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NEURONAL CONTROL OF SWIMMING IN THE MEDICINAL LEECH

V. CONNEXIONS BETWEEN THE OSCILLATORY INTERNEURONES AND THE MOTOR NEURONES

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SUMMARY

A network of intra- and intersegmental synaptic connexions has been identified in the ventral nerve cord of the leech that links the set of oscillatory interneurones of the central swim oscillator to the motor neurones commanding the swimming rhythm. Excitatory connexions lead from oscillatory interneurones to both excitatory and inhibitory motor neurones, whereas inhibitory connexions lead from oscillatory interneurones to only the inhibitory motor neurones. Connexions leading from a motor neurone back to the oscillatory interneurones were found in only one exceptional case, an inhibitory motor neurone previously known to have access to the central swim oscillator. This network of identified connexions can account reasonably well for the mechanism by which the oscillatory interneurones drive their follower motor neurones into the phasic activity pattern characteristic of the swimming movement.

INTRODUCTION

It was reported in the preceding paper (Friesen, Poon & Stent, 1978) that the segmental ganglia of the ventral nerve cord of the leech, *Hirudo medicinalis*, contain a set of oscillatory interneurones, cells 123, 28, 27 and 33, that appear to generate the basic swimming rhythm of the leech. Bilateral and serial homologues of these interneurones exist on right and left sides of all abdominal ganglia of the cord, with the possible exception of the very frontmost and rearmost. These interneurones are interconnected in a manner such that they form a series of intersegmentally concatenated neuronal rings. These rings constitute an oscillatory network because they incorporate the property of recurrent cyclic inhibition (Székely, 1965).

Theoretical analysis and electronic analogue modelling of this network have shown (Friesen & Stent, 1977) that it is capable of generating the observed segmental activity rhythm of the four oscillatory interneurones summarized schematically in

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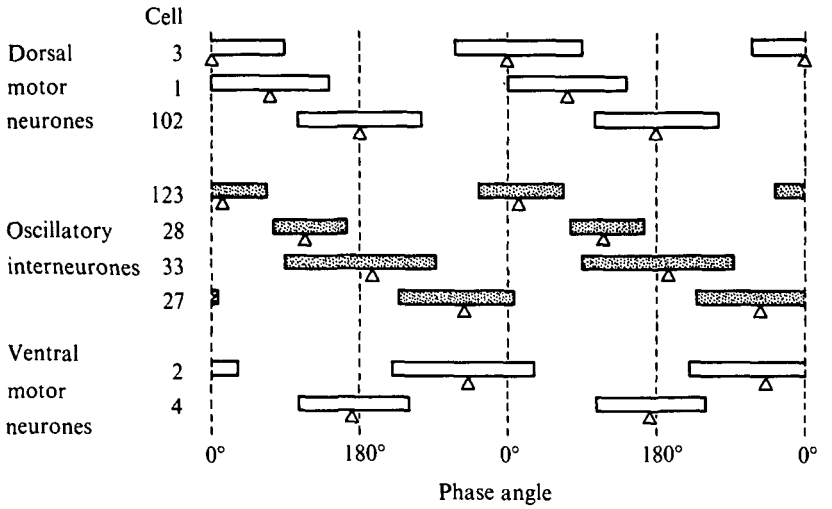

Fig. 1. Summary diagram of the swimming activity cycles of the oscillatory interneurones and of a representative subset of the motor neurones. Each bar indicates the duration of the impulse burst of the cell, and the triangle under each bar points to the burst 'midpoint' (median spike). The burst midpoint of cell 3 has been arbitrarily assigned the phase angle 0°.

Fig. 1. Moreover, the network can generate also the intersegmental, rostro-caudal phase lag of the duty cycles of serially homologous interneurones necessary for generating the metachronal body wave characteristic of the swimming movement. However, before the conclusion that oscillatory interneurones constitute the central swim oscillator of the leech (Kristan & Calabrese, 1976) can be accepted, it must be shown that they make appropriate output connexions to the segmental motor neurones commanding the rhythmic contraction of the segmental musculature.

The dorso-ventrally antiphasic contractile rhythm of each body segment – the troughs and crests of the body wave – is generated by phasic synaptic input provided to the longitudinal muscle fibres by an ensemble of bilaterally and serially homologous excitatory and inhibitory motor neurones in the ganglia of the nerve cord. This ensemble includes (Ort, Kristan & Stent, 1974) four excitatory motor neurones to the dorsal longitudinal muscles, the *dorsal excitors*, cells 3, 5, 7 and 107; three excitatory motor neurones to the ventral longitudinal muscles, the *ventral excitors*, cells 4, 8 and 108; two inhibitory motor neurones to the dorsal longitudinal muscles, the *dorsal* inhibitors, cells 1 and 102; and one inhibitor to the ventral longitudinal muscles, the *ventral inhibitor*, cell 2. In addition to their peripheral inhibitory connexions to the longitudinal muscles, the dorsal and ventral inhibitors make central inhibitory connexions with the dorsal and ventral excitors, respectively. During a swimming episode, the membrane potential of these motor neurones oscillates between a depolarized and a hyperpolarized state, with a burst of action potentials arising in the depolarized state. Fig. 1 presents in schematic form the activity rhythm of the five motor neurones studied in the work to be reported here, namely of the dorsal excitor cell 3, the ventral excitor cell 4, the dorsal inhibitors, cells 1 and 102, and the ventral inhibitor, cell 2. As can be seen, the phase relation of the activity cycles of the motor neurones is such that

as would be expected, the impulse bursts of the dorsal and ventral excitors seem out of phase with each other and out of phase with the impulse bursts of their respective inhibitors. Detailed examination of these phase relations reveals, however, that this rhythm of motor neurone activity, just like the oscillatory interneurone rhythm, is characterized by four rather than only two cycle-phases. That is to say, if the phase angle of 0° is arbitrarily assigned to the median impulse (burst 'midpoint') of the cell 3 burst, then the midpoint of the burst of cell 123 also occurs at a phase angle of about 0°, that of cells 1 and 28 at about 90°, that of cells 102, 33 and 4 at about 180°, and that of cells 27 and 2 at about 270°.

Recently, the identification of an additional ventral inhibitor, cell 119, has been reported (Sawada *et al.* 1976). Contrary to the previous report that cell 119 does not participate in the swimming rhythm (Ort *et al.* 1974), a re-examination of cell 119 has now shown that during swimming episodes its membrane potential does oscillate between a depolarized and a hyperpolarized state, with the impulse burst midpoint occurring at a phase angle of about 0°. Moreover, cell 119 makes a direct inhibitory synaptic connexion to the ventral excitor, cell 4 (Poon, 1976). Thus, cell 119 should be added to the roster of motor neurones driving the swimming movement. In being truly antiphasically active with its homonymous excitors, cell 119 apparently plays the inhibitory role on the ventral side that the dorsal inhibitor, cell 102, plays on the dorsal side.

This paper reports the identification of a network of intra- and intersegmental output connexions from the oscillatory interneurones to a representative subset of the motor neurones, consisting of cells 1, 2, 3, 4 and 102. The midpoints of the bursts of the dorsal excitors cells 5, 7 and 107 not included in this subset occur at exactly the same phase angle of 0° as the burst midpoint of cell 3 (Kristan, Stent & Ort, 1974*b*). All four dorsal excitors are linked by electrical junctions (Ort *et al.* 1974), and, moreover, cell 3 and cell 5 receive inhibitory synaptic potentials from a common presynaptic source (Poon, 1976). Hence it seems highly likely that the oscillatory interneurones impose the rhythm on all dorsal excitors by a common pathway. The midpoints of the bursts of the ventral excitors, cells 8 and 108, not included in the representative subset, occur at nearly the same phase angle of about 180° as the burst midpoint of cell 4. All three ventral excitors are linked via electrical junctions and it is likely that the oscillatory interneurones make similar, but not identical, output connexions to the three ventral excitors. Since the output connexions found in this study can give a reasonably good account of the generation of the representative motor neurone subset, the present results support the inference that the identified oscillatory interneurones represent the central swimming oscillator. A preliminary report of these results has been previously published (Friesen, Poon & Stent, 1976).

