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MORPHOLOGY AND ELECTROPHYSIOLOGY OF THE OVULATION HORMONE PRODUCING NEURO-ENDOCRINE CELLS OF THE FRESHWATER SNAIL *LYMNAEA STAGNALIS* (L.)

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SUMMARY

The ovulation hormone producing neuro-endocrine cells of *Lymnaea stagnalis*, the caudo-dorsal cells (CDC), are comparable to the bag cells of *Aplysia*. Both cell types are capable of the production of a long-lasting activity (afterdischarge) during which an ovulation hormone is released. The CDC (30 cells in the left cerebral ganglion and 70 cells in the right) are usually electrically silent but an afterdischarge can be brought about in all cells of both groups by direct, repetitive electrical stimulation of single CDC. This is not possible in every preparation, indicating that the CDC can be in different states of excitability.

All cells participate in the afterdischarge and fire approximately synchronously. All CDC are electrotonically connected. Results of experiments in which neurones were injected with horseradish peroxidase suggest that the demonstrated electrotonic connexions between the two opposite groups of CDC are brought about by 10–12 special axons.

INTRODUCTION

The central nervous system of the freshwater pulmonate snail *Lymnaea stagnalis* contains several neurosecretory cells which differ in histochemical and ultrastructural characteristics. These cells are often found in groups which occur at characteristic locations in the ganglion ring (cf. Wendelaar Bonga, 1970, 1971) and which can be recognized in the living ganglia (Joosse, 1964). The axons of the neurosecretory cells run to the periphery of nerves, commissures or connectives where they terminate with many neurohaemal endings which release the products into the haemolymph by exocytosis (Roubos, 1975).

The function of some of the groups is known. The caudo-dorsal cells (CDC), located in the cerebral ganglia, constitute one of these groups, and produce an ovulation hormone (Geraerts, 1976; Geraerts & Bohlken, 1976). Cells producing ovulation hormone are also known in opisthobranchs: the 'bag cells' of *Aplysia* (Kupfermann, 1970). Bag cells are usually electrically silent. However, a typical repetitive firing pattern, the 'afterdischarge', can be elicited nearly synchronously in all cells by a

strong electrical stimulus applied to the connectives (Kupfermann & Kandel, 1970). The afterdischarge lasts for several minutes up to half an hour, after which the solution bathing the preparation contains a factor that induces egg laying when injected into responsive animals (Kupfermann, 1970; Pinsker & Dudek, 1977).

In the present paper, an electrophysiological study is made of the CDC of *L. stagnalis*. To interpret the results, the branching pattern of the cells was examined using horseradish peroxidase (Muller & McMahan, 1976; Luiten, 1976).

MATERIAL AND METHODS

Laboratory bred adult specimens (shell heights 27–23 mm) were used. The snails were kept under 12/12 h light-dark regime. Dissection, recording, and iontophoresis of HRP, were carried out in snail Ringer (30 mM-NaCl, 1·5 mM-KCl, 2 mM-$MgCl_2$, 4 mM-$CaCl_2$, 0·25 mM-Na_2HPO_4, 18 mM-$NaHCO_3$). The central ganglion ring was pinned down on a Xantopren (Bayer) layer in a Perspex dish. The outer connective tissue sheath was removed. In some cases a few grains of Pronase (Calbiochem, B grade) were applied to the inner sheath for 15–30 s, after which the brain was washed 3 times in Ringer.

Horseradish peroxidase (HRP) (Merck, 100 u/mg) was injected into the cells through micropipettes containing a solution of 0·2 M-KCl and 5% HRP in 0·5 M Tris-HCl (pH 8·2). Depolarizing current pulses (200 ms, 4 Hz) of 80 nA were used to drive HRP into the cells. After 10–45 min of filling, the brain was left to stand for periods of up to 14 h (mostly 30 min), permitting the peroxidase to enter the fine branches. The brain was then washed thoroughly in Ringer and incubated in a reaction medium containing 20 μl/l H_2O_2 (30%) and 0·04 mM benzidine-diHCl, buffered to pH 7·4 with 0·1 mM Tris-HCl. Incubation lasted 20–45 min. After fixing in 3% formaldehyde in 0·5 M Tris-HCl (pH 7·4), dehydrating in acetone and clearing in creosote, the brain was embedded in Depex mounted on a hollow slide.

Extracellular application of HRP was done by inserting a blunt microelectrode between the cells or into the neuropile and ejecting HRP with a current of 1 μA for 30 min. The preparation was then developed as described above.

For intracellular recordings K_2SO_4-filled glass microelectrodes (5–50 MΩ) were connected to a DC-amplifier permitting current injection up to 4 nA through the recording electrode via a bridge balance system. For membrane resistance measurements one cell was impaled with two electrodes: one for current injection and the other for recording.

Extracellular recordings were made with glass micropipettes, bevelled to a tip diameter of about 200 μm, filled with Ringer, and connected to an AC-coupled amplifier. The same type of electrode was used for extracellular electrical stimulation, using a stimulus isolation unit.

