-channel ink recorder. Glass suction electrodes were used for extracellular recording and stimulation. Conventional amplifying and stimulating equipment was used.

Movements of the buccal mass were monitored by attaching the radular sac to an isotonic force transducer by a nylon thread. Movements were recorded as potential changes.

The serotonin neurones were identified by fluorescence histochemistry (Falck & Owman, 1965). The cerebral ganglia were freeze dried, exposed to formaldehyde gas at 65% relative humidity and 80 °C for 1–3 h, and subsequently embedded in paraffin wax. Serial 10 μm sections were examined with a Leitz Ortholux fluorescence microscope. To distinguish autofluorescence from the fluorescence caused by serotonin or catecholamines, sections were immersed in 0·1% sodium borohydride in 98% isopropanol. This quenches the specific fluorescence, which can then be restored by exposure to formaldehyde gas (Corrodi, Hillarp & Jonsson, 1964). The influence of ultraviolet light on fluorescence was also observed, and sections of tissues not exposed to formaldehyde gas were examined for comparison.

RESULTS

Identification of the serotonin neurones

In sections of cerebral ganglia examined with the fluorescence microscope the serotonin neurones were readily identifiable (Figs. 1 and 2) by their pale yellow

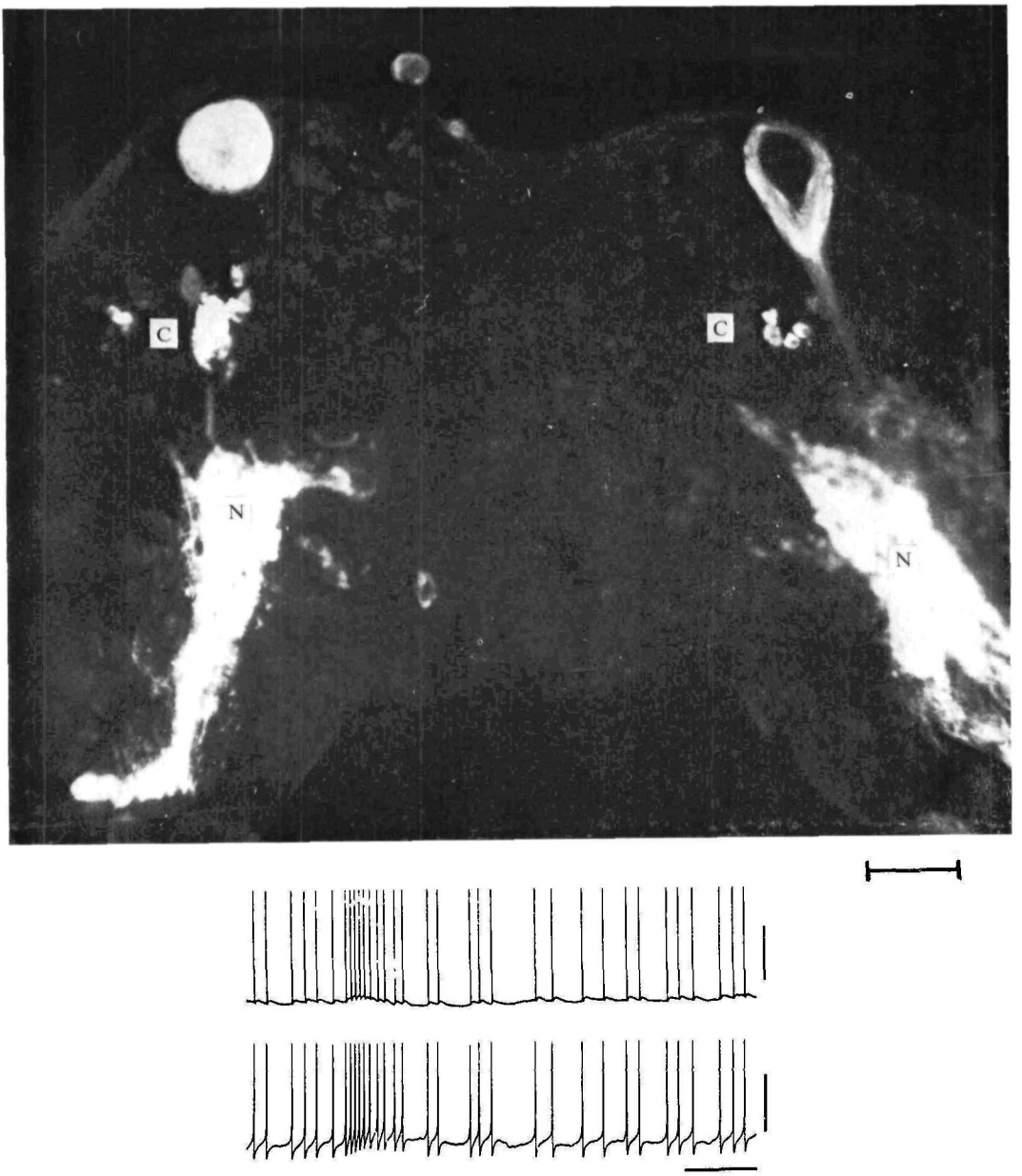

Fig. 1. Fluorescence micrograph of a 10 μm frontal section through the cerebral ganglia, showing the electrically coupled giant serotonin neurones (the large fluorescent (yellow) neurones on the dorsal (upper) surface of the ganglia). Fluorescence indicating the presence of catecholamines is seen in a few small neurones (C) and in large areas of neuropile (N). The scale represents 100 μm. The simultaneous intracellular recordings from the serotonin neurones were made immediately before freeze-drying, and demonstrate the coupling between them. The neurones were firing spontaneously, with the left neurone (lower) driving the right neurone (upper). The left neurone was briefly depolarized to produce the burst of spikes. Time scale, 5 s. Voltage calibration, 25 mV. The full amplitude of the action potentials was not recorded.

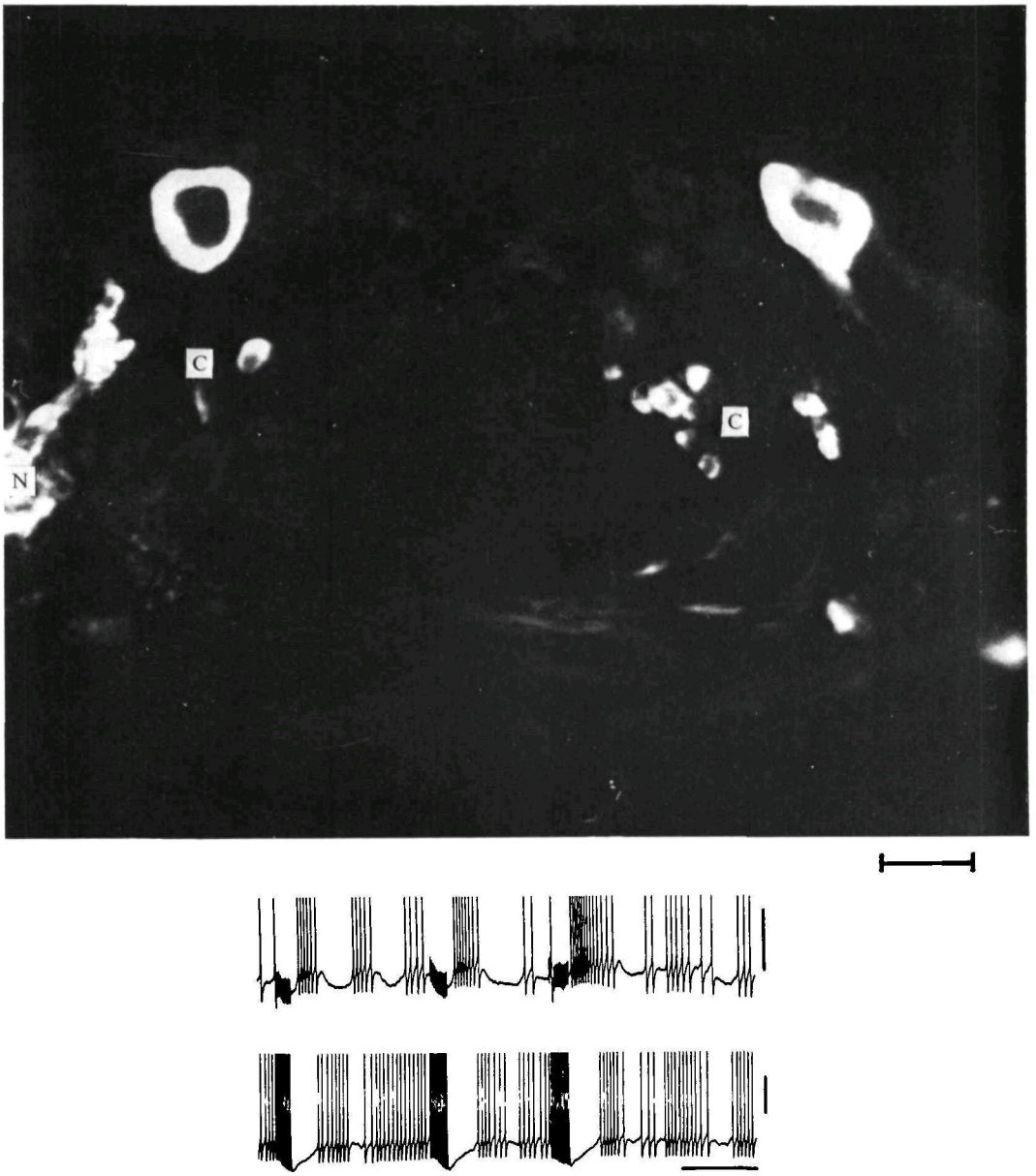

Fig. 2. Fluorescence micrograph of a 10 μm frontal section through the cerebral ganglia, showing non-coupled giant serotonin neurones. The section is in a similar position and plane to that illustrated in Fig. 1, and the labelled fluorescent structures are comparable. The non-coupled serotonin neurones show the same fluorescence colour and intensity as the coupled neurones (Fig. 1). The scale represents 100 μm. The absence of coupling is shown by the simultaneous intracellular recordings from the serotonin neurones which were made immediately before freeze-drying. The neurones were firing spontaneously. Three short bursts of stimuli, applied to the proximal end of the cut left cerebro-buccal connective, produced antidromic spikes in the left neurone (lower). The stimulus artifacts are visible in the upper trace. Time scale, 5 s. Voltage calibration, 25 mV.