MATERIALS AND METHODS

The methods of dissecting and mounting the isolated ventral nerve cord preparation, of taking intracellular recordings from nerve cell bodies by means of glass capillary microelectrodes and extracellular recordings from segmental nerves by means of glass-tipped suction electrodes, of computer averaging of synaptic signals, of passing current into nerve cells, and of numbering segments and designating the

components of the segmental nerve system of *H. medicinalis*, were those previously described (Kristan, Stent & Ort, 1974*a*; Ort *et al.* 1974; Kristan & Calabrese, 1976; Friesen *et al.* 1978).

RESULTS

The oscillator network described in the preceding paper (Friesen *et al.* 1978) imposes the swimming rhythm on the leech motor neurones through excitatory and inhibitory connexions. These connexions were identified by simultaneous intracellular recordings from an interneurone and a motor neurone of an isolated ventral nerve cord of *H. medicinalis*. Any such cell pair is inferred to be connected if passage of current into one cell via the inserted microelectrode evokes a change in membrane potential, or in impulse frequency, in the other cell. Moreover, the connexion is considered to be direct if an impulse in the presynaptic cell is followed with constant delay by a synaptic potential in the postsynaptic cell. To keep the number of such pairwise recordings within reasonable bounds, this survey was limited to the output of the four oscillatory interneurones and to only five of the eleven known swimming motor neurones: a dorsal excitor, a ventral excitor, two dorsal inhibitors and a ventral inhibitor. The inference of every connexion reported in the following is based on experiments carried out with at least two different isolated nerve cord preparations. With all simultaneous electrode penetrations of an interneurone and a motor neurone the effects of passing hyperpolarizing and depolarizing current into one member of the cell pair on the membrane potential or impulse frequency of the other member of the pair were tested. However, in the following we report only those results where passage of current produced an effect, or where the absence of an effect has some special significance, such as implying the presence of a rectifying electrical junction.

(1) *Output of the interneurone, cell 123*

An excitatory link leads from cell 123 to the dorsal excitor, cell 3 (Fig. 2). The delay of about 20 ms between an impulse recorded from cell 123 and an excitatory synaptic potential (amplitude about 1·5 mV) in cell 3 of the next posterior ganglion is in full accord with the previous estimate of an intersegmental impulse conduction time of about 20 ms per segment for the axons of the oscillatory interneurones, and the previous finding that the axon of cell 123 projects rearward along the nerve cord (Friesen *et al.* 1978). Thus it can be inferred that the excitatory link from cell 123 to cell 3 of the next posterior ganglion is a direct one. However, within the same ganglion, the connexion between this cell pair may not be a direct one, since there was no correspondence between impulses in the presynaptic cell and synaptic potentials in the postsynaptic cell. Results not presented here indicate that evoked depolarization of cell 123 causes an increase in the impulse frequency of cell 3 homologues as far as eight segments rearward.

The results exemplified in Fig. 2 agree with the finding that the two cells are active in very nearly the same phase during a swim episode (Fig. 1). Thus far no other output connexion of cell 123 has been found. Negative evidence (not presented here) indicates that cell 123 is linked neither to the ventral excitor, cell 4, nor to the ventral inhibitor, cell 2.

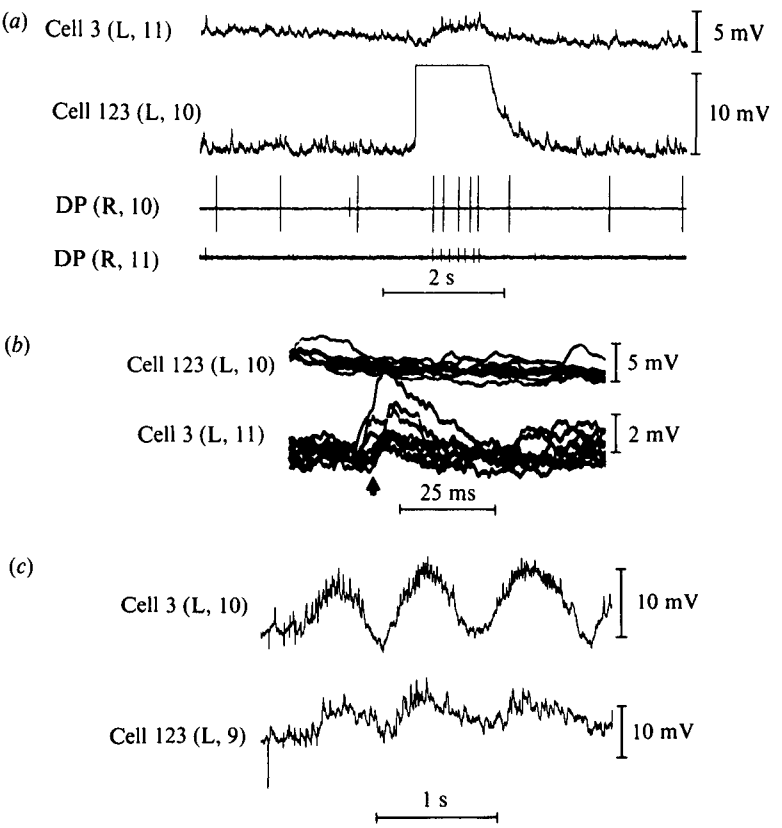

Fig. 2. Excitatory connexion from cell 123 to the dorsal excitor, cell 3. (*a*) Simultaneous intracellular recordings taken from the two cells in adjacent ganglia of an isolated nerve cord preparation. Passage of depolarizing current into cell 123 depolarizes and increases synaptic activity and impulse frequency of cell 3 of the next posterior ganglion and in the same ganglion. (*b*) Composite superimposed oscilloscope sweeps of recordings taken from cell 123 and cell 3 of the more posterior ganglion, triggered by impulse in cell 123. Each impulse in cell 123 is followed by an excitatory potential in cell 3 with a constant delay of about 20 ms. (*c*) Polarization rhythms of cell 3 and cell 123 during a swim episode. In this, and in all following electrophysiological records presented here, the letters R or L following in parentheses the designation of a cell or segmental nerve indicate right or left side, respectively, and the number indicates the abdominal segment from which the recording was taken. A sharp upward or downward deflexion of the intracellular traces marks passage of depolarizing or hyperpolarizing current, respectively, of no more than 5×10^{-9} A into the cell. The time of initiation of an inhibitory and excitatory potential in composite superimposed oscilloscope sweeps is indicated by an arrow. The traces labelled DP represent extracellular suction electrode recordings taken from the dorsal branch of a segmental posterior nerve. The large amplitude spikes in the DP records represent impulses of the dorsal excitor, cell 3.

(2) *Output of the interneurone, cell 28*

The output connexions of the interneurone cell 28 are much more extensive than those found for cell 123, in that they reach not only ventral excitors, but also dorsal and ventral inhibitors. A direct inhibitory link was demonstrated to lead from cell 28 to its ipsilateral cell 2 in the same ganglion (Fig. 3). The records of Fig. 3 show also that cell 28 has an inhibitory effect on cell 2 of the next anterior ganglion. However,

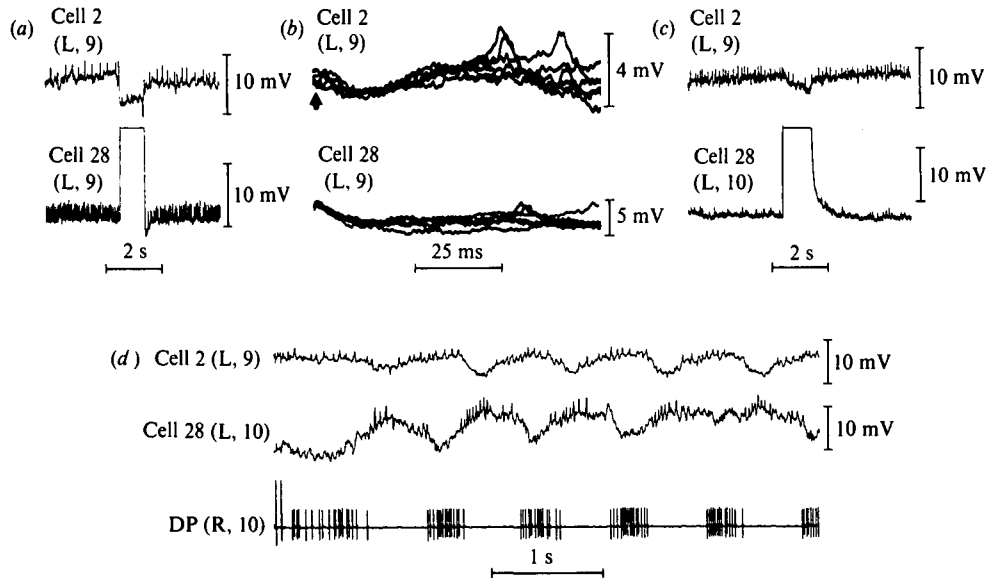

Fig. 3. Inhibitory connexion from cell 28 to the ventral inhibitor, cell 2. (*a*) Passage of depolarizing current into cell 28 hyperpolarizes cell 2 of the same ganglion. (*b*) Composite superimposed oscilloscope sweeps of recordings from cell 28 and cell 2 of the same ganglion, triggered by impulses in cell 28. Individual impulses in cell 28 are followed by an inhibitory potential in cell 2 with a constant delay of less than 5 ms. (*c*) Passage of depolarizing current into cell 28 hyperpolarizes cell 2 of the next anterior ganglion. (*d*) Polarization rhythms of cell 2 and cell 28 during a swim episode.