RESULTS

The CDC are situated ventromedially in the dorsal part of each cerebral ganglion. They can be identified *in vivo* by their location and colour, which ranges from pale grey to white (Fig. 1). They can easily be distinguished from the adjacent (growth hormone producing) neurosecretory cells, which have a pale orange colour. The CDC

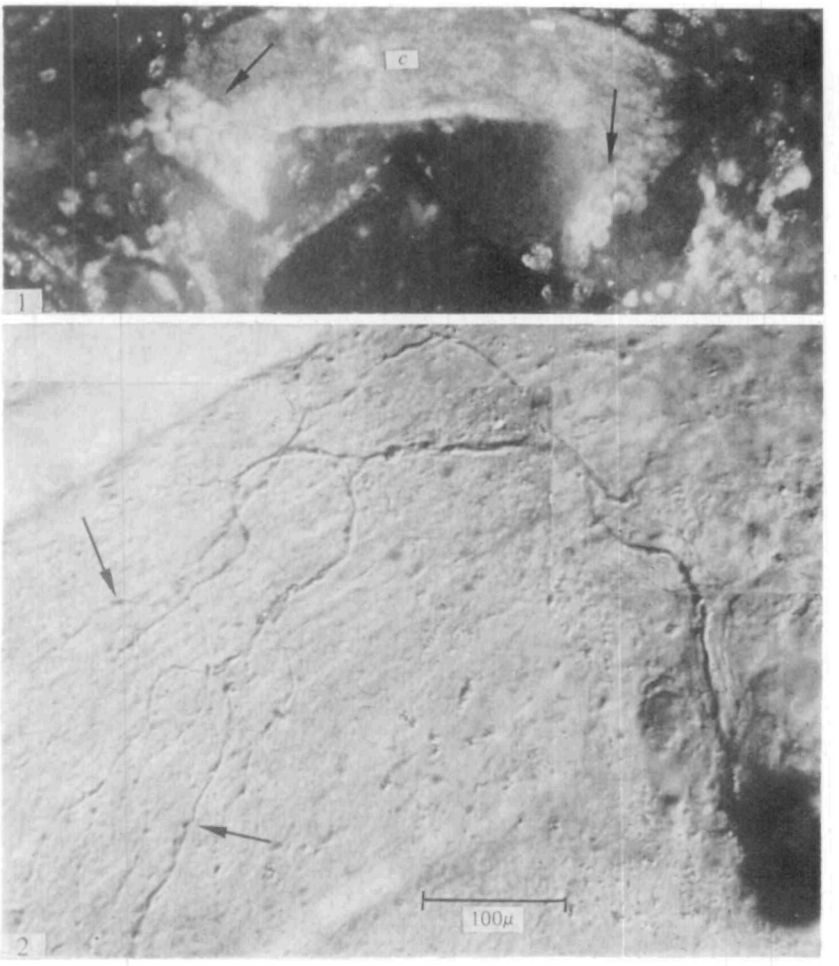
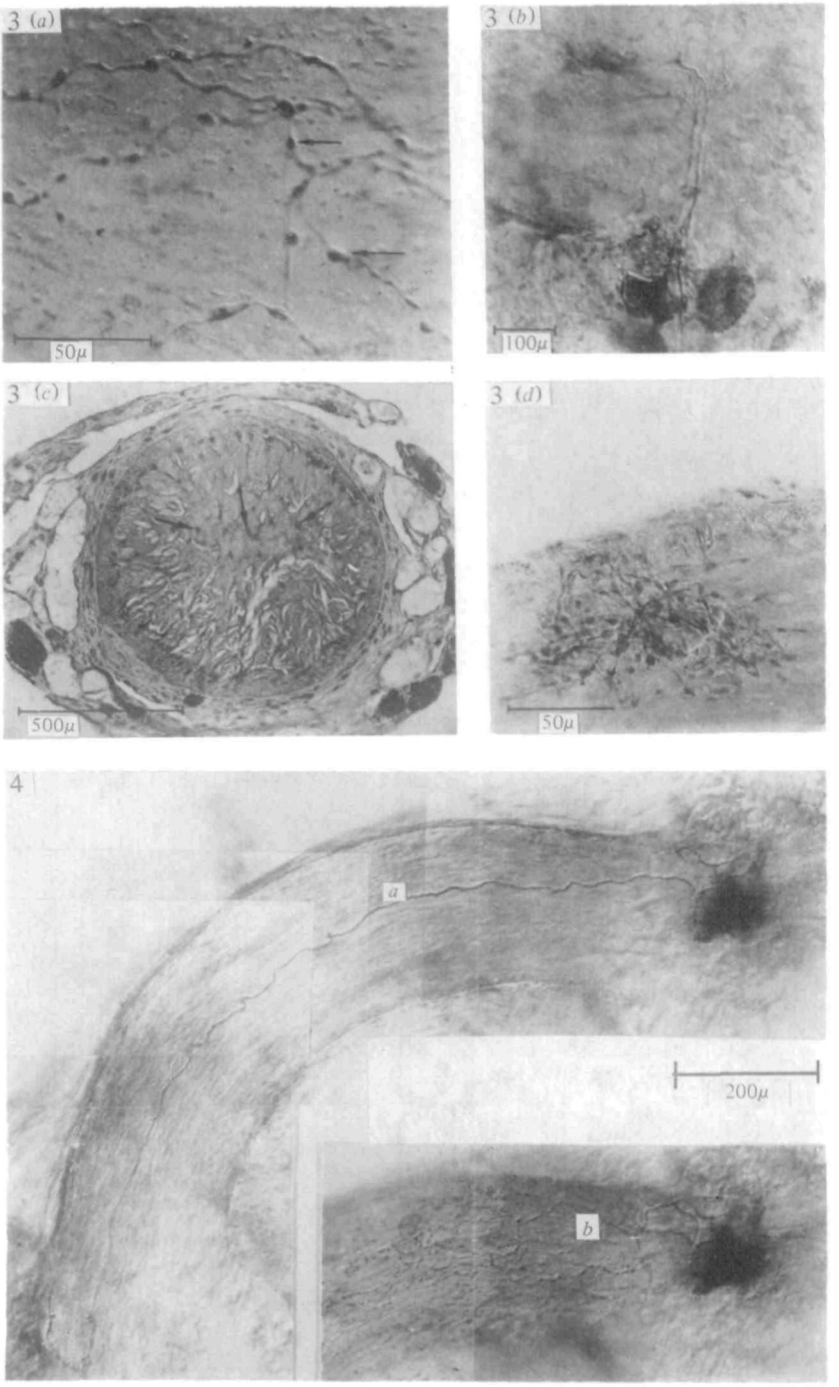