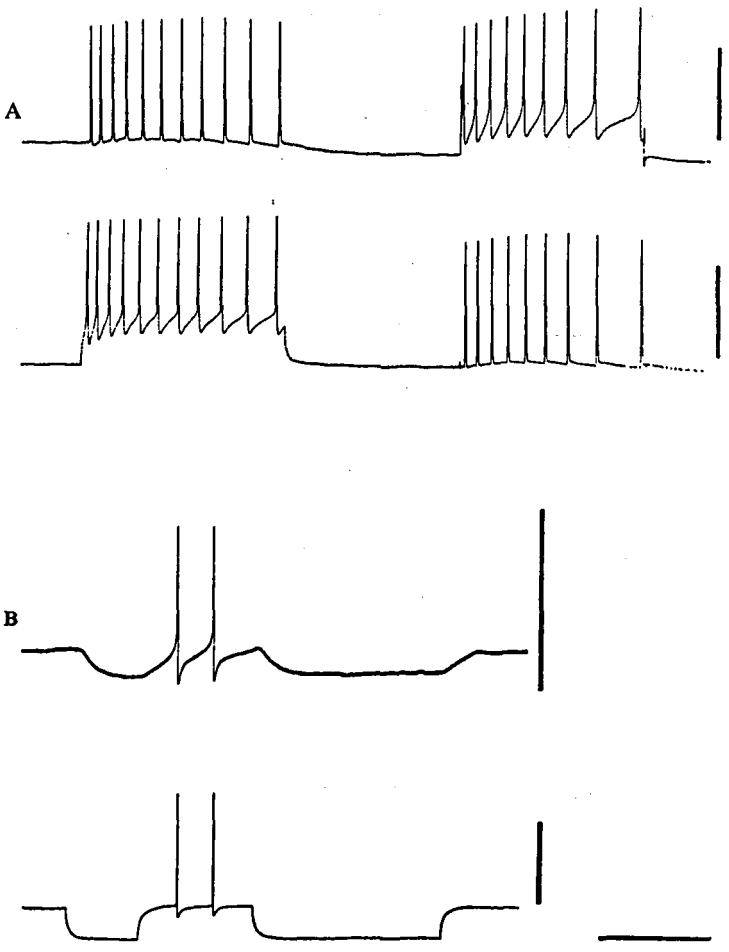

Fig. 3. Simultaneous intracellular recordings from the serotonin neurones illustrating the two-way electrical transmission. (A) The first train of spikes was elicited by depolarizing the right neurone (lower trace) and the second train by depolarizing the left neurone (upper trace). Each action potential elicited by the depolarization produced an action potential in the partner neurone with a constant latency of about 20 ms. (B) Hyperpolarizing current pulses applied to the right neurone (lower) produce a potential change in the left neurone (upper). Applied hyperpolarizing or depolarizing pulses only rarely produced measurable potential changes in the partner, which is unusual in view of the efficacy of spike transmission. However, applied pulses are conducted decrementally whereas spikes are propagated actively, so that the amplitude of transmitted pulses does not necessarily give an indication of the strength of coupling, particularly if the somata are connected by long, small-diameter processes. Time scale: A, 1 s, B, 5 s. Voltage calibrations: 50 mV.

fluorescence which was judged specific for serotonin by the usual criteria of colour, reducibility with sodium borohydride, and rapid fading with exposure to ultraviolet light. They were the only large fluorescent neurones in the ganglia. By comparing reconstructions of serial sections with the living ganglia it was obvious that the neurones were visually identifiable in living preparations by virtue of their relatively large size and characteristic position on the antero-dorsal surface of the ganglia. Confirmation of visual identification by intracellular recording was particularly easy

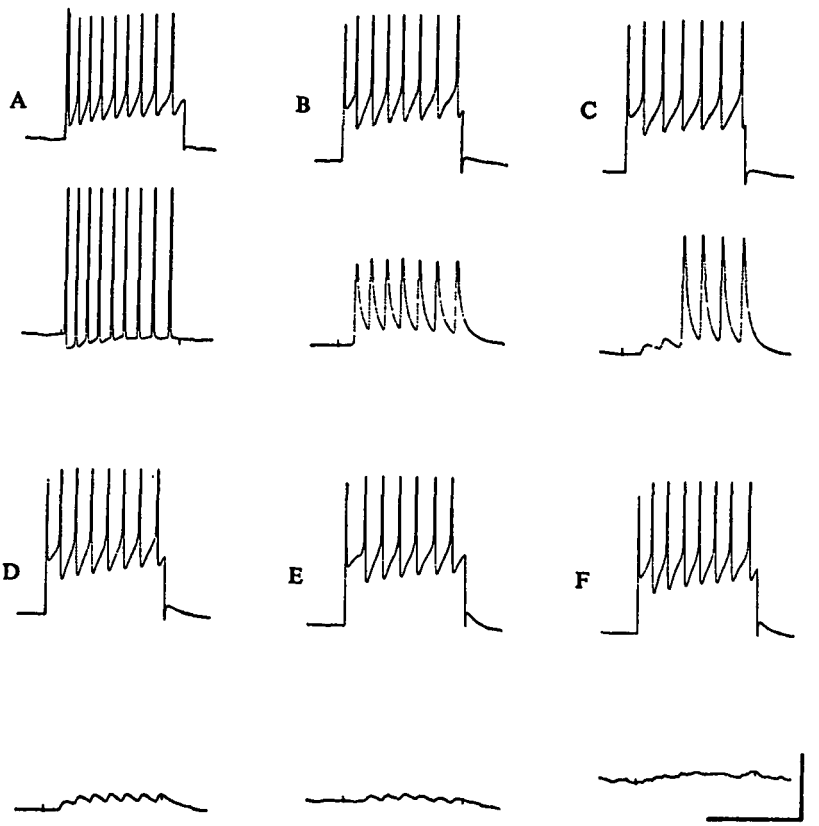

Fig. 4. Effect of progressively increasing hyperpolarization of the postsynaptic neurone on transmission between the serotonin neurones. In each pair of records the presynaptic neurone (upper) was made to fire repetitively by a depolarizing pulse while the postsynaptic neurone (lower) was hyperpolarized to various levels (A, resting potential, −70 mV; B, −85 mV; C, −100 mV; D, −115 mV; E, −130 mV; F, −145 mV). A, Each presynaptic spike produces a postsynaptic soma spike; B, the soma spikes disappear to reveal a second component; C, the second component is potentiated, but shows two failures which reveal a third component; D, E, F, the second component disappears, and the third component is progressively reduced in amplitude. The results indicate that transmission between the neurones is electrotonic and that the synapse is not close to the cell body. Time scale, 1 s. Voltage calibration, 50 mV (upper), 30 mV (lower).

when the neurones were electrically coupled (see next section). An extensive search revealed no connexions between the serotonin neurones and other neurones in either cerebral ganglion, and no connexions were found between the other large cerebral neurones. The presence of the coupling therefore served as an absolute criterion of identification of the serotonin neurones in living ganglia. In preparations where the coupling was absent, care had to be taken in identification because there was always the possibility that one or both neurones were in different positions and other neurones were being studied. In many cases the ganglia were subsequently observed under the fluorescence microscope for final confirmation. However, the neurones could quite easily be identified by their characteristic axon distribution, synaptic input, and effects on buccal ganglion neurones and the buccal mass.

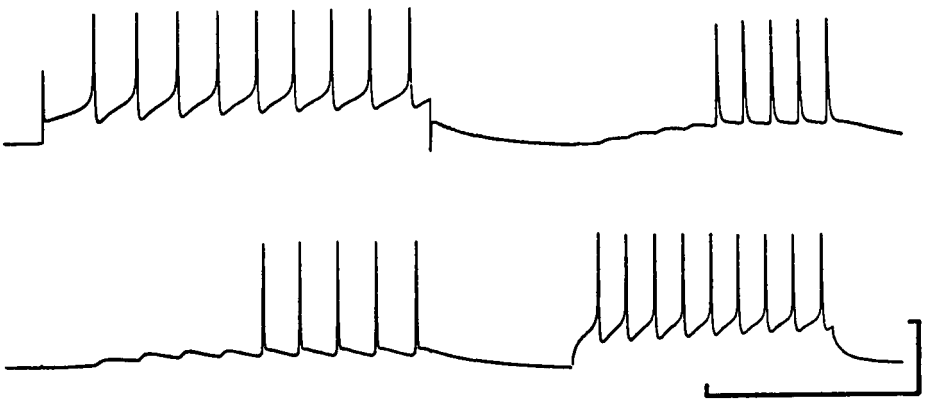

Fig. 5. Effect of Ca-free physiological solution on transmission between the serotonin neurones. The records were made 45 min after the substitution for normal saline of Ca-free saline containing 30 mM-Mg and 1 mM-EGTA. The left neurone (upper) was first depolarized and then the right neurone (lower). The first few presynaptic spikes failed to produce postsynaptic soma spikes. Before the addition of the Ca-free solution every presynaptic spike produced a postsynaptic soma spike, and full recovery from the Ca-free solution occurred about 2 min after the re-introduction of normal saline. Time scale, 2 s. Voltage calibration, 50 mV.