this effect may be indirect, since it is weaker than the homologous intraganglionic effect, and impulses of cell 28 are not followed with constant delay by a synaptic potential in the anterior cell 2. The inhibitory effect is in agreement with the finding (Fig. 1) that these two cells are antiphasically active.

Cell 28 was also found to have an excitatory connexion to cell 4 in the same and anterior ganglia. The delay time between impulses recorded from cell 28 and the postsynaptic potentials indicated that the links to anterior ganglia were direct (Fig. 4), the conduction time of about 15 ms per segment being in good agreement with a previous estimate (Friesen *et al.* 1978). However, impulses of cell 28 are not followed with constant delay by a synaptic potential in cell 4 of the same ganglion; so this link may be indirect, possibly via the tandem inhibitory connexions from cell 28 to cell 2 and from cell 2 to cell 4. The inference of a direct excitatory link from cell 28 to anterior cell 4 homologues is consonant with the previously inferred frontward projection of the cell 28 axon, and with the observation that these two cells are only slightly out of phase during the swim cycle (Fig. 1).

An additional excitatory link from cell 28 was found to the dorsal inhibitor, cell 1, of the same ganglion (Fig. 5), and these cells are also nearly in phase during the swim cycle (Fig. 1). The connexion was inferred to consist of a rectifying electrical junction since it permitted only depolarizing current to flow from cell 28 to cell 1 and only hyperpolarizing current in the reverse direction (Fig. 5). Since cell 28 impulses are not followed by excitatory potentials in cell 1 homologues of anterior ganglia, it appears that these cells are linked only intraganglionically.

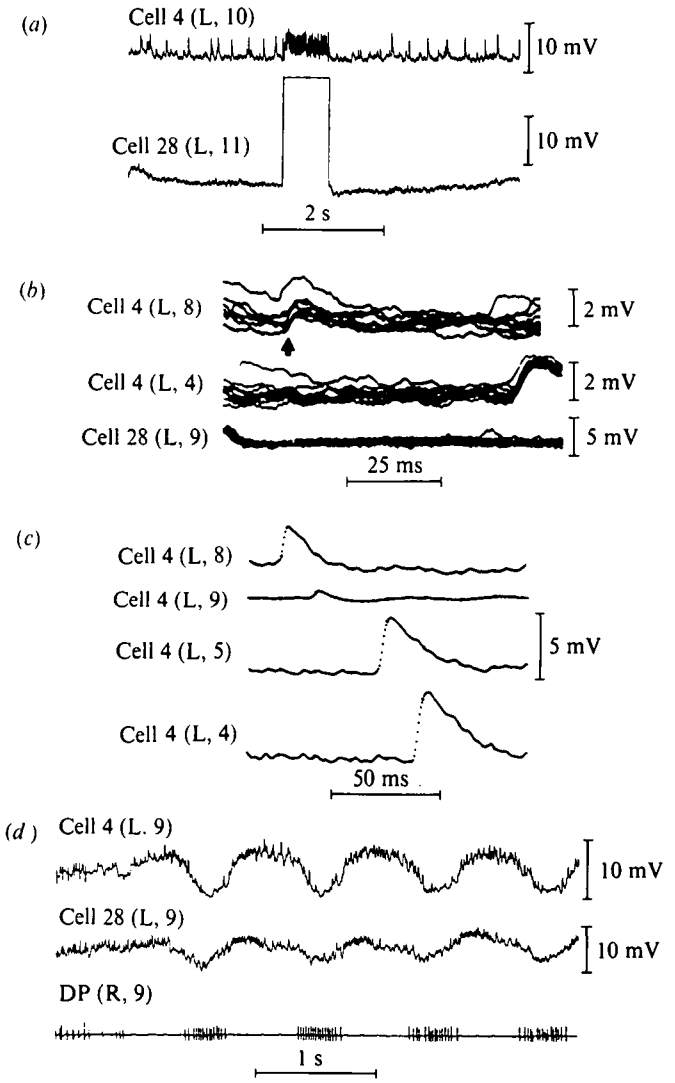

Fig. 4. Excitatory connexion from cell 28 to the ventral excitor cell 4. (*a*) Passage of depolarizing current into cell 28 depolarizes and increases the impulse frequency of cell 4 of the next anterior ganglion. (*b*) Composite superimposed oscilloscope sweeps of recordings taken from cell 28 and cell 4, triggered by impulses of cell 28. Individual impulses in cell 28 are followed by an excitatory potential in cell 4, one ganglion and five ganglia anterior to cell 28, with a constant delay of about 20 ms and 80 ms, respectively. (*c*) Computer-averaged signals recorded from cell 4 triggered by cell 28 impulses showing an increase of the delay between impulse and excitatory potential with intersegmental distance. The potential in the top trace is triggered by cell 28 (L, 9), the second trace by cell 28 (L, 11), the third trace by cell 28 (L, 9) and the bottom trace by cell 28 (L, 9). (*d*) Polarization rhythm of cell 4 and cell 28 during a swim episode.

The dorsal inhibitor, cell 102, was also found to have an excitatory input from cell 28 (Fig. 6). This input appeared direct to anterior ganglia since impulses from cell 28 were followed by excitatory potentials in cell 102 after a constant delay of about 18 ms. Data not presented here show that evoked depolarization of cell 28 also has a weak