Fig. 1. Ventral view of the two groups of CDC (arrows) in the cerebral ganglia of *Lymnaea stagnalis*. C: intercerebral commissure.

Fig. 2. A whole mount preparation of a HRP-stained CDC. The cell body is located in the cerebral ganglion. The axon branches in the region near the anterior lobe and enters the intercerebral commissure where it branches extensively. Note the beads (arrows). These are absent on the proximal part of the axon. Montage of micrographs taken with interference contrast.



Figs. 3 and 4. For legends see opposite.

form two homogeneous clusters; one of about 30 cells in the left, and another of about 70 cells in the right cerebral ganglion. Their neurohaemal area is the periphery of the intercerebral commissure (Joose, 1964).

HRP staining

Out of 58 injections of single cells, 30 were considered successful in that the peroxidase remained inside the cell and could be seen in fine axonal branches in the intercerebral commissure. After 15 min of current injection (80 nA) the cells were inexcitable. As the cells can withstand a current of 15 nA for 2 h, and as they were damaged after current injection of 80 nA through a microelectrode filled with 2 M-K_2SO_4, the damage done in the standard filling procedure probably results from the high current involved and not from the peroxidase.

The shortest possible staining procedure which produced good results (but only very occasionally) consisted of current injection at 80 nA for 10 min, immediately followed by incubation. The HRP had then travelled 3·5 mm, giving a transport rate of 2·1 cm/h, which is similar to the axonal transport rates of [^{14}-C]glutamate in *Helix* nerves, 3 cm/h, measured by Kerkut, Shapira & Walker (1967).

The deposit found in the cell after developing was brown; it was seen as a uniform layer or as large vesicles. The former does not indicate damaged cells, and the latter intact ones, as suggested by Nauta, Pritz & Lasek (1974), because some intracellularly filled cells contained vesicles, whereas, occasionally cells that had taken up extracellularly applied HRP were evenly stained. The observations that (*a*) in some intracellularly injected cells the distal end of the axon had a granular appearance, whereas the cell body and the proximal part of the axon were evenly stained, and (*b*) in extracellularly applied HRP preparations, in which both forms can be seen, the cells that lie at some distance from the injection spot were granular, whereas, cells at the injection spot were evenly stained, suggest that in *Lymnaea* neurones the differences are concentration-dependent.

Morphology

Intracellular staining with HRP shows that the fine branches of the CDC axons in the periphery of the intercerebral commissure have a beaded appearance (Figs. 2, 3 *a*, *d*). The varicosities are secretory endings: they occur in large numbers in the periphery of the commissure, whereas they are absent in the proximal part of the axon; the size of the beads (about 4 μm) corresponds with the size of the widened neurohaemal

Fig. 3. Morphological details of axonal branches of CDC. (*a*) HRP-stained axonal branches with beads (arrows) in the periphery of the commissure. Interference contrast. (*b*) Three HRP-stained, dorsally located, CDC. The axons run towards the anterior lobe before entering the commissure. Near the anterior lobe fine branches intermingle. Interference contrast (*c*) A phloxin-stained cross-section of the intercerebral commissure. The periphery is studded with widened endings. In the central part some axons of the second type are denoted by arrows. (*d*) Oblique section of the commissure. A number of cells were filled extracellularly. The axonal branches form a mat right under the connective tissue surrounding the commissure.

Fig. 4. Two montages of micrographs of the same ventrally located CDC, taken at different planes of focus. The second axon (*a*) runs through the intercerebral commissure. Note the absence of beads upon this axon. Beads are present upon the branches of other axons (*b*). Interference contrast.