Electrotonic coupling between the serotonin neurones

Each action potential elicited in either serotonin neurone produced an action potential in the partner at the highest frequencies of firing (Fig. 3A). Only when there were signs of damage did the one for one correspondence of spikes break down. This strong two-way transmission is indicative of an electrotonic synapse, but in only a very few preparations did current injected into one neurone produce a potential change in the other (Fig. 3B). The following experiments were performed to obtain more information on the nature of the synapse in preparations where an electrotonic connexion could not be directly demonstrated.

The effects of hyperpolarizing the postsynaptic neurone were observed on transmission between the serotonin neurones (the term 'postsynaptic' refers to the serotonin neurone in which action potentials were indirectly elicited by intracellular stimulation of the partner). The results of progressively increasing hyperpolarization are shown in Fig. 4. First the soma spike disappeared, revealing a second component which had a similar rise time but was smaller and more prolonged than the soma spike. This component became slightly larger until at about 30 mV hyperpolarization it too suddenly disappeared to reveal a small, slowly rising deflexion which was progressively reduced in amplitude with increasing hyperpolarization. These results are interpreted to indicate the presence of an electrical synapse some distance from the cell body. The second component presumably represents the axon spike which becomes larger with hyperpolarization because the membrane potential of the axon is increased. This is then blocked as the hyperpolarization is increased further (see Tauc, 1962; Tauc & Hughes, 1963). The smallest component may represent the electrotonic postsynaptic potential. There are other possible interpretations, but the fact that the responses are reduced or abolished by hyperpolarization indicates that they are not chemical EPSPs.

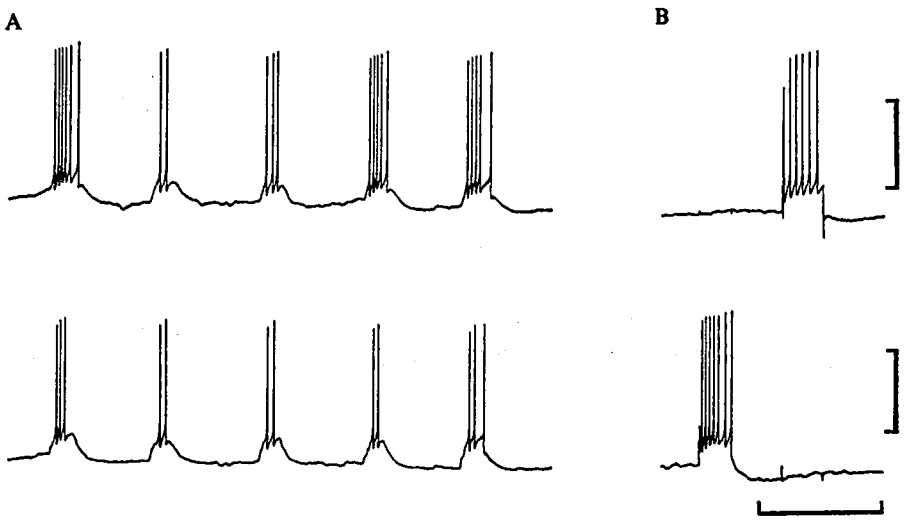

Fig. 6. Simultaneous recordings from a pair of non-coupled serotonin neurones. (A) The neurones fire more or less in phase as a result of synchronous bursts of excitatory input but there is not an exact one for one correspondence of spikes. (B) Intracellular stimulation of either neurone produces no sign of input to the partner. Time scale, 5 s. Voltage calibrations, 50 mV.

To determine whether this reduction of the postsynaptic responses could be due to the anomalous rectification which was found in these cells, the effect of hyperpolarization was observed on EPSPs produced by stimulating a cerebral nerve trunk. These EPSPs were progressively potentiated, reaching a maximum at the level of hyperpolarization at which the input from the partner cell disappeared. This potentiation of presumed chemical input shows that the reduction of input from the partner neurone was unlikely to be caused by a reduction in membrane resistance.

Ca-free solution containing 30 mM-Mg and 1 mM-EGTA abolished all observed chemical transmission in the ganglia. The effect of 45 min exposure to this solution on transmission between the serotonin neurones is illustrated in Fig. 5. Transmission was reduced but not abolished, demonstrating the electrotonic nature of the connexion. The reduction in transmission may be due to the decrease in membrane resistance caused by the Ca-free solution.

Serotonin neurones showing weak or no coupling

In some preparations stimulation of either serotonin neurone produced no observable synaptic input to the partner and no change in its firing rate (Figs. 2, 6). To determine whether the coupling may have been abolished by damage caused by microelectrode penetration, one or both of a pair of coupled neurones was damaged by the electrode until the action potentials were reduced to a few millivolts. Although this abolished the one for one correspondence of spikes, in no case did it abolish signs of input from the partner. The absence of coupling was therefore not an artifact caused by damage.

Although both coupled and non-coupled serotonin neurones could be found at any time of year, it was observed that during the winter there were few coupled neurones

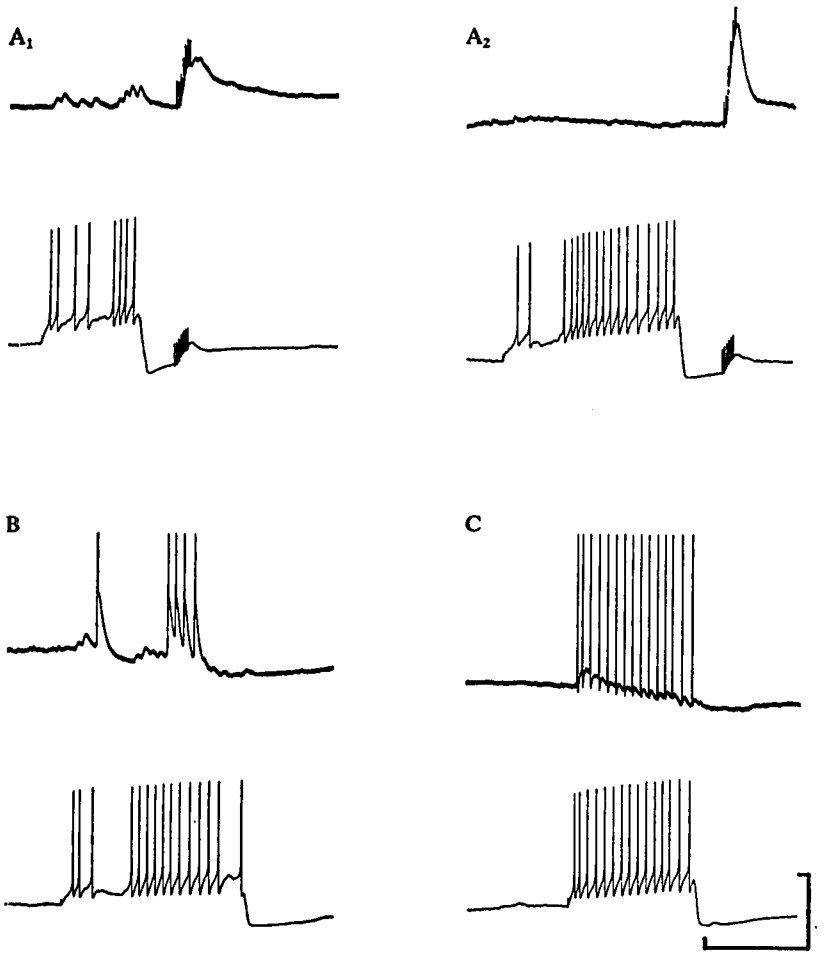

Fig. 7. Simultaneous recordings from a pair of weakly coupled serotonin neurones. A_1, Spikes elicited in the left neurone (lower) by intracellular depolarization produce small depolarizing deflexions in the right neurone (upper). After the train of spikes a short burst of stimuli (see stimulus artifacts) was applied to a cerebral nerve trunk to produce a compound EPSP in both neurones. A_2, Hyperpolarization of the postsynaptic neurone (upper) by 40 mV causes abolition of input from the partner but potentiation of the input elicited by stimulating the nerve trunk (see artifacts). This indicates that the input from the partner is not chemical in nature. B, C, Recordings from the same pair of neurones taken 15 min (C) and 25 min (B) after record A. The presynaptic neurone was depolarized to fire a burst of spikes, and the postsynaptic neurone recorded at the resting membrane potential. Note the differences in the effectiveness of transmission. The different postsynaptic responses resemble those produced by hyperpolarizing the postsynaptic member of a pair of strongly coupled serotonin neurones. Time scale, 5 s. Voltage calibration, 13 mV (upper), 50 mV (lower).

but during the spring and early summer most of the serotonin neurones were coupled. Some preparations showed weak coupling but this was found in only seven of more than a hundred preparations studied.