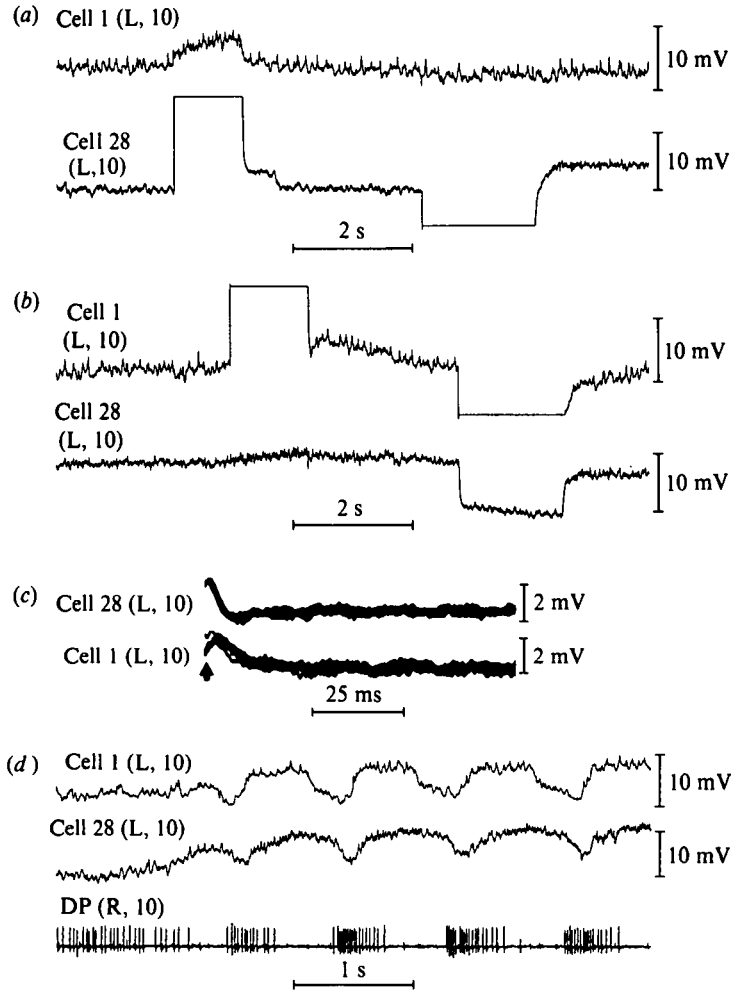

Fig. 5. Excitatory connexion from cell 28 to the dorsal inhibitor, cell 1. (*a*) Passage of depolarizing current into cell 28 depolarizes cell 1 of the same ganglion. Passage of hyperpolarizing current has little, if any, effect. (*b*) Passage of depolarizing current into cell 1 slightly increases the impulse activity of cell 28. Passage of hyperpolarizing current strongly hyperpolarizes cell 28. (*c*) Composite superimposed oscilloscope sweeps of recording from cell 28 and cell 1 triggered by impulse in cell 28. Each impulse in cell 28 is followed by an excitatory potential in cell 1 with a constant delay of less than 2 ms. (*d*) Polarization rhythms of cell 1 and cell 28 during a swim episode.

excitatory effect on cell 102 of its own ganglion. This effect is likely to be mediated by an indirect link (probably via cell 1, which is known to be joined via an electrical junction to cell 102), since cell 28 impulses are not followed by excitatory potentials in cell 1 of the same ganglion. The records shown in Fig. 6 confirm the previous observation that the oscillation of cell 28 leads that of cell 102 by a phase angle of about 90° (Fig. 1).

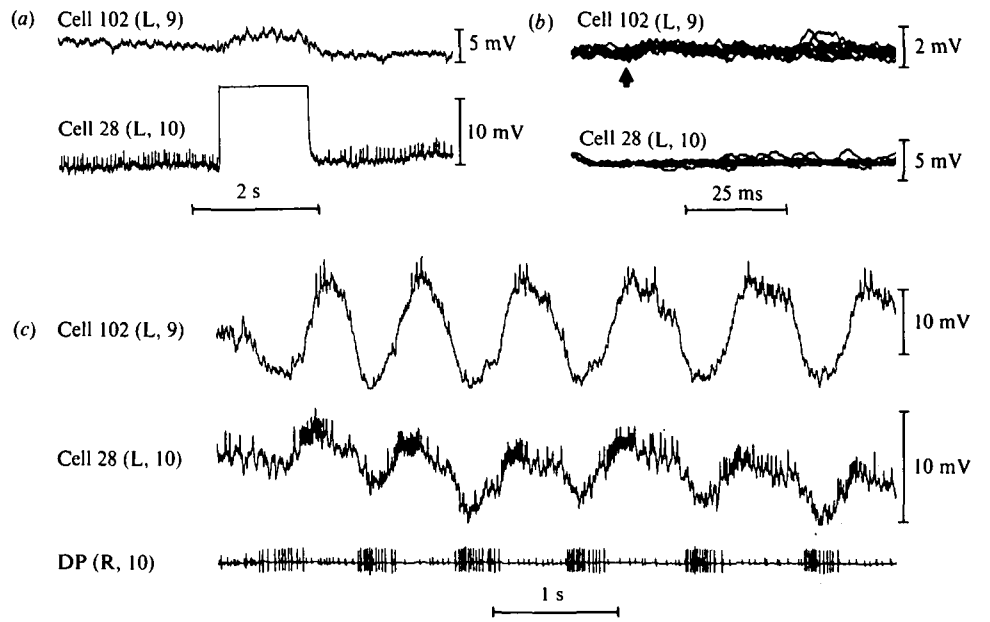

Fig. 6. Excitatory connexion from cell 28 to the dorsal inhibitor, cell 102. (*a*) Passage of depolarizing current into cell 28 depolarizes cell 102 of the next anterior ganglion. (*b*) Composite superimposed oscilloscope sweeps of recordings taken from cell 28 and cell 102, triggered by impulses in cell 28. Each impulse in cell 28 is followed by an excitatory potential in cell 102 with a constant dealy of about 20 ms. (*c*) Polarization rhythms of cell 102 and cell 28 during a swim episode.

(3) *Output of the interneurone, cell* 33

As shown in Fig. 7, passage of depolarizing current into cell 33 depolarizes, and increases the electrical activity of, the dorsal excitor, cell 3, in the next anterior ganglion. Individual impulses recorded from cell 33 are seen to be followed by an excitatory synaptic potential in homologues of cell 3 of the next anterior ganglion and of a ganglion 5 segments to the anterior, after constant delays of about 20 ms and 60 ms, respectively. Thus there exists a direct excitatory link from cell 33 to the cell 3 homologue of anterior ganglia. This excitatory link appears to consist of an electrical rather than a chemical synapse, since the excitatory synaptic potential in cell 3 can be observed while the preparation is bathed in saline containing 35 mM-Mg^{2+}. This concentration of Mg^{2+} blocks chemical synaptic transmission in the leech nerve cord (Nicholls & Purves, 1970). The finding of such an excitatory link is rather unexpected, however, since, as is evident in Figs. 1 and 7, the oscillations of the two cells are separated by a phase angle of about 180°, suggesting an inhibitory rather than excitatory link. This finding of a paradoxical excitatory link between antiphasically active neurones resembles the previous encounters with electrical junctions between the dorsal and ventral excitors, cell 1 and cell 2 (Ort *et al.* 1974), as well as between some oscillatory cells in the stomatogastric ganglion of the lobster (Mulloney & Selverston, 1974). No really satisfactory explanation of the role of these paradoxical links is as yet available, except the proposal that their function is the modulation of amplitude rather than control of phase in the oscillatory activity.

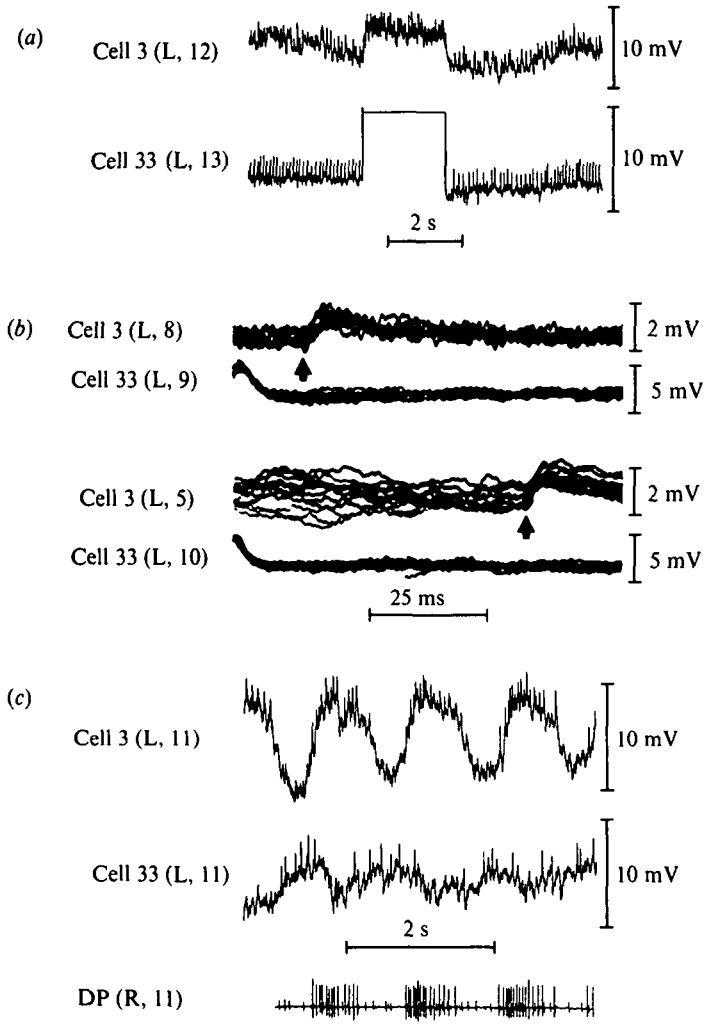

Fig. 7. Excitatory connexion from cell 33 to the dorsal excitor, cell 3. (*a*) Passage of depolarizing current into cell 33 depolarizes cell 3 of the next anterior ganglion. (*b*) Composite superimposed oscilloscope sweeps of recordings taken from cell 33 and cell 3, triggered by impulses of cell 33. Each impulse in cell 33 is followed by an excitatory potential in cell 3, one ganglion and five ganglia more anterior to cell 33, with a constant delay of about 20 and 60 ms, respectively. (*c*) Polarization rhythms of cell 3 and cell 33 during a swim episode.