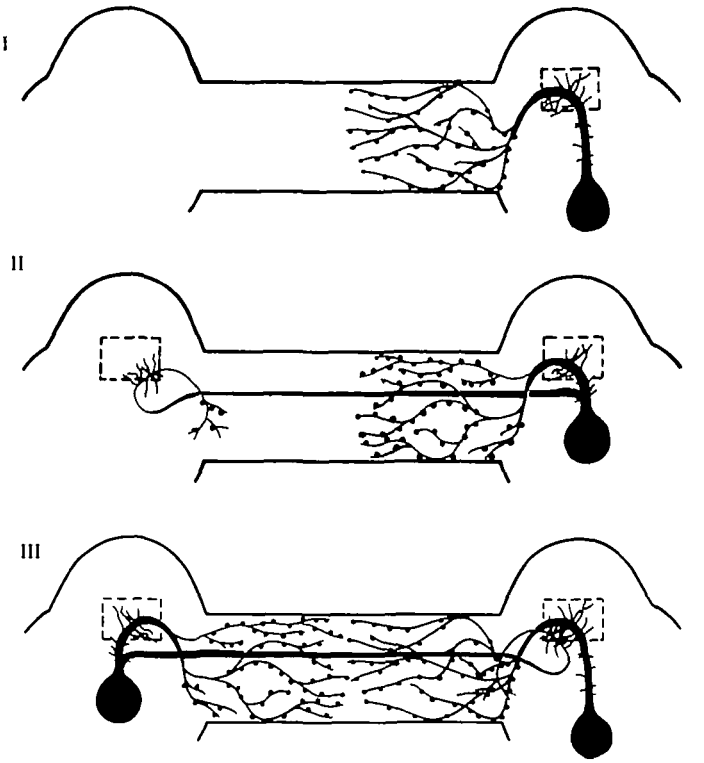


Fig. 5. Schemes of the branching patterns of the two types of CDC. The axons of all CDC end with many fine branches in the periphery of the intercerebral commissure. These branches have a beaded appearance. The axons of all CDC come in close contact at a special location near the anterior lobe. This area is shown by a broken line. I: Axonal branching pattern of the majority of the CDC. II: Axonal branching pattern of a CDC with an additional second axon. Note that this axon branches off without entering the area of contact in the ipsilateral ganglion, but does so in the contralateral ganglion. This cell type can only be found in the part of the cluster nearest the anterior lobe. They form a minority in both clusters: approximately 12 out of a total number of 100 CDC have a second axon. III: Scheme of the interaction between the two cell types.

endings seen in Gomori-stained sections (Fig. 3*c*) and in electron micrographs of the neurohaemal area; the number of beads per cell (about 300) is in the same range as the estimate given by Wendelaar Bonga (1971).

The proximal part of the CDC axon has many fine branches, suggesting synaptic contacts (Benjamin & Ings, 1972). Most of these branches intermingle in the neuropile directly underlying the cells. Fig. 3*b* shows that the axonal branching patterns of the CDC are remarkably similar to each other.

A number of CDC in the ventral part of the cluster have a second axon which runs through the commissure and loops through the neuropile ventral to the contralateral CDC (Fig. 4). It then returns to the commissure and, like the other axon, ends with beads in the periphery, though branching less extensively. The commissural part of this second axon is devoid of varicosities. The characteristics of the two types of CDC are illustrated in the scheme of Fig. 5. The number of cells of the second type was assessed in preparations in which groups of cells were stained by inserting a blunt

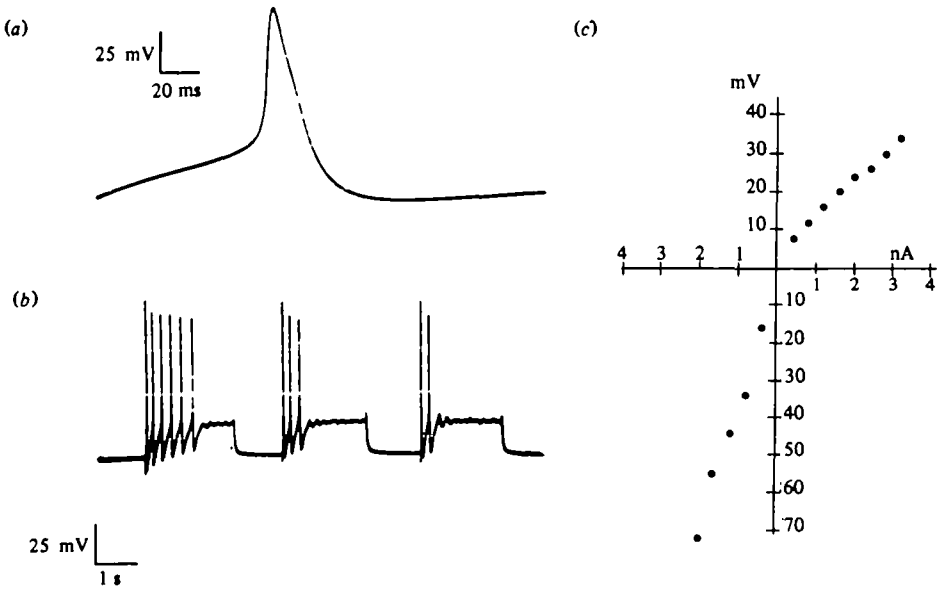


Fig. 6. Some electrical characteristics of CDC. (*a*) Evoked action potential of a silent CDC. Membrane potential about 60 mV. (*b*) Repeated depolarization with square current pulses of 1·6 nA. Rapid accommodation. (*c*) Current to voltage relationship, obtained by injecting current through one electrode and measuring membrane potential with another. The membrane shows rectification.

microelectrode into the neuropile just under the ventral part of the cluster and permitting the cells to take up the HRP. The maximum number of the second type of axon seen in these preparations was 8. In phloxin-stained serial cross-sections of the commissure a number of axons are seen running through the central part. In sections halfway between the cerebral ganglia 10–12 axons were counted (8 preparations). As axons of the first type never project beyond the middle of the commissure this range is a fair estimate of the number of axons of the second type.