The features of the weak connexion are illustrated in Fig. 7. There was no transmission of injected current of either polarity but the connexion appeared to be electrotonic since transmission was abolished by postsynaptic hyperpolarization

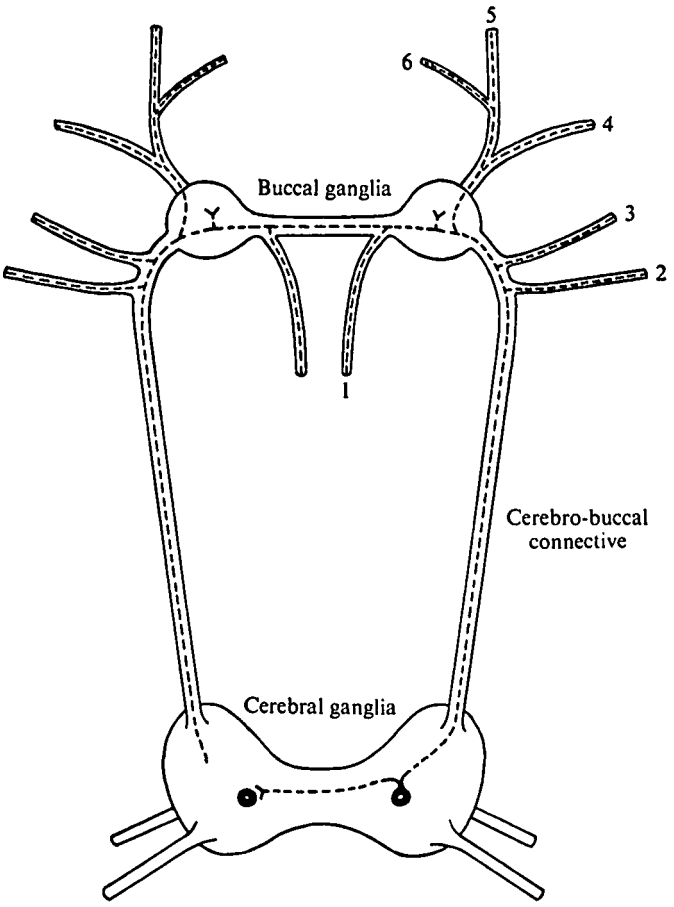

Fig. 8. Diagram of the cerebral and buccal ganglia, illustrating the axon distribution of the serotonin neurones. The axons have been drawn for only the right neurone but the left neurone shows a pattern of branching which is a mirror image of the right. There is an axon in each of the major buccal nerve trunks and probably also in some cerebral nerve trunks though these have not yet been mapped. The diagram shows coupled neurones; the pattern is the same for non-coupled neurones except that it is not known whether these send an axon directly to the contralateral cerebral ganglion. Synapses are made with neurones in both buccal ganglia. Note that one axon is sent via the buccal ganglia to the contralateral cerebral ganglion. This has not been described in other gastropods, but in *Helix* and *Tritonia* an axon branch runs in the contralateral connective towards the buccal ganglia (Dorsett, 1967; Pentreath & Cottrell, 1974). For simplicity, each principal buccal nerve has been numbered.

(Fig. 7A). However, it was also considerably reduced by Ca-free solution. There was sometimes wide variation in the effectiveness of transmission from time to time in any one preparation (Fig. 7B, C). This may have been the result of variation in synaptic control of the electrotonic coupling (cf. Spira & Bennett, 1972), or changes in post-synaptic membrane potential towards or away from threshold. During the short periods when spikes were transmitted on a one for one basis, this differed from the normal strong coupling in being rapidly fatigued and very easily abolished by post-synaptic hyperpolarization of only a few millivolts. There was no periodic variation in effectiveness of transmission between strongly coupled neurones.

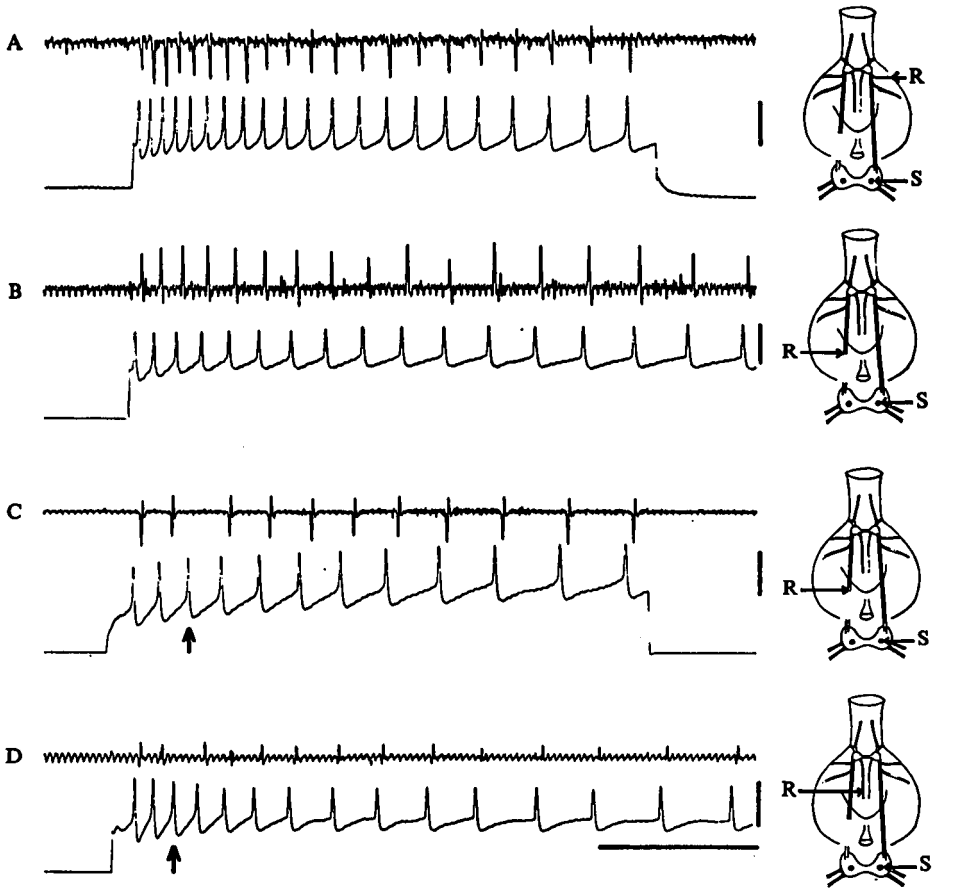

Fig. 9. Extracellular recordings from buccal nerves and a cerebro-buccal connective during orthodromic stimulation of a serotonin neurone. In each pair of records, which are from different preparations, the upper trace is the extracellular recording and the lower trace from one of the serotonin neurones which was made to fire by intracellular depolarization. The inset diagrams (of the buccal mass and cerebral and buccal ganglia) show the particular serotonin neurone stimulated (S) and the position of the extracellular recording electrode (R). In each preparation the serotonin neurones were coupled but the contralateral cerebro-buccal connective was cut to avoid recording the spike in the contralateral serotonin neurone. A, Each spike in the serotonin neurone is followed at constant latency by a spike in ipsilateral buccal nerve 3; B, there is fairly constant latency of the spike in the contralateral cerebro-buccal connective; C, the latency of the spike in the contralateral connective is more variable in this preparation and there is the occasional loss of response (arrow); D, there is variation in the latency of spikes in contralateral nerve 1 (radular nerve) and the occasional lost response (arrow). Time scale, 500 ms. Voltage calibrations, 50 mV.

Axon distribution of the serotonin neurones

Electrophysiological methods were used to trace the axons of the serotonin neurones. The results are illustrated in Fig. 8. Each neurone was found to send an axon into the ipsilateral cerebro-buccal connective and into ipsilateral and contralateral buccal nerves supplying the buccal mass and oesophagus. Unlike the situation in *Helix* no axon was found running to the buccal ganglia in the contralateral cerebro-buccal connective, but there did appear to be an axon in this connective running back from the buccal ganglia towards the contralateral cerebral ganglion.

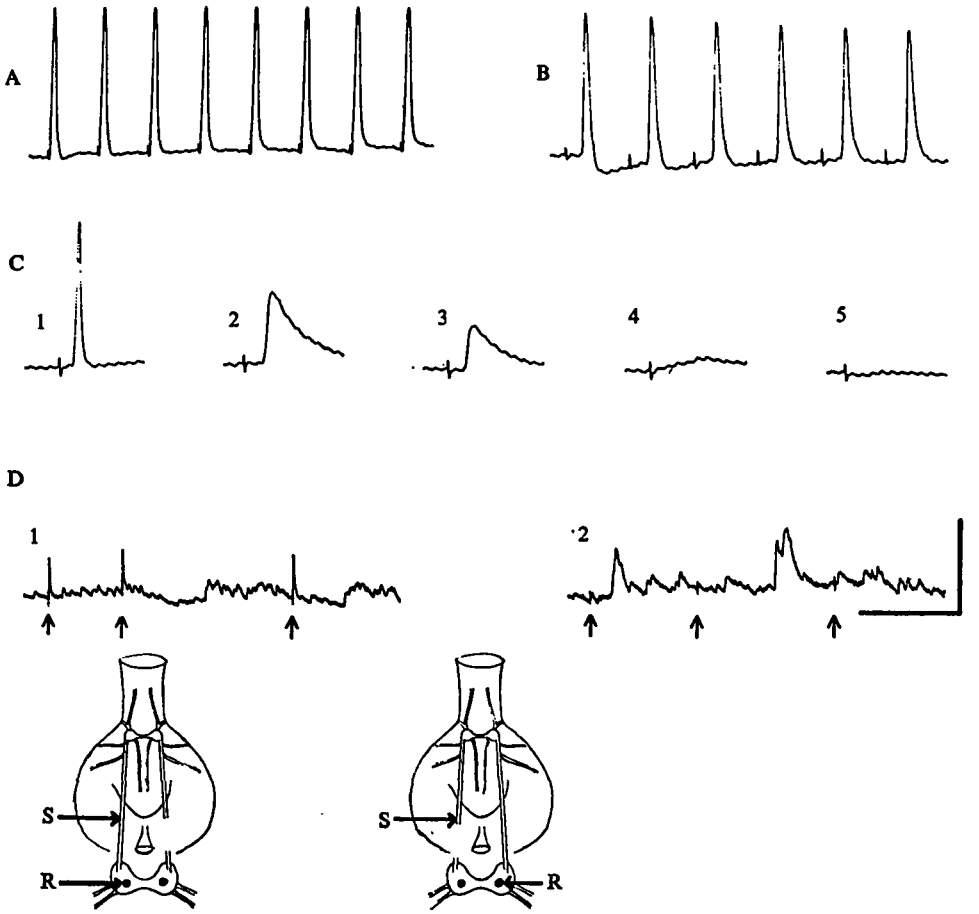

Fig. 10. Antidromic responses of the serotonin neurones to stimulation of the ipsilateral and contralateral cerebro-buccal connectives. In each record the stimulus artifact can be seen as the small rapid deflexion which precedes the action potential. A, The ipsilateral connective was repetitively stimulated after section of the contralateral connective (see left inset diagram) to avoid stimulating the partner neurone antidromically. Each stimulus produced a soma spike with short, constant latency. Records B–D are from a different preparation. The contralateral cerebro-buccal connective was cut and then repetitively stimulated as shown in the right inset diagram. B, Each stimulus produced a soma spike with fairly constant latency. C, Progressive hyperpolarization of the soma from the resting potential (C1, −70 mV) to −140 mV (C5) resulted in a reduction and eventual abolition of the responses, indicating that they were true antidromic responses and not the result of synaptic activation. D, Antidromic responses recorded at −90 mV (D1) and −120 mV (D2). Arrows indicate the stimulus artifacts. In D2 the antidromic responses were abolished whereas the spontaneous synaptic input was generally potentiated, indicating that the abolition of the antidromic responses was not the result of a decrease in membrane resistance. Time scale, 200 ms (A–C), 5 s (D). Voltage calibration, 50 mV.