In addition, cell 33 has inhibitory links to the ipsilateral dorsal inhibitor, cell 1, of its own ganglion and anterior ganglia (Fig. 8). The constant delay before the appearance of the postsynaptic impulses indicates that these links are direct. It is to be noted, however, that passage of depolarizing, or of hyperpolarizing, current into cell 1 is seen to increase, or decrease, respectively, the impulse frequency of cell 33 of its own ganglion. Thus the two cells are linked also by a pathway other than the direct inhibitory connexion. This pathway could be a direct electrical junction between the two cells, but it might also be indirect. For instance, hyperpolarizing current might flow from cell 1 to cell 33 via the known rectifying electrical junctions leading from

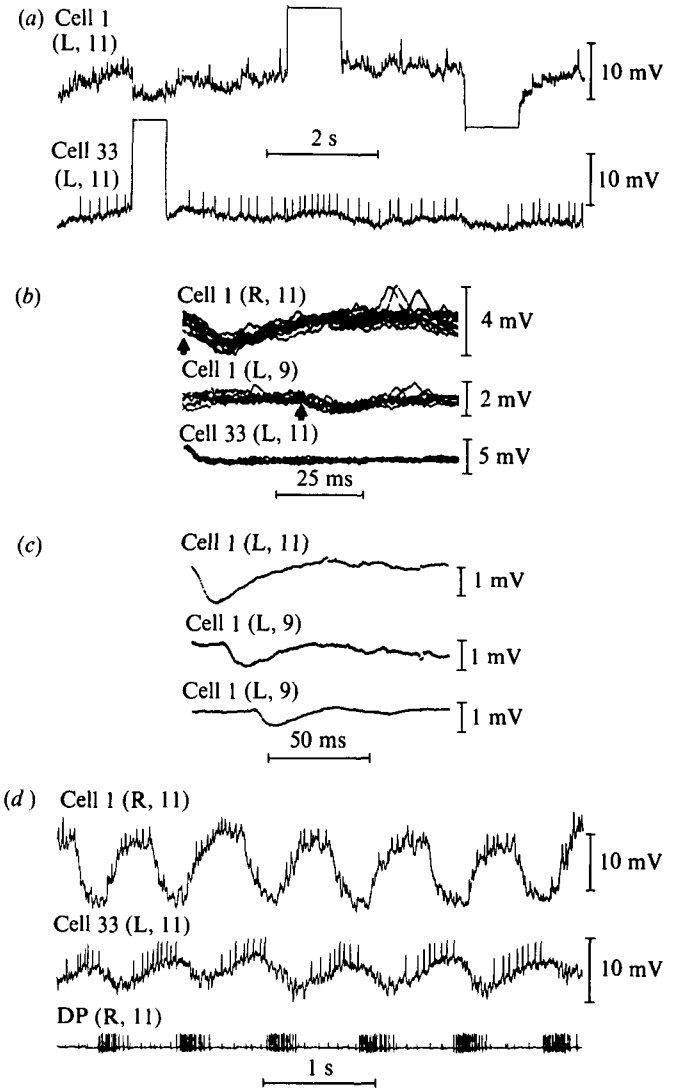

Fig. 8. Inhibitory connexion from cell 33 to the dorsal inhibitor, cell 1. (*a*) Passage of depolarizing current into cell 33 hyperpolarizes cell 1 of the same ganglion. Passage of depolarizing current into cell 1 slightly depolarizes and increases impulse activity of cell 33. Passage of hyperpolarizing current into cell 1 turns off the impulses of cell 33. (*b*) Composite superimposed oscilloscope sweeps of recordings from cell 1 and cell 33, triggered by impulses in cell 33. [The cell 1 (R, 11) trace was triggered by impulses in cell 33 (R, 11), whereas the cell 1 (L, 9) trace was triggered by impulses in cell 33 (L, 11), as shown]. Each impulse of cell 33 is followed by an inhibitory potential in cell 1 of the same ganglion and two ganglia more anterior to cell 33, with a constant delay of less than 5 ms and about 40 ms, respectively. (*c*) Computer averaged signal recorded in cell 1 homologues in three different ganglia, triggered by cell 33 impulses showing a progression of the delay between impulse and inhibitory potential. The inhibitory potential in the top trace is triggered by cell 33 (R, 11), the middle trace by cell 33 (L, 10) and the bottom trace by cell 33 (L, 11). (*d*) Polarization rhythms of cell 1 and cell 33 during a swim episode.

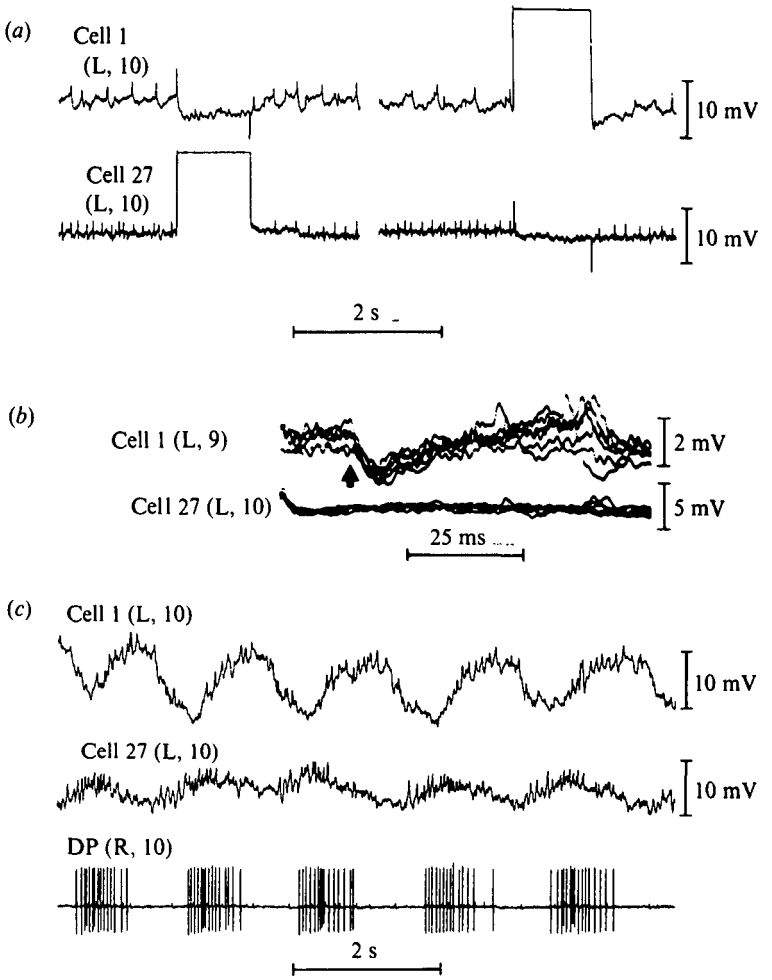

Fig. 9. Inhibitory connexion from cell 27 to the dorsal inhibitor, cell 1. (*a*) Passage of depolarizing current into cell 27 hyperpolarizes cell 1 of the same ganglion. (*b*) Composite superimposed oscilloscope sweeps of recordings taken from cell 27 and cell 1 of the next anterior ganglion. Each impulse of cell 27 is followed by an inhibitory potential in cell 1 with a constant delay of about 20 ms. (*c*) Polarization rhythms of cell 1 and cell 27 during a swimming episode.

cell 1 to cell 28 and onward from cell 28 to cell 33. However, no indirect pathway is as yet known that would readily account for the passage of depolarizing current from cell 1 to cell 33. The records taken during a swim episode confirm the previous observation that the oscillation of cell 1 leads that of cell 33 by about 90°.