Electrophysiology

The CDC are usually silent and have a steady membrane potential devoid of spontaneous PSPs. Only after special stimulation (see below) or when central nervous systems were studied from animals which were due to start egg laying (see below) could a sustained firing activity be observed. When depolarized, the CDC rapidly accommodate firing an action potential (Fig. 6*a*) or a short burst (Fig. 6*b*). Thresholds vary considerably between but not within preparations, probably reflecting the physiological state of the animals. Antidromic spikes can be elicited only by electrical stimulation of the intercerebral commissure, which confirms the morphological results. The resistance for depolarizing and for hyperpolarizing currents was different (Fig. 6*c*), indicating rectification by the membrane. The range of resistances was 20–60 MΩ for hyperpolarizing currents, and 12–24 MΩ for depolarizing currents (measurements from 20 cells). However, these measurements have a restricted significance, because of shunting due to electrotonic coupling (see below).

The occurrence of axons projecting towards the contralateral cluster is confirmed

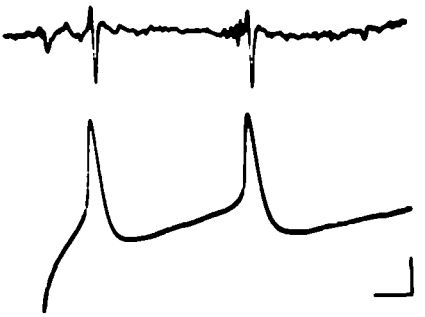


Fig. 7. Contralaterally recorded spikes generated by action potentials evoked in a ventral cell. Calibration: time, 20 ms; upper trace voltage, 50 μV; lower trace, 20 mV.

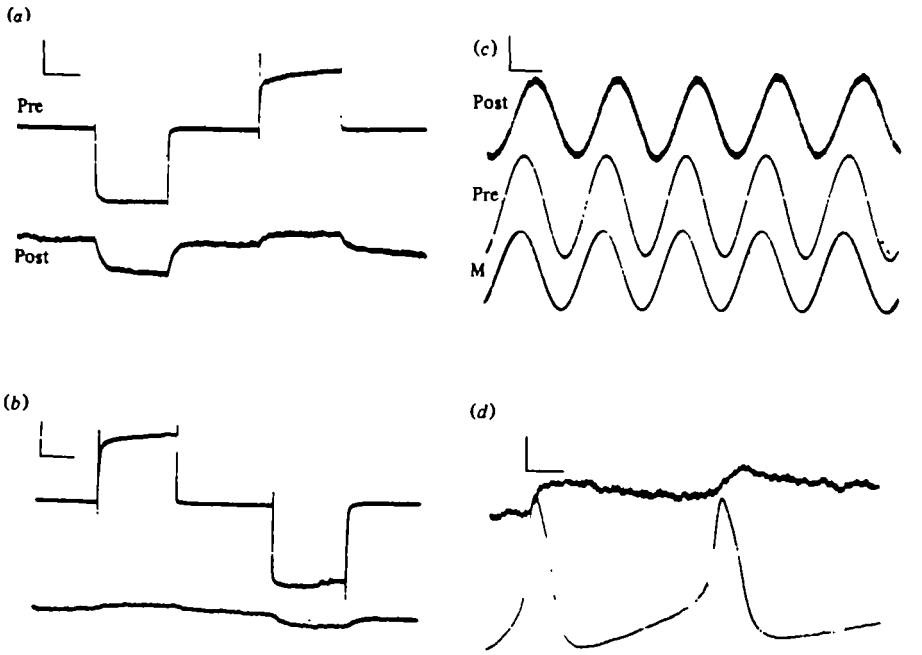


Fig. 8. Electrotonic coupling of the CDC. (*a*) Coupling between two ipsilateral CDC. Current injected via the recording electrode. Electrode resistance balanced by a Wheatstone bridge. Trace Pre: presynaptic membrane potential (calibration, 50 mV), Trace Post: postsynaptic (calibration, 5 mV). (*b*) Coupling between cells in opposite clusters. Scale and current injection as in (*a*). Coupling weaker than between ipsilateral pairs. (*c*) Ipsilateral pair. Transmission of a 1 Hz sine-wave M: current monitor (calibration, 2 nA) Pre: membrane potential of presynaptic cell (calibration, 25 mV). Post: membrane potential of postsynaptic cell (calibration, 2 mV). Phase of presynaptic cell shifted compared with current monitor, due to membrane resistance and capacitance and possibly to loading by the coupled cells. Presynaptic cell leads postsynaptic cell by 30 degrees. (*d*) Unitary EPSP's in postsynaptic cell (upper trace calibration, 2 mV) generated by action potentials of the presynaptic element (lower trace calibration, 25 mV). Time calibration; 2 s in (*a*), (*b*) and (*c*), 10 ms in (*d*).