Evidence obtained from orthodromic stimulation is illustrated in Fig. 9. When one of the serotonin neurones was stimulated intracellularly to fire at high frequency each action potential was followed at constant latency by a spike recorded in the ipsilateral cerebro-buccal connective and buccal nerves (Fig. 9A), indicating the presence of an axon branch. Similar results were obtained in the contralateral buccal nerves and connective in many preparations (Fig. 9B). In other preparations, however, there was

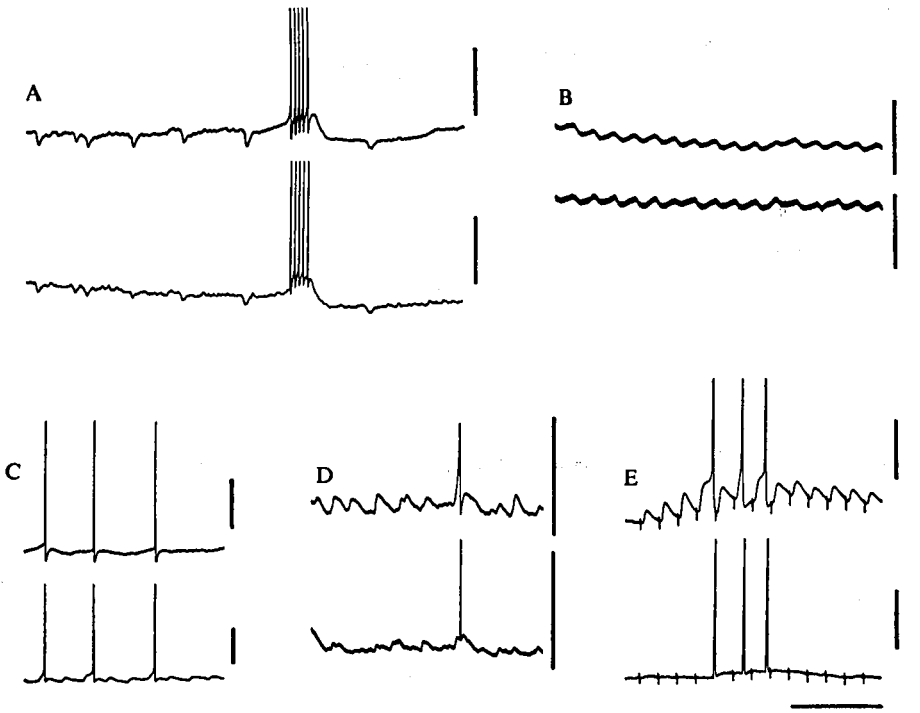

Fig. 11. Synaptic input to the serotonin neurones. Simultaneous recordings were made from electrically coupled cells. A, B, Spontaneous common inhibitory input; C, D, spontaneous independent excitatory input. In C note the large EPSPs in the lower record which are not seen in the upper. In D some of the input is common but some appears in only the upper record or is very much larger in this record. E, During stimulation of a cerebral nerve (note stimulus artifacts) EPSPs were produced in only the ipsilateral serotonin neurone (upper). Note that in spite of unilateral input the two cells always fire in synchrony. Time scale, 5 s (A, C, D), 1 s (B, E). Voltage calibrations, 25 mV (A, C, D, E), 5 mV (B).

variation in the latency and the occasional loss of an extracellular spike in the contralateral nerves and connective during high frequency firing (Fig. 9C, D). This was never seen in the ipsilateral buccal nerves and connective, and may indicate that there is a synapse between the stimulating electrode and contralateral recording electrode. An alternative explanation is the block of axonal spike at high frequency, perhaps at a branch point (see Parnas, 1972; Siegler, Mpitsos & Davis, 1974). The loss of spikes only occurred at fairly high frequency, and no loss was observed at firing rates of about 7 per sec for many minutes.

As a second test for the presence of peripheral axons, antidromic responses were observed to stimulation of ipsilateral and contralateral buccal nerves and cerebrobuccal connectives (Fig. 10A, B). With progressively increasing hyperpolarization, the antidromic spike was reduced in amplitude and eventually disappeared (Fig. 10C). This was presumably due to the blockage of the axon spike further and further from the soma (Tauc & Hughes, 1963). It is unlikely that the presumed axon spike was an EPSP, as spontaneous and evoked EPSPs were progressively increased in amplitude by the hyperpolarization (Fig. 10D). Again there was some variation in the latency of the contralateral responses and the occasional loss of response which did not occur

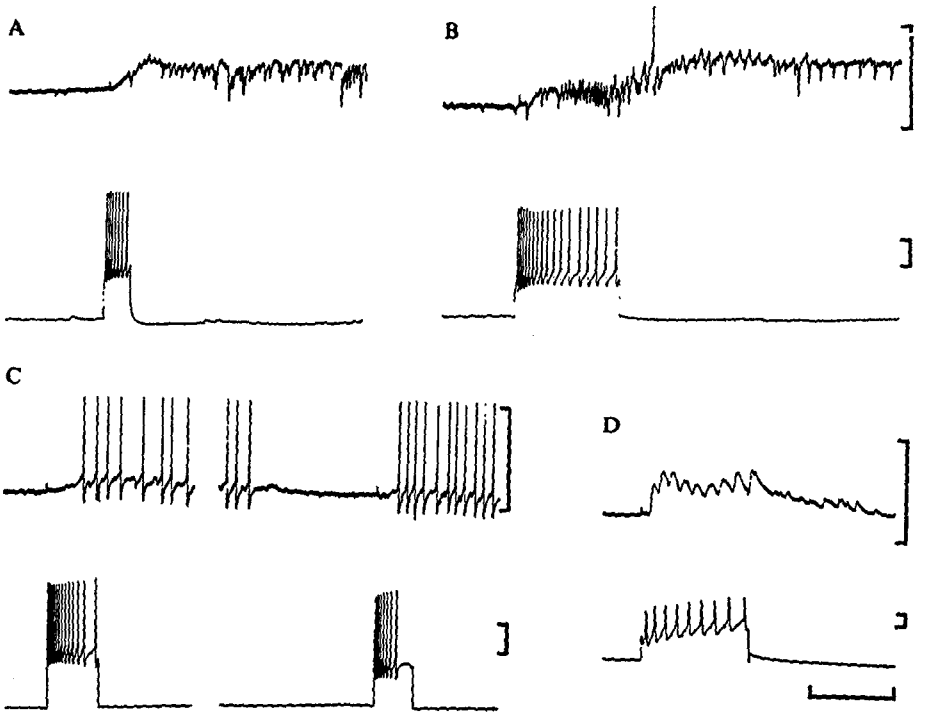

Fig. 12. Synaptic input from the serotonin neurones to neurones in the buccal ganglia. The upper traces of records A, B and C are from three neurones in the same ipsilateral buccal ganglion, recorded at their resting potential. D is from a different preparation. In A and B, stimulation of the serotonin neurone (lower) produced a prolonged depolarization of the buccal ganglion neurones, with superimposed IPSPs. The activity continued for more than a minute in both cells. A common feature of postsynaptic responses was the additional, rebound depolarization seen at the end of stimulation of the serotonin neurone in B. In this instance a single spike was generated but there was frequently a burst of up to 10 spikes. In C the gap in the record represents 10 s. D, A small contralateral buccal ganglion neurone received large, short-latency EPSPs from the serotonin neurone. The cell was hyperpolarized by 30 mV. In both preparations, which were isolated from the buccal mass, the serotonin neurones were coupled. Time scale, 5 s (A, B, C), 1 s (D). Voltage calibrations, 25 mV.

with the ipsilateral buccal nerves and connective. Since this type of antidromic response with occasional failure could have been produced either if there was an electrotonic synapse in the pathway or an axonal branch point with high safety factor, no positive conclusions can be drawn. Identical results using orthodromic and antidromic stimulation were obtained in the presence of low concentrations of Ca (1 mM) and high concentrations of Mg (30 mM) which greatly reduced chemical transmission. Coupled and non-coupled serotonin neurones both showed the same distribution of peripheral axons.

Synaptic input to the serotonin neurones

The spontaneous input to the serotonin neurones was extremely variable, consisting of both common and independent EPSPs and IPSPs (Figs. 6, 11, 15). Stimulation of cerebral nerve trunks elicited some common input and also other input which was seen in only the ipsilateral neurone (Fig. 11E). Coupled neurones always fired in synchrony irrespective of whether the input was unilateral or bilateral (Fig. 11C, D, E).