(4) *Output of the interneurone, cell 27*

Like cell 33, cell 27 is linked by inhibitory connexions to cell 1 homologues of its own ganglion and of anterior ganglia (Fig. 9). However, unlike for cell 33, the connexion might not be direct, since individual cell 27 impulses are not followed with constant delay by an inhibitory synaptic potential in the homoganglionic cell 1. Cell 27 also differs from cell 33 in that it undergoes a reduction, not an increase, in impulse

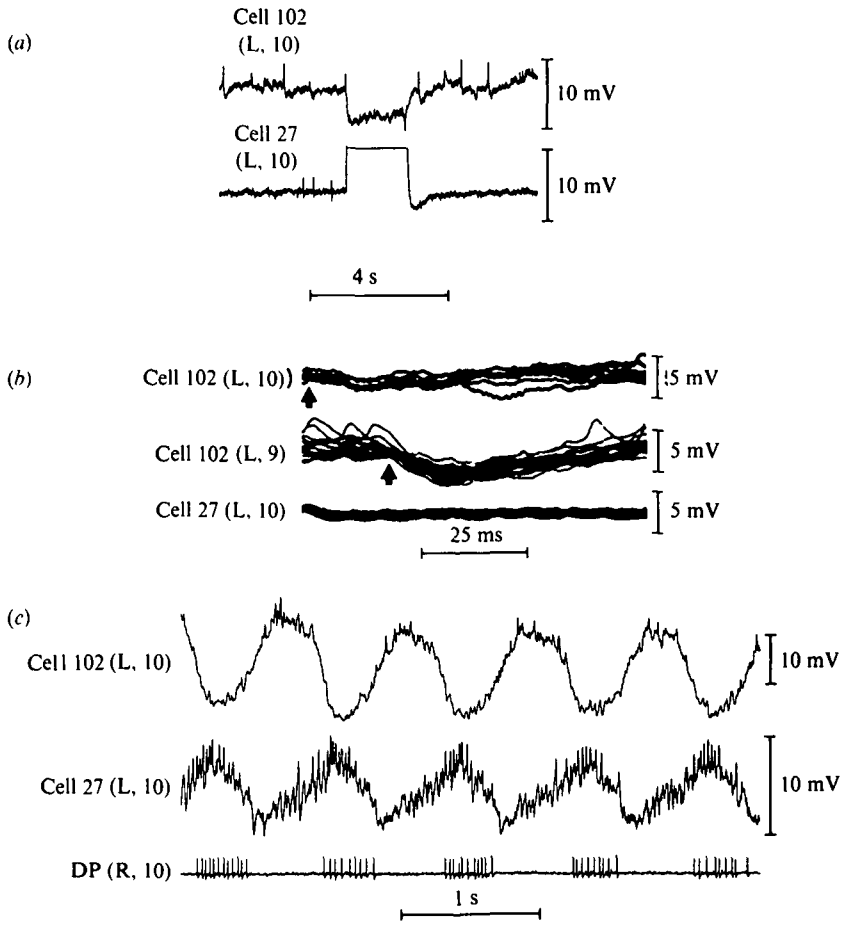

Fig. 10. Inhibitory connexion from cell 27 to the dorsal inhibitor, cell 102. (*a*) Passage of depolarizing current into cell 27 hyperpolarizes cell 102 of the same ganglion. (*b*) Composite superimposed oscilloscope sweeps of recordings taken from cell 27 and cell 102. Each impulse of cell 27 is followed by an inhibitory potential in cell 102 of the same and the next anterior ganglion, with a constant delay of less than 5 ms and about 20 ms, respectively. (*c*) Polarization rhythms of cell 102 and cell 27 during a swim episode.

frequency following depolarization of cell 1. Thus there exists an inhibitory pathway, possibly indirect, leading back from the dorsal inhibitor cell 1 to the oscillatory interneurone, cell 27. This inhibitory pathway is in agreement with the observation that the oscillations of the two cells are separated by a phase angle of 180° (Fig. 1).

Cell 27 also has an inhibitory connexion to the ipsilateral dorsal inhibitor, cell 102, of the same ganglion (Fig. 10). Records not presented here show also a similar inhibition of the cell 102 homologue of the next anterior ganglion. Delay times indicate the links to be direct. The records agree with the previous observation that the polarization rhythm of cell 102 leads that of cell 27 by a phase angle of about 90° (Fig. 1).

A similar phase angle is found between the rhythms of cell 27 and the ipsilateral dorsal excitor, cell 3, of the next anterior ganglion, but here the connexion is excita-

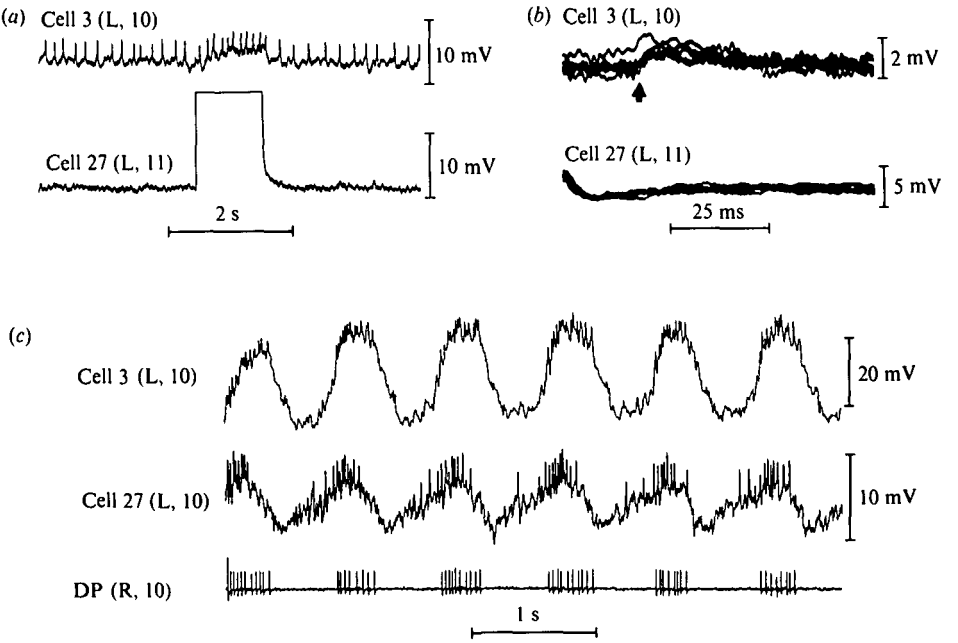

Fig. 11. Excitatory connexion from cell 27 to the dorsal excitor, cell 3. (*a*) Passage of depolarizing current into cell 27 depolarizes and increases impulse activity of cell 3 of next anterior ganglion. (*b*) Composite superimposed oscilloscope sweeps of recordings taken from cell 27 and cell 3. Each impulse of cell 27 is followed by an excitatory potential in cell 3 of the next anterior ganglion, with a constant delay of about 25 ms. (*c*) Polarization rhythms of cell 3 and cell 27 during a swim episode.

tory (Fig. 11). The constant delay with which impulses in cell 27 are followed by postsynaptic potentials indicates that this connexion is direct.

(5) *The exceptional dorsal inhibitor, cell* 1

The dorsal inhibitor, cell 1, has the exceptional property for a motor neurone that passage of depolarizing current into it can shift the phase of the swimming rhythm in the entire nerve cord (Kristan & Calabrese, 1976). All other known leech motor neurones lack this feature (Ort *et al.* 1974), which has been used as the criterion for identifying a cell as a candidate component of the central swimming oscillator (Friesen *et al.* 1976, 1978). Accordingly, prior to the discovery of the oscillatory interneurones, cell 1 was put forward as the first identified candidate for an oscillator component (Kristan & Stent, 1976). To ascertain whether this unusual property of the dorsal inhibitor is reflected in its anatomy, cell 1 was stained by intracellular injection of horseradish peroxidase (Muller & McMahan, 1971), as shown in Fig. 12. In accord with previous fine-structural visualizations of motor neurones to the longitudinal musculature (Muller & McMahan, 1976), cell 1 is seen to be a monopolar neurone whose processes form an extensive, bilateral arborization in the neuropil and whose axon projects peripherally via the contralateral segmental nerves. In further accord with earlier physiological findings (Ort *et al.* 1974), the axon of cell 1 enters the root of the contralateral posterior nerve. However, most importantly for the present con-