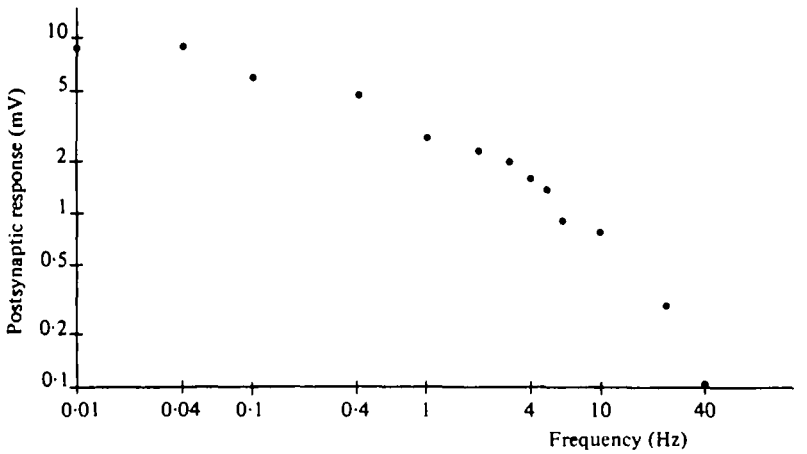


Fig. 9. Frequency-filtering curve of 5 ipsilateral pairs. Only the means are given. The junction clearly acts as a low-pass filter.

by the observation of a one for one correlation between spikes recorded intracellularly in one cluster and spikes recorded extracellularly in the contralateral cluster (Fig. 7). This was possible only when the intracellular electrode was inserted into a cell located ventrally in the cluster.

Electrotonic coupling appears to be a common feature of *Lymnaea* neuro-endocrine cell types (Benjamin, 1977). Pairs of cells belonging to either ipsilateral or contralateral clusters were impaled. A current of 4 nA was injected into one cell and the resulting potential change was measured in the other (Fig. 8*a*). Thereafter the procedure was reversed (Fig. 8*a*, *b*). The experimental procedure was as follows. One cell was penetrated and the other microelectrode was introduced successively into as many cells as possible (usually 4–8) evenly distributed over both clusters. This procedure was then repeated in the next preparation. Measurements were obtained from 20 ipsilateral pairs and from 25 contralateral pairs of cells. The average potential change after injection of 4 nA was 6·33 mV (S.E. 1·63) in ipsilateral pairs, and 2·99 mV (S.E. 2·02) in contralateral pairs. As could be expected coupling is stronger within a cluster than between clusters. The junctions appeared to be non-rectifying and no pairs of CDC were found which did not show electrical coupling, It is concluded that all CDC are electrotonically coupled.

With injections of sinusoidal current it was found that the junctions act as low-pass filters (Figs. 8, 9). In agreement with this is the finding that there is poor transmission of evoked action potentials of CDC that are not spontaneously active, although transmission was better than one would expect from the frequency-filtering curve (Figs. 8*d*, 9). The same phenomenon was found by Getting (1974) in the TGN of *Tritonia diomedea*. The explanation given by this author is that the junctional sites are on the axons of the cells and that there is an active conduction of the spike towards this junctional region, whereas the frequency response is determined by passive conduction from soma to soma. In the CDC of *L. stagnalis* the junctions probably are also between axons as there is a delay between the spike in one cell and the unitary electrotonic EPSP recorded in another (Fig. 8*d*).

It is of interest to note that due to the low-pass characteristics of the junctions, the transmission of spikes of spontaneously active CDC is better. As will be described below these spikes have a much longer duration and can be conceived as half cycles of a 2·5 Hz sine wave. Fig. 9 shows that in this frequency range there is a good transmission.

The firing pattern

In one series of experiments during which 150 preparations were used, none of the CDC were found to be spontaneously active. Spontaneously active CDC were only found during experiments designed to synchronize egg-laying behaviour in a number of animals. This can be achieved by keeping snails for a time in polluted water and then suddenly supplying fresh and oxygenated water (cf. van der Steen, 1967). This results in a high percentage of animals producing egg capsules within 3 h. Animals were taken during a 2 h period following the water change; their nervous systems were isolated and their genital tracts were inspected for ovulated eggs. CDC were spontaneously active only in animals which had ovulated or were ovulating. The chance occurrence of such activity in the experimental series mentioned above was probably prevented by some feature of the experimental procedure.

In about 50% of the 150 preparations, the CDC only responded with one spike per stimulus upon direct stimulation and with inhibitory synaptic potentials (results on this synaptic input will be published elsewhere) when peripheral nerves were electrically stimulated. The direct electrical stimuli could be applied either via an external electrode to the axon endings in the neurohaemal area (antidromic) or by means of an intracellular electrode in one cell (orthodromic).

In about 40% of the preparations a long-lasting activity could be evoked by a short period of direct electrical stimulation. A typical example of the induction of long-lasting CDC activity by orthodromic stimulation is shown in Fig. 10. At first the cell responds with one action potential per stimulus (Fig. 10*a*). After about 18 s the cell starts to respond with more than one action potential per stimulus and simultaneously the extracellular recording shows that an increasing number of CDC are activated in the opposite cluster. When stimulation is terminated (arrow) spike activity is sustained and develops further in a special way. This is shown (in another preparation) in Fig. 10*b*. The record begins, just after termination of the stimulation, with about 10 regularly spaced spikes. Then there is a sharp increase in firing rate followed by a silent period with a hyperpolarizing wave, after which a new burst develops on top of a depolarizing wave. After the burst, the cell is more depolarized than before and starts firing regularly. The extracellular record shows that the opposite CDC have a similar pattern of firing. Fig. 10*c* was made 10 min after the recording of Fig. 10*b* and shows (on an expanded time base) that the firing is very regular and nearly synchronous in both clusters. Finally the firing rate declines, becomes irregular (Fig. 10*d*, note compressed time base) and ultimately the cell becomes silent.