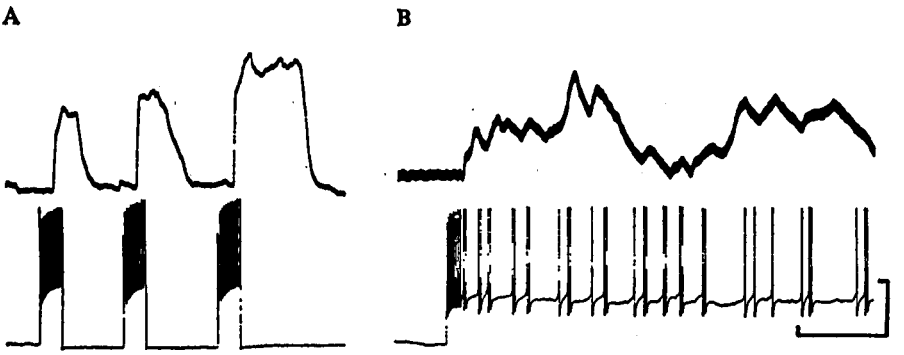

Fig. 13. Movements of the buccal mass elicited by stimulating one of the serotonin neurones in two semi-intact preparations. The upper traces show movements of the buccal mass, recorded by attaching a piece of thread to the radular sac, and the lower traces are from a serotonin neurone which was coupled to its partner. A, Each burst of spikes produced a short series of contractions in the buccal mass which was otherwise quiescent. The responses became larger with repetition but in many other preparations they remained constant or declined with repetition. B, In an originally quiescent buccal mass a long train of spikes in the serotonin neurone produced prolonged rhythmic activity. The activity usually ceased a few seconds after the serotonin neurone stopped firing, but in some preparations it continued for several minutes. Time scale, 5 s. Voltage calibrations, 50 mV (A), 45 mV (B).

In non-coupled serotonin neurones, common input produced similar firing patterns (Fig. 6A), but unilateral input resulted in completely independent firing.

Synaptic input from the serotonin neurones to buccal ganglion neurones

It was hoped that the serotonin neurones would make monosynaptic connexions with identifiable neurones in the buccal ganglia so that their precise central actions could be studied, and also the possible transmitter role of serotonin (cf. Cottrell & Macon, 1974; Gerschenfeld & Paupardin-Tritsch, 1974). Although apparently direct connexions were observed (Fig. 12D), the postsynaptic neurones were small and very difficult to find in different preparations. There were no synapses onto the two largest buccal ganglion neurones but several of the larger motoneurones received input from the serotonin neurones (see Berry, 1972*b*). Some of this input may have been direct but most of it was obviously indirect and often very complex and long-lasting (Fig. 12A, B, C). A burst of spikes elicited in a serotonin neurone produced a variable train of action potentials in some postsynaptic neurones, or more usually a sustained subthreshold depolarization with superimposed IPSPs. This depolarization lasted for a few seconds up to several minutes, and a similar response could again be initiated.

Role of the serotonin neurones in feeding behaviour

Depolarization of either serotonin neurone to produce a burst of spikes resulted in movements of the buccal mass in semi-intact preparations (Fig. 13). The movements appeared to be due to the excitation of motoneurones rather than the direct action of the serotonin neurones on the buccal musculature; the recorded muscle action potentials were not correlated on a one for one basis with serotonin neurone spikes (Fig. 14). Movements occurred mainly in the supramedian radular retractor

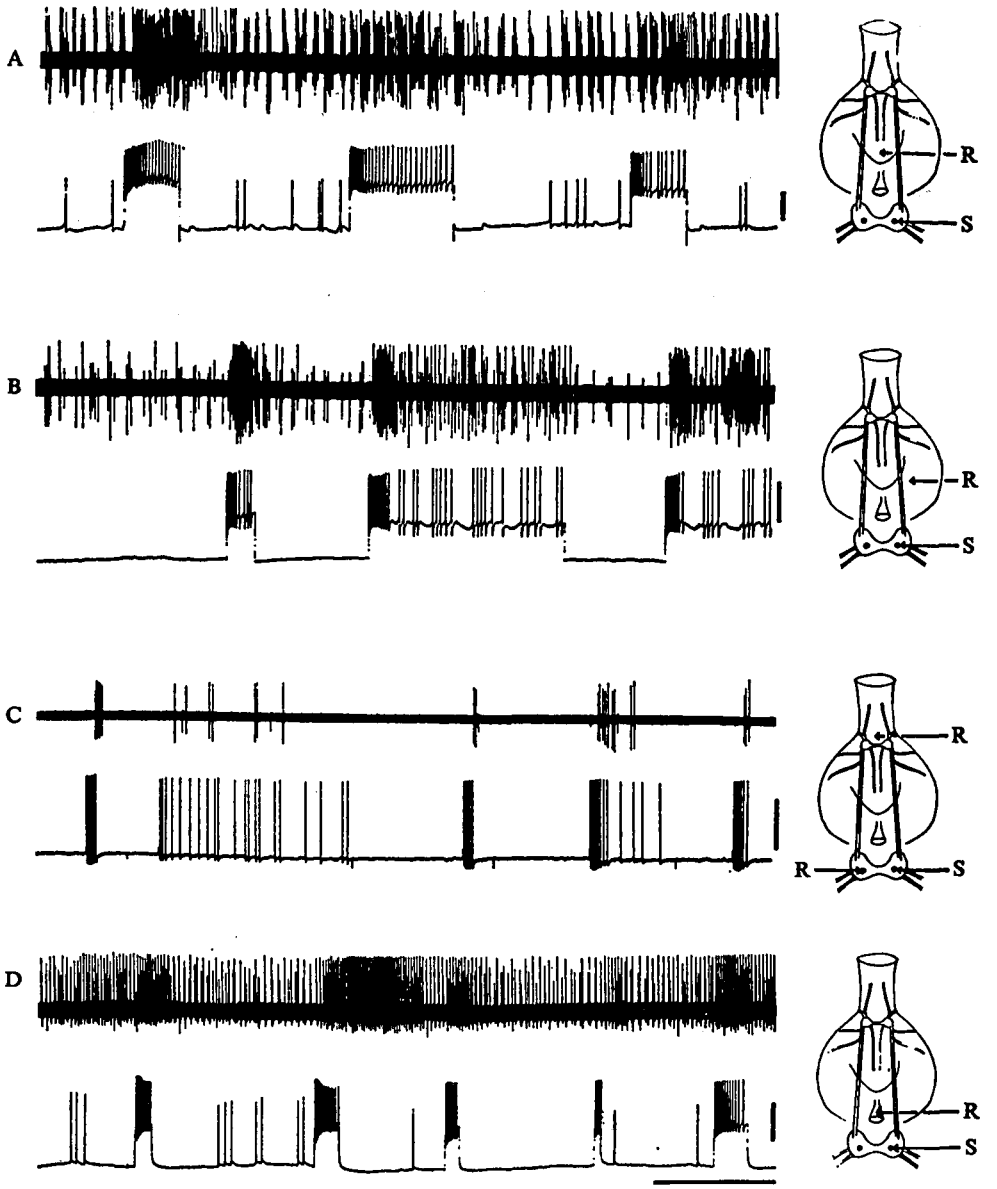

Fig. 14. Muscle action potentials elicited by stimulation of one of the serotonin neurones in a semi-intact preparation. In each pair of records the upper trace was made with a suction electrode placed on the muscle indicated by the arrow (R) in the inset diagram. The lower trace is from a serotonin neurone (S on the inset diagram) which was stimulated by depolarizing pulses. Records were made from the suspensor muscle of the radular sac (A) and from the supramedian radular retractor (B). The buccal musculature at the neck of the oesophagus was recorded in C; the lower trace is from the partner neurone (R, left ganglion) which was coupled to the stimulated serotonin neurone (S). Record D is from the aorta. All the records were made from different preparations, and in each the serotonin neurones were coupled. The neurones fired spontaneously in A and D but were silent until stimulated in B and C. Time scale, 10 s. Voltage calibrations, 50 mV.

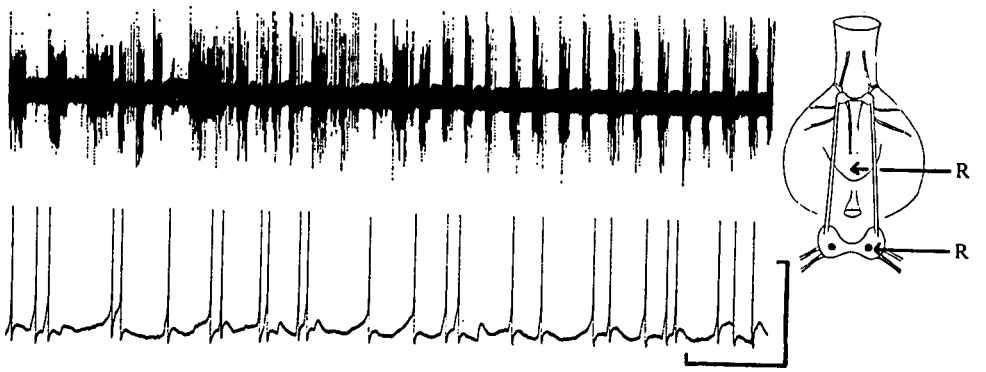

Fig. 15. Spontaneous activity in a serotonin neurone during rhythmic movements of the buccal mass in a semi-intact preparation. The upper trace is a recording from the suspensor muscle of the radular sac (see inset diagram) using a suction electrode; the bursts of electrical activity were coordinated with the movements of the buccal mass. Note that there is no obvious correlation between activity in the muscle and that in the serotonin neurone (lower). There was a small change in firing pattern of the serotonin neurone during the increased activity in the muscle, but this was not a regular occurrence. Activity sometimes appeared coordinated for short periods but there were very much longer periods during which no phase relationship was apparent. Time scale, 5 s. Voltage calibration, 50 mV. The full amplitude of the action potentials was not recorded.

muscle and in the suspensor muscle of the radular sac (see Hembrow, 1973, for structure of the buccal mass); muscle action potentials were readily recorded from these muscles with a suction electrode (Fig. 14A, B). Increased activity during stimulation of the serotonin neurones was also recorded from the muscle beneath the buccal ganglia (Fig. 14C) and from the aorta (Fig. 14D). No change in activity was seen in the oesophagus, although each oesophageal nerve (buccal nerves 5 and 6) receives an axon from both serotonin neurones. Similar movements and electrical activity in the muscles were recorded during stimulation of either coupled or non-coupled serotonin neurones.