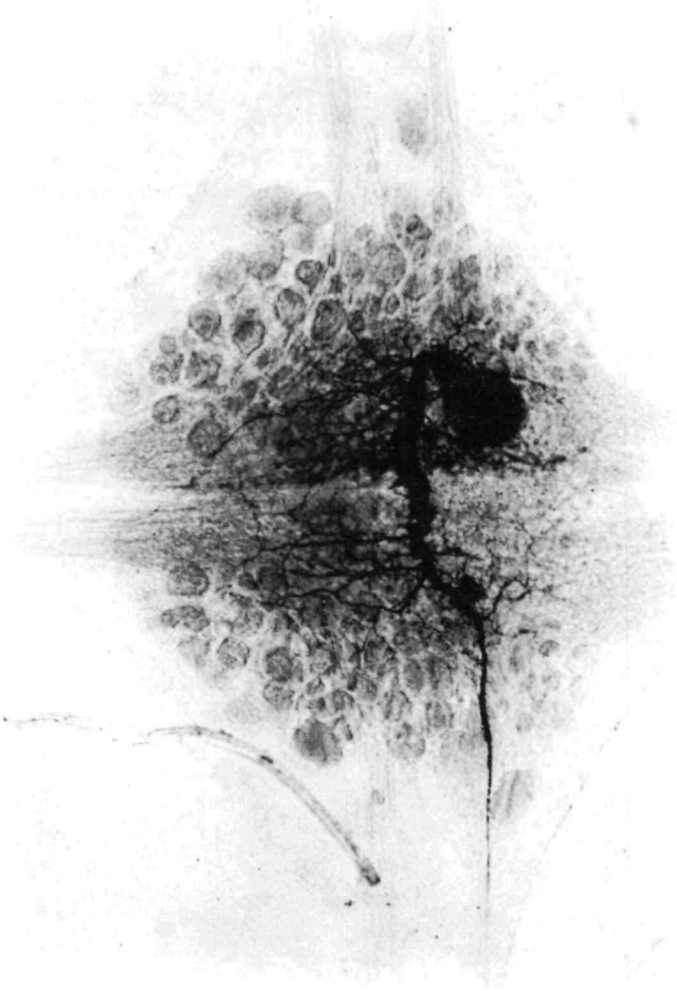

Fig. 12. Photomicrograph of the dorsal inhibitor cell 1, stained by intracellular injection of horseradish peroxidase. The axon of the motor neurone is seen to exit to the periphery via the segmental nerve. The anterior connective is at the left edge of this picture.

siderations, there is no indication that any process of cell 1 projects into either anterior or posterior connectives, a characteristic of the osillatory interneurones (Friesen *et al.* 1978). Hence the anatomy of cell 1 is that of an ordinary motor neurone. Its nerve-cord-wide effects on the swimming rhythm must therefore be attributable to indirect connexions via neurones whose axons project intersegmentally.

Such indirect connexions could be those to the oscillatory interneurones brought to light by the present study. For as was seen in Figs. 5, 8 and 9, transient passage of depolarizing current into cell 1 depolarizes cells 28 and 33, and hyperpolarizes cell 27. Hence such evoked depolarization of cell 1 can readily cause a transient arrest of the swim cycle by its effect on the oscillatory interneurone network. Consequently, cell 1 need no longer be considered as a component element of the central swim oscillator. But this exclusion is quite arbitrary. It is justified merely by the recurrent cyclic inhibition model by which the oscillation of the interneuronal network has been explained and in which cell 1 plays no necessary part. In any case, even if cell 1 is not an integral part of the central swim oscillator, it differs from all other known motor neurones in having access to the component elements of the oscillator.

DISCUSSION

The identified direct intra- and interganglionic output connexions from oscillatory interneurones to motor neurones participating in the swimming rhythm have been summarized schematically in Fig 13. As can be seen, cell 123 has the most sparse output: it merely provides excitation to the dorsal excitor cell 3 homologues of posterior ganglia. In line with its being the only oscillatory cell with a rearward projecting axon, cell 123 is also the only interneurone that provides direct output to posterior motor neurones. The other three interneurones, cell 28, cell 33 and cell 27, provide some output to motor neurones of both their own ganglion and of anterior ganglia. Moreover, their output to the motor neurone ensemble is both excitatory as well as inhibitory.

Although a frontward output to the anterior motor neurones from oscillator interneurones has been identified over a distance of five segments only for the excitatory connexions from cell 28 to cell 4 and from cell 33 to cell 3, it seems likely that the frontward excitatory connexion from cell 28 to cell 102 and the frontward inhibitory connexion from cell 33 to cell 1 similarly extend for at least five segments. Moreover, since the axon of cell 27 projects frontward for at least four segments (Friesen *et al.* 1978), it seems likely that the frontward connexions of cell 27 to cells 1, 3 and 102, so far demonstrated to reach only the next anterior ganglion, also extend for at least four, if not five, segments. Finally, since the axon of cell 123 projects rearward for at least two segments (Friesen *et al.* 1978), it seems likely that the excitatory link from cell 123 to posterior cell 3 homologues so far demonstrated to reach only the next posterior ganglion is iterated in a series of posterior ganglia.

Consideration of the data in Fig. 13 shows that generalizations may be made about the manner in which the motor neurones are driven into the swimming activity rhythm. First, with the possible exception of cell 2, all motor neurones receive phasic synaptic input from more than one cell. Second, the inhibitory motor neurones receive both excitatory and inhibitory phasic input from the oscillator interneurones.

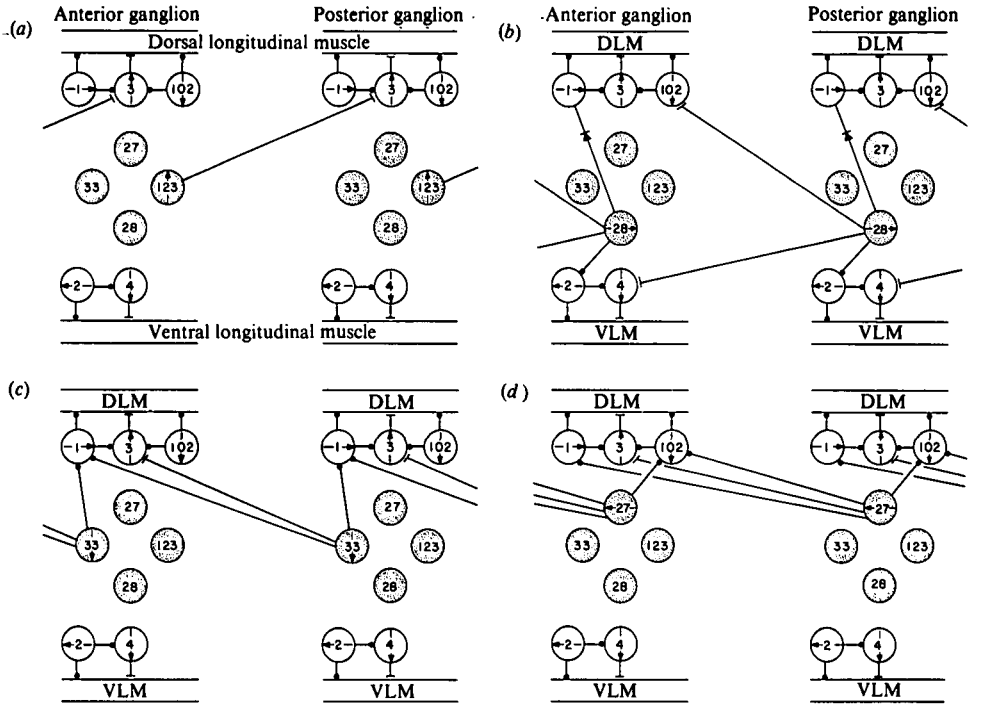

Fig. 13. Summary diagram of the direct output of the oscillator interneurones, cells 123, 28, 33 and 27. The arrow indicates the phase angle of the impulse burst midpoint of each cell: upward and downward pointing arrows signify 0° and 180°, respectively. Left and right pointing arrows signify 270° and 90°, respectively. Meaning of symbols: T joint = excitatory synapse; filled circle = inhibitory synapse; diode = rectifying electrical junction.