As a rule it was not possible to re-initiate long-lasting activity in CDC; their electrical behaviour was then comparable to those CDC in which no long-lasting activity could be evoked. It seems appropriate to distinguish 'excitability states' in CDC.

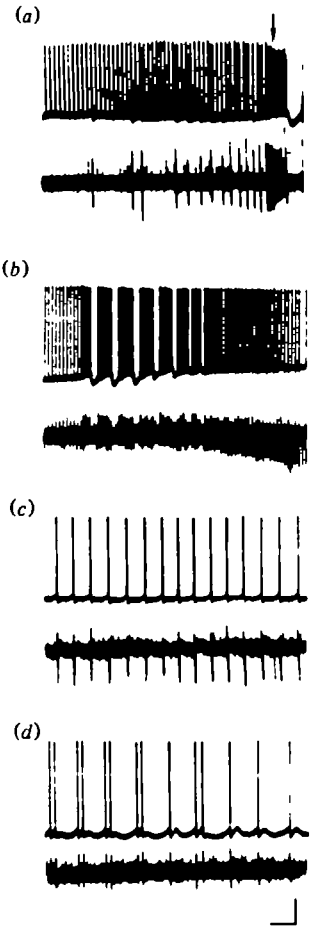


Fig. 10. Initiation of the CDC afterdischarge. Simultaneous recordings made intracellularly from a CDC in the right cluster (upper traces) and extracellularly from CDC axons in the intercerebral commissure (lower traces). (*a*) The CDC is stimulated with depolarizing pulses, until arrow, once per s. The cell initially responds with one spike per stimulus, but after about 18 s more spikes per stimulus are elicited. Simultaneously the extracellular record shows recruitment of other CDC. (*b*) Another preparation just after termination of stimulation. The afterdischarge begins with a period of bursting activity after which regular firing occurs. (*c*) Same preparation, recorded 10 min after recordings in (*b*). The firing is very regular (note expanded time base). (*d*) Same preparation, recorded 40 min after recordings in (*b*). Decline of firing rate leads to the end of the afterdischarge (note compressed time base). Voltage calibration: in (*a*), upper trace, 20 mV; lower trace, 50 μV; in (*c*) and (*d*), upper trace, 25 mV; lower trace, 100 μV. Time calibrations: 5 s in (*a*), 16 s in (*b*), 4 s in (*c*) and 20 s in (*d*).

In preparations in which the CDC were engaged in this long-lasting activity not a single silent CDC was ever encountered. It is concluded that it is possible in excitable CDC to bring about activity of all CDC by electrical stimulation of only one CDC. In view of the similarities between the long-lasting activity of *Lymnaea* CDC and *Aplysia* bag cells the CDC long-lasting activity is also called afterdischarge. The time histograms of Fig. 11 show that there are three phases in this afterdischarge: (1) initial high frequency and bursting activity, (2) a period of regular firing and (3) decline in

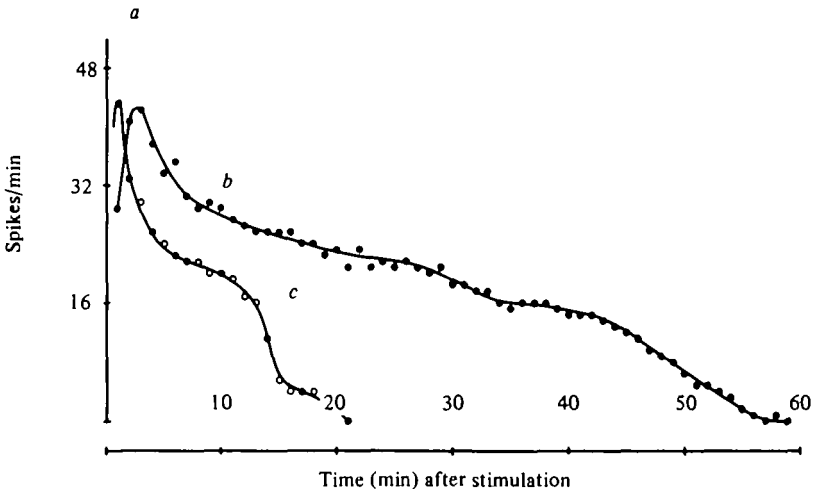


Fig. 11. Frequency–time relationship of two afterdischarges, evoked by a short period of repetitive stimulation, in two different preparations, made by counting action potentials in one CDC. There is variation in duration of the afterdischarge but always three phases can be distinguished: (*a*) initial high frequency and bursting mode of firing, (*b*) slow decline of firing rate, and (*c*) rapid decline of the rate of firing.