There was wide variation in the strength of the movement and electrical responses. In some preparations very little activity was recorded even with intense stimulation of the serotonin neurones. In other preparations activity was initiated by quite low frequency stimulation and often continued for several seconds, sometimes for several minutes, after the end of stimulation. If one of the cerebro-buccal connectives was cut, the effects of stimulating a serotonin neurone were greatly enhanced (this of course did not occur for a non-coupled neurone when the ipsilateral connective was cut since this severed its input to the buccal ganglia, but with coupled cells the input to the buccal ganglia was maintained after cutting the ipsilateral connective by excitation of the partner). The buccal mass generally became more active after section of one or both cerebro-buccal connectives.

Although the serotonin neurones could initiate or increase buccal mass activity, their input and output did not appear to be correlated with the spontaneous rhythmic movements of the buccal mass (Fig. 15). Rhythmically repeating bursts of EPSPs and IPSPs did occur in the serotonin neurones but they showed no obvious phase relation to the cyclic buccal mass activity, and usually continued unaltered after cutting both cerebro-buccal connectives. When the buccal mass was mechanically

stimulated with a glass rod or distended by cannulating the oesophagus and injecting saline under pressure with a syringe, there was no associated input to the serotonin neurones. Also, stimulation of motoneurones in isolated or semi-intact preparations never produced any observable input to the serotonin neurones. Weak excitatory input could be elicited, however, by touching the skin, particularly around the mouth. The serotonin neurones thus appear not to be involved with the normal generation of the feeding cycles, but are capable of modulatory activity the precise function and mechanism of which are unknown.

DISCUSSION

Electrotonic coupling between the serotonin neurones

In many different nervous systems synchronization of action potentials in different neurones is aided by electrotonic coupling, but exact one for one firing usually occurs only when the cells have common excitatory input or synchronized pacemaker depolarizations, i.e. when the cells are close to threshold (see Berry & Pentreath, 1976). There are some examples of exceptionally strong connexions (e.g. Bennett, Pappas, Giménez & Nakajima, 1967) but these are at present few in number. The serotonin neurones in *Planorbis* were synchronized at the highest frequencies of firing, and showed no fatigue even during the presence of tonic inhibitory input. Postsynaptic hyperpolarization of at least 25 mV was required to block the production of a postsynaptic axon spike by presynaptic stimulation. The reason for such a strong connexion is not at present clear; common input generally serves to synchronize the output of symmetric neurones in molluscs (Gardner, 1971). None of the many other examples of electrical synapses in *Planorbis* show such effective spike-transmission properties (Berry, 1972*a*, *b*; Berry & Pentreath, 1975). Connexions between the serotonin neurones do not occur in *Aplysia*, *Tritonia* or *Helix* (Dorsett, 1967; Cottrell & Macon, 1974).

Another peculiar feature is the variability of the coupling in different preparations. The predominance of coupling in spring and early summer, and of non-coupling in winter suggests that there may be a seasonal variation in the connexion. It is possible that there is a hormonal or other mechanism for switching the coupling on and off; perhaps the weak coupling found in a few preparations represents a transition stage. Synaptic modulation of the coupling (Spira & Bennett, 1972) is probably not responsible because Ca-free solution, which should abolish such an action, did not increase the strength of weak coupling. Alternatively, in some animals the connexions may not be formed during the development of the nervous system, and the anatomical connexion may simply not exist in non-coupled serotonin neurones.

Axon distribution of the serotonin neurones

The electrophysiological mapping experiments show the serotonin neurones to have processes in both cerebro-buccal connectives and in the major nerve trunks of both buccal ganglia. However, in view of the occasional loss of the orthodromic or antidromic response of the contralateral cerebro-buccal connective and buccal nerves it is difficult to exclude the possibility that these responses reflect activity in an additional neurone, coupled electrically to the serotonin neurone. But even if the recorded contralateral spikes do represent activity in a neurone other than the sero-

tonin neurone, the two neurones act for the most part as a single unit, and the anatomical distinction between direct and indirect pathways has little apparent functional significance. An alternative explanation is the occasional failure of the spike to invade the contralateral axons. Siegler *et al.* (1974) found that soma spikes in neurones in the buccal ganglia of *Pleurobranchaea* sometimes failed to invade the axons in the buccal nerves, although dye injection had clearly shown the presence of these axons. It is noteworthy that an important criterion for determining the monosynaptic nature of a connexion (i.e. the following of every presynaptic soma spike by a postsynaptic potential) could be seriously confused by this failure of spikes to enter the axon, for a direct connexion would have the characteristics of an indirect connexion. Also, electrotonic coupling of the strength shown by the serotonin neurones would make an indirect connexion appear direct.

An extensive axon distribution is a feature of the giant serotonin cells of *Aplysia*, *Tritonia* and *Helix* (Dorsett, 1967; Kupfermann & Weiss, 1974; Pentreath & Cottrell, 1974) and of many other molluscan neurones (Willows, Dorsett & Hoyle, 1973*a*; Siegler *et al.* 1974; Woollacott, 1974; Dorsett, 1975). For the serotonin neurones, which do not seem to be either motoneurones or sensory neurones, the presence of so many peripheral axons is puzzling. In *Aplysia* the serotonin cells have a direct effect on buccal muscles, causing an increase in the force of contraction (Weiss *et al.* 1975*a*, *b*). The peripheral endings of the serotonin neurones in *Planorbis* are obviously a promising area for study.

Behavioural role of the serotonin neurones

Experiments with semi-intact preparations showed that some of the buccal ganglion cells which are influenced by the serotonin neurones are motoneurones involved in feeding. This is also the situation in *Aplysia* and *Limax* (Weiss *et al.* 1975*b*; Gelperin, 1975). In *Planorbis* a common feature of the postsynaptic responses was a tonic, subthreshold depolarization which outlasted the stimulation of the serotonin cell and was accompanied by discrete inhibitory potentials. The depolarization resembles the excitation plateau that occurs in many brain cells in *Tritonia* (Dorsett, Willows & Hoyle, 1973; Willows, Dorsett & Hoyle, 1973*b*). This is a maintained depolarization of from 2 to 10 mV that takes place during the escape-swimming sequence in *Tritonia*, and has superimposed volleys of excitation and inhibition. It is believed to be produced by positive feedback and regenerative activity between the different groups of neurones involved in swimming. The origin of the depolarization in *Planorbis* is unknown.

In *Aplysia* the excitatory input from the serotonin neurones to buccal ganglion motoneurones was generally subthreshold and did not result in movements of the buccal mass (Weiss *et al.* 1975*a*, *b*), whereas in *Planorbis* stimulation of the serotonin neurones often produced powerful contractions of the buccal mass. Also, whereas spontaneous biting or swallowing activity in *Aplysia* is associated with phasic excitatory input to the serotonin neurones (Kupfermann & Weiss, 1974), in *Planorbis* there was generally an absence of any phasic input to the serotonin neurones during buccal mass activity, and when phasic input was present it was not coordinated with buccal mass movements.

The behavioural data, although incomplete, do show that measurable changes in

activity are produced by stimulating the serotonin neurones. The total repertoire of actions of the serotonin neurones may be difficult to elucidate; feeding behaviour is complex, and involves integration between several ganglia (Davis, Siegler & Mpitsos, 1973; Dawkins, 1974; Davis *et al.* 1974; Kater, 1974; Kupfermann, 1974*a*, *b*). The ability of the serotonin neurones to initiate rhythmic buccal mass activity in many preparations suggests that they may act as command neurones (i.e. neurones that elicit patterned behavioural output, in this case feeding). However, the resulting muscle activity appeared generally uncoordinated and often required intense and perhaps non-physiological firing rates of the serotonin neurones. Most of the input to buccal ganglion neurones from the serotonin neurones was subthreshold. Kupfermann & Weiss (1974) found that continuous firing of the serotonin neurones in *Aplysia* sometimes elicited rhythmic bursts of activity in buccal ganglion neurones but generally produced direct, smoothly summating synaptic potentials with slow rise and decay. Weiss *et al.* (1975*a*, *b*) concluded that the serotonin neurones in *Aplysia* are not command cells, and that the slow synaptic potentials they produce in buccal ganglion neurones makes them unsuited to be part of the pattern-generating system which produces the rhythmic feeding output. Rather, the serotonin neurones are modulatory cells, producing alterations in the intensity of activity but not in its pattern. The data suggest that the serotonin cells in *Planorbis* also serve such a modulatory role.