Third, the excitatory motor neurones receive only excitatory phasic input from the oscillator interneurones, with their phasic inhibitory input being provided by the inhibitory motor neurones innervating the same longitudinal muscles. The observed phase relations and durations of the bursts of impulses of the motor neurones can now be accounted for on the basis of the phases of the cycles of activity of the oscillator interneurones, the connexions of those interneurones with the motor neurones, and the connexions of the inhibitory motor neurones with the excitatory motor neurones (Figs. 1 and 13).

The dorsal inhibitor, cell 1, receives excitatory input from cell 28, and inhibitory input from cells 33 and 27. Thus, while cell 28 is active, it depolarizes cell 1, but as cell 33 enters its active phase, its output begins to repolarize and eventually terminates the active phase of cell 1. As cell 27 enters its active phase, it joins cell 33 in the hyperpolarization of cell 1, and cell 1 remains in its active phase until the most posterior of the cell 27 serial homologues which provide inhibitory input to cell 1 has entered its own inactive phase. Upon release from inhibition by cell 27, cell 1 begins to receive excitatory input from cell 28, and thus produces its impulse burst. The impulse burst of the dorsal inhibitor, cell 102, is initiated and maintained by the *inter*ganglionic excitatory input from cell 28. The active phase of cell 102 is terminated and the inactive phase maintained by *intra-* and *inter*ganglionic inhibitory input from the posterior serial homologues of cell 27. The active phase of the dorsal excitor, cell 3,

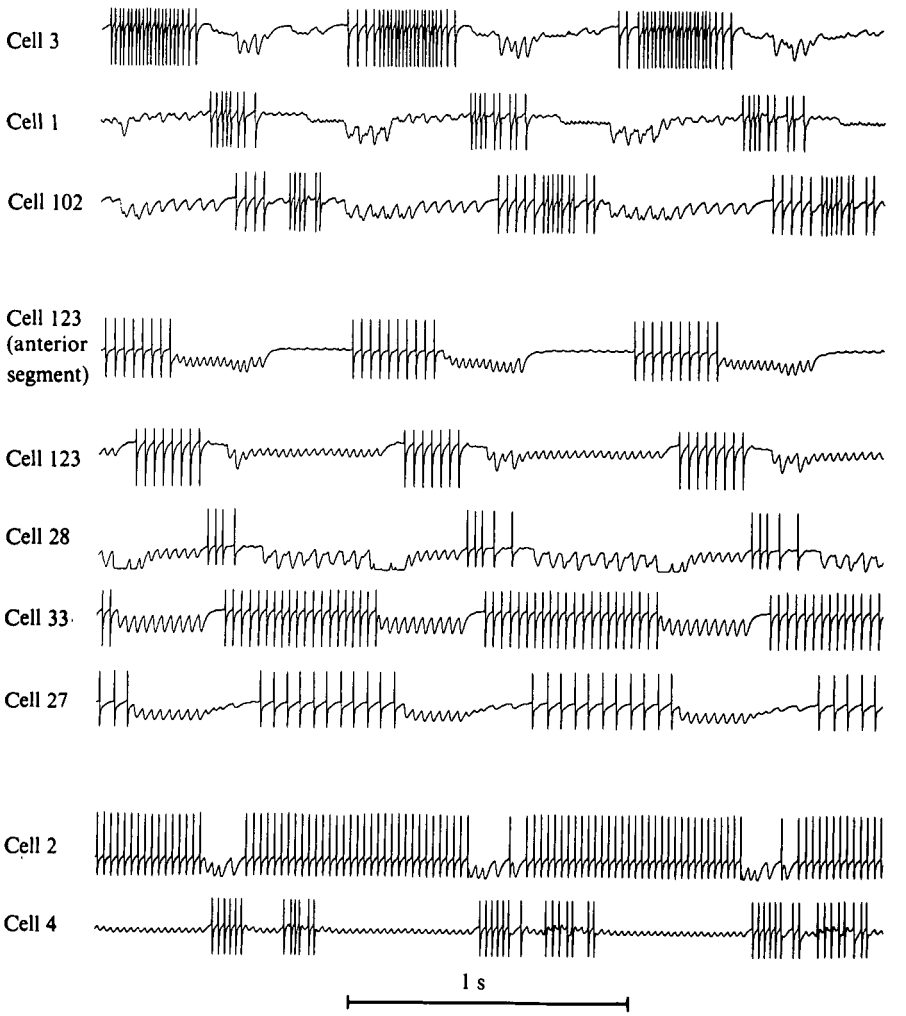

Fig. 14. Generation of the motor neurone rhythm by an electronic analogue model. Impulse bursts generated by five neuromimes representing the segmental motor neurones, cells 1, 2, 3,4 and 102. The motor neurone analogues are driven by play-back of a tape-recorded impulse burst rhythm of neuromimes representing the oscillatory interneurones, cells 123, 28, 33 and 27 of one ganglion and cell 123 in a ganglion four segments to the anterior. The neuromimes are linked to each other and to the appropriate output terminals of the tape recorder via the connexions indicated in Fig. 13. To mimic the interganglionic synaptic inputs reaching a motor neurone from oscillator neurones of a ganglion more posterior by four segments, the connexion from the appropriate tape recorder terminal to the synaptic input terminal of the motor neurone analogue included a transmission delay of 270 ms, so as to mimic both a phase delay of 190 ms and an intersegmental conduction time of $4 \times 20 = 80$ ms. To mimic the excitatory input reaching cell 3 from a cell 123 homologue four segments to the anterior, the cell 3 analogue was connected to the appropriate tape recorder terminal with a transmission delay of 80 ms. The impulse transmission delays were modelled by means of a shift register. Sufficient tonic excitation was provided to each motor neurone analogue to produce an impulse frequency of about 60 Hz at the height of its active phase. The level of tonic excitation needed to achieve this impulse frequency was different for different motor neurone analogues, because of differences in excitatory synaptic input provided to them by the interneuronal network. For instance, the cell 3 analogue, which (according to Fig. 13) receives excitation from anterior cells 123 and from posterior cells 27 and 33, was not provided with any tonic excitation at all, whereas the cell 2 analogue, for which no excitatory synaptic input has yet been identified, had to be provided with maximum tonic excitation.

is brought on and maintained by the interganglionic phasic excitatory input from both cells 27 and 123. The inactive phase of cell 3 is brought on by hyperpolarization from the dorsal inhibitor, cell 1, and maintained by input from dorsal inhibitor, cell 102. The impulse burst of the ventral excitor, cell 4, is initiated and maintained by the interganglionic phasic excitatory input provided by the serial homologues of cell 28. The impulse burst of cell 4 is terminated by the active phase of the ventral inhibitor, cell 2.

The ventral inhibitor, cell 2, is the only motor neurone that does not have any known excitatory input. Thus cell 2 derives its excitation either from an endogenous source of membrane depolarization or from an as yet unidentified exogenous source. Cell 2 receives phasic inhibitory input from cell 28.

To ascertain whether the foregoing considerations give a reasonable account of the generation of the actual motor neurone rhythm, an analogue circuit of the identified neuronal network was constructed according to the connexions diagrammed in Fig. 13. This circuit was intended to model an ensemble of dorsal and ventral excitors and inhibitors, cells 1, 2, 3, 4 and 102 of a single ganglion which receive both intra- as well as interganglionic excitatory and inhibitory synaptic input from oscillatory interneurones. To simplify the analogue circuitry, the *inter*ganglionic synaptic input was modelled to originate from only a single, 'average' ganglion, either four segments to the anterior in the case of cell 123, or four segments to the rear in the case of cells 28, 33 and 27. In the analogue circuit, each of the five motor neurones was represented by an electronic 'neuromime' element (Lewis, 1968), as described in the preceding paper of this series (Friesen *et al.* 1978). Rhythmic synaptic input for the five motor neurone analogues was provided by tape recordings of synthesized rhythmic bursts of impulses like those from oscillatory interneurones, cells 123, 28, 33 and 27 of one ganglion and cell 123 of a ganglion four segments anterior. (The synthesis was by an electronic analogue of the oscillatory interneurone network.)

The output of the five motor neurone analogues, as well as the pre-recorded output of the interneurone analogues driving the system, is presented in Fig. 14. As can be seen, the entire model network runs with a cycle period of about 1000 ms, and the phase relations of the impulse bursts of the analogues of both inter- and motor neurones