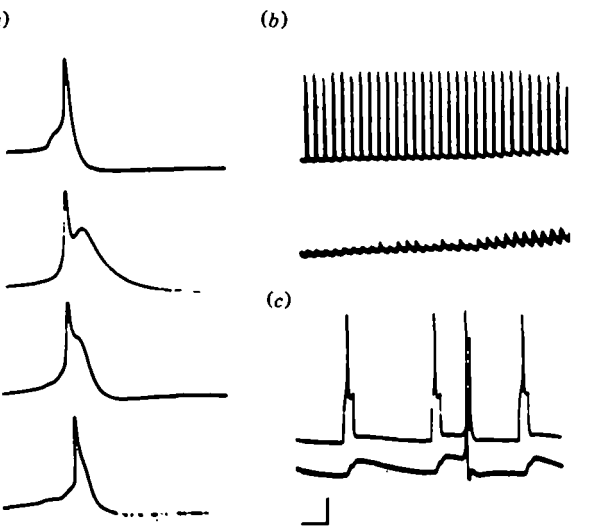


Fig. 12. Effects of intracellular stimulation of CDC and changes in shape of spikes of CDC during afterdischarge. (*a*) Change of shape of spike during afterdischarge. Spikes recorded (from top to bottom), after a few s, 7 min, 30 min and 55 min after beginning of afterdischarge. (*b*) and (*c*) Simultaneous intracellular recordings of two CDC from opposite clusters. Intracellular stimulation in one CDC (upper traces) results in postsynaptic activity in the other (lower traces). (*b*) Repetitive stimulation of one cell resulting in slow depolarization of both cells. (*c*) As in (*b*) but the second postsynaptic potential elicits an action potential in the presynaptic cell (due to the fact that current was supplied without bridge balance, the synaptically evoked spike is easily distinguished from the spikes evoked by imposed current). Voltage calibration: in (*a*) 20 mV; in (*b*) and (*c*); upper trace, 20 mV; lower trace 10 mV. Time calibration; 50 ms in(*a*) 500 ms in (*b*) and (*c*).

frequency. Although there is much variation of the frequencies within the phases and of their duration (especially the duration of phase 2) it was always possible to distinguish these three phases.

A third category of responsiveness to electrical stimulation was found in about 10% of the preparations. They appeared to be intermediate between the two former ones. In these preparations it was possible to evoke more than one spike per stimulus by using repetitive stimulation but an afterdischarge was never produced.

During the period of electrical stimulation of excitable CDC phenomena were found in the afterdischarge that suggest a role for the electrotonic junctions. Firstly, there is a clear increase in spike duration during the first part of the afterdischarge (Fig. 12*a*). This increase in duration will facilitate transmission across the junctions as described above. Secondly, simultaneous intracellular recordings of two CDC, whereby one cell was rhythmically stimulated, showed the occurrence of subthreshold potentials in the unstimulated cell. Fig. 12*b* shows a gradual depolarization of both cells accompanied by a gradual increase in amplitude of these potentials. Fig. 12*c* shows what happens in a more excited preparation (when more stimuli have already been given). The evoked action potentials in one cell result in post synaptic potentials in the follower cell. The second post synaptic potentials even leads to a spike in the follower cell. This spike in its turn gives rise to a spike in the presynaptic cell. This mutual interaction is very commonly found among pairs of excited CDC.

DISCUSSION

The results show that the hormone producing CDC of *L. stagnalis* closely resemble the bag cells of *Aplysia* (Kupfermann & Kandel, 1970) for which the correlation between egg-laying and electrical activity has been demonstrated by Pinsker & Dudek (1977). The CDC are usually electrically silent and spontaneous activity was only found in preparations from animals having ovulated eggs in the genital duct. In other preparations long-lasting activity could be induced by a short period of repetitive electrical stimulation of one (or more) CDC.

Kandel (1976) argues that the bag cells of *Aplysia* have a 'triggering' function in the all or none act of egg-laying. Also usually silent, these cells can respond to strong electrical shock to the connectives with an afterdischarge in which all cells participate and during which the hormone is released. In the present experiments the afterdischarge was brought about by direct stimulation of CDC. Input from other neurones could well have been masked by pronounced inhibition evoked by the extracellular stimulation of nerves and connectives (De Vlieger & Roubos, 1978).

Kandel (1976) suggests that a major factor determining the massive discharge of the bag cells is electrotonic coupling by 'remote' axonal processes. This coupling has been directly established only in *Apylsia dactylomela* (Blankenship & Haskins, 1979). In *L. stagnalis* electrotonic coupling appeared to be quite pronounced between all CDC and could very well explain that excitation in one cell spreads to all others, especially since in active cells there is a progressive increase in spike width, facilitating transmission across the junctions.

The importance of electrical coupling is underlined by the results on the morphology of the CDC. With the HRP injection it was found that although all CDC have

the same endocrine function, two morphological types can be distinguished. One of them is 'secretory' only, sending its axon directly towards the neurohaemal area, whereas the other one has an additional axon running towards the opposite cell cluster. It is clear that the latter axons are essential for the electrical coupling of both clusters of CDC. This way of coupling obviates the need for a common interneurone to explain synchrony of firing. In addition electrotonic junctions could affect the dynamic properties of the CDC (Getting, 1974; Berry & Pentreath, 1977).

There are two remaining problems. The first is that of the identity of the input normally operating to initiate the afterdischarge. The second is the nature of the variability of excitability in CDC. In this respect an interesting finding is that egg-laying in *L. stagnalis* is highly dependent on the light regime given: 'long day' animals show an enormous increase in egg-laying behaviour (Bohlken, 1977). Preliminary electrophysiological experiments have shown that a much larger proportion of CDC are capable of producing an afterdischarge in long day animals than in the animals used in the present study. It is tempting to speculate that the CDC have one input for setting the level of excitability, and another one for triggering the afterdischarge, while the electrotonic couplings ensure that all cells participate in the discharge.

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