Homologues of the serotonin neurones in other gastropod molluscs

A symmetric pair of large serotonin-containing neurones which send axons into the cerebro-buccal connectives and buccal nerves is present in the cerebral ganglia of *Helix*, *Limax*, *Aplysia*, *Tritonia* and *Archidoris* (Weinreich *et al.* 1973; Cottrell & Macon, 1974; Dorsett, 1967, 1974; Gerschenfeld & Paupardin-Tritsch, 1974; Gelperin, 1975), and probably also in *Pleurobranchaea* (Davis *et al.* 1974). Weiss and Kupfermann (1974) compared some of the properties of the serotonin neurones in *Helix* and *Aplysia* and concluded that the neurones are true homologues. The serotonin neurones in *Planorbis* share the following properties with those in *Aplysia* and *Helix*: they show anomalous rectification, receive a dual excitatory-inhibitory input following stimulation of certain cerebral nerves, and are depolarized by iontophoretic application of acetylcholine and hyperpolarized by glutamate (unpublished data). They produce synaptic potentials in neurones in both buccal ganglia, and have axons in ipsilateral and contralateral buccal nerves.

On the basis of their size, position in the cerebral ganglia, content of serotonin, axon distribution, synaptic connexions, anomalous rectification, and responses to drugs, the serotonin neurones in *Planorbis* appear homologous to those in *Aplysia* and *Helix*.

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REFERENCES

BENNETT, M. V. L., PAPPAS, G. D., GIMÉNEZ, M. & NAKAJIMA, Y. (1967). Physiology and ultrastructure of electrotonic junctions. IV. Medullary electromotor nuclei in gymnotid fish. *J. Neurophysiol.* **30**, 236–300.

BERRY, M. S. (1972*a*). A system of electrically coupled small cells in the buccal ganglia of the pond snail, *Planorbis corneus*. *J. exp. Biol.* **56**, 621–37.

BERRY, M. S. (1972*b*). Electrotonic coupling between identified large cells in the buccal ganglia of *Planorbis corneus*. *J. exp. Biol.* **57**, 173–85.

BERRY, M. S. & PENTREATH, V. W. (1975). Problems associated with the use of tetraethylammonium to test for monosynaptic connexions. *J. exp. Biol.* **62**, 797–803.

BERRY, M. S. & PENTREATH, V. W. (1976). Criteria for distinguishing between monosynaptic and polysynaptic transmission. *Brain Res.* **105**, 1–20.

CORRODI, H., HILLARP, N. A. & JONSSON, G. (1964). Fluorescence methods for the histochemical demonstration of monoamines: 3. Sodium borohydride reduction of the fluorescent compounds as a specific test. *J. Histochem. Cytochem.* **12**, 582–6.

COTTRELL, G. A., BERRY, M. S. & MACON, J. B. (1974). Synapses of a giant serotonin neurone and a giant dopamine neurone: studies using antagonists. *Neuropharmacol.* **13**, 431–9.

COTTRELL, G. A. & MACON, J. B. (1974). Synaptic connexions of two symmetrically placed giant serotonin-containing neurones. *J. Physiol., Lond.* **236**, 435–64.

COTTRELL, G. A. & OSBORNE, N. N. (1970). Subcellular localization of serotonin in an identified serotonin-containing neurone. *Nature, Lond.* **225**, 470–2.

DAVIS, W. J., MPITSOS, G. J., SIEGLER, M. V. S., PINNEO, J. M. & DAVIS, K. B. (1974). Neuronal substrates of behavioural hierarchies and associative learning in *Pleurobranchaea*. *Am. Zool.* **14** 1037–50.

DAVIS, W. J., SIEGLER, M. V. S. & MPITSOS, G. J. (1973). Distributed neuronal oscillators and efference copy in the feeding system of *Pleurobranchaea*. *J. Neurophysiol.* **36**, 258–74.

DAWKINS, M. (1974). Behavioural analysis of coordinated feeding movements in the gastropod *Lymnaea stagnalis* (L.). *J. comp. Physiol.* **92**, 255–71.

DORSETT, D. A. (1967). Giant neurones and axon pathways in the brain of *Tritonia*. *J. exp. Biol.* **46**, 137–51.

DORSETT, D. A. (1974). Neuronal homologies and the control of branchial tuft movements in two species of *Tritonia*. *J. exp. Biol.* **61**, 639–54.

DORSETT, D. A. (1975). Some aspects of neural organization in molluscs. In '*Simple*' *Nervous Systems* (ed. P. N. R. Usherwood and D. R. Newth), pp. 265–322. London: Edward Arnold.

DORSETT, D. A., WILLOWS, A. O. D. & HOYLE, G. (1973). The neuronal basis of behaviour in *Tritonia*. IV. The central origin of a fixed action pattern demonstrated in the isolated brain. *J. Neurobiol.* **4**, 287–300.

FALCK, B. & OWMAN, C. (1965). A detailed methodological description of the fluorescence method for the cellular demonstration of biogenic monoamines. *Acta Univ. Lund, Sec.* **11**, **7**, 1–23.

GARDNER, D. (1971). Bilateral symmetry and interneuronal organization in the buccal ganglia of *Aplysia*. *Science* **173**, 550–3.

GELPERIN, A. (1975). An identified serotonergic input has reciprocal effects on two electrically coupled motoneurons in the terrestrial slug, *Limax maximus*. *Biol. Bull. Woods Hole*, **149**, 426–7.

GERSCHENFELD, H. M. & PAUPARDIN-TRITSCH, D. (1974). On the transmitter function of 5-hydroxytryptamine at excitatory and inhibitory monosynaptic junctions. *J. Physiol., Lond.* **243**, 457–81.

HEMBROW, D. (1973). Observations on the structure and function of the buccal mass of *Planorbarius corneus* (L.). *Proc. malac. Soc. Lond.* **40**, 505–21.

KATER, S. B. (1974). Feeding in *Helisoma trivolvis*: the morphological and physiological bases of a fixed action pattern. *Am. Zool.* **14**, 1017–36.

KUPFERMANN, I. (1974*a*). Feeding behaviour in *Aplysia*: a simple system for the study of motivation. *Behav. Biol.* **10**, 1–26.

KUPFERMANN, I. (1974*b*). Dissociation of the appetitive and consummatory phases of feeding behaviour in *Aplysia*: a lesion study. *Behav. Biol.* **10**, 89–97.

KUPFERMANN, I. & WEISS, K. R. (1974). Functional studies on the metacerebral cells of *Aplysia*. *Abstr. Soc. Neurosci.* **4**, 297.

MARSDEN, C. & KERKUT, G. A. (1970). The occurrence of monoamines in *Planorbis corneus*: a fluorescence microscope and microspectrometric study. *Comp. gen. Pharmacol.* **1**, 101–16.

OSBORNE, N. N. & COTTRELL, G. A. (1971). Distribution of biogenic amines in the slug, *Limax maximus*. *Z. Zellforsch.* **112**, 15–30.

PARNAS, I. (1972). Differential block at high frequency of branches of a single axon innervating two muscles. *J. Neurophysiol.* **35**, 903–14.

PENTREATH, V. W. (1973). Effect of stimulating a central giant serotonin-containing neurone on peripheral muscles in the snail *Helix pomatia*. *Experientia* **29**, 540–2.

PENTREATH, V. W. & COTTRELL, G. A. (1974). Anatomy of an identified serotonin neurone studied by means of injection of tritiated 'transmitter'. *Nature, Lond.* **250**, 655–8.

PENTREATH, V. W., OSBORNE, N. N. & COTTRELL, G. A. (1973). Anatomy of giant serotonin-containing neurones and other neurones in the cerebral ganglia of *Helix pomatia* and *Limax maximus*. *Z. Zellforsch.* **143**, 1–20.

SIEGLER, M. V. S., MPITSOS, G. J. & DAVIS, W. J. (1974). Motor organization and generation of rhythmic feeding output in buccal ganglion of *Pleurobranchaea*. *J. Neurophysiol.* **37**, 1173–96.

SPIRA, M. E. & BENNETT, M. V. L. (1972). Synaptic control of electrotonic coupling between neurons. *Brain Res.* **37**, 294–300.

TAUC, L. (1962). Site of origin and propagation of spike in the giant neuron of *Aplysia*. *J. gen. Physiol.* **45**, 1077–97.

TAUC, L. & HUGHES, G. M. (1963). Modes of initiation and propagation of spikes in the branching axons of molluscan central neurons. *J. gen. Physiol.* **46**, 533–49.

WEINREICH, D., MCCAMAN, M. W., MCCAMAN, R. E. & VAUGHN, J. E. (1973). Chemical, enzymatic and ultrastructural characterization of 5-hydroxytryptamine-containing neurons from the ganglia of *Aplysia californica* and *Tritonia diomedia*. *J. Neurochem.* **20**, 969–76.

WEISS, K. R., COHEN, J. & KUPFERMANN, I. (1975*a*). Modulatory command function of the metacerebral cell on feeding behaviour in *Aplysia*. *Fed. Proc.* **34**, 418.

WEISS, K. R., COHEN, J. & KUPFERMANN, I. (1975*b*). Potentiation of muscle contraction: a possible modulatory function of an identified serotonergic cell in *Aplysia*. *Brain Res.* **99**, 381–86.

WEISS, K. R. & KUPFERMANN, I. (1974). Homology of giant cerebral cells in *Aplysia* to the metacerebral cells of pulmonate molluscs. *Abstr. Soc. Neurosci.* **4**, 471.

WILLOWS, A. O. D., DORSETT, D. A. & HOYLE, G. (1973*a*). The neuronal basis of behaviour in *Tritonia*. I. Functional organization of the central nervous system. *J. Neurobiol.* **4**, 207–37.

WILLOWS, A. O. D., DORSETT, D. A. & HOYLE, G. (1973*b*). The neuronal basis of behaviour in *Tritonia*. III. Neuronal mechanism of a fixed action pattern. *J. Neurobiol.* **4**, 255–85.

WOOLLACOTT, M. H. (1974). Patterned neural activity associated with prey capture in *Navanax* (Gastropoda, Aplysiacea). *J. comp. Physiol.* **94**, 69–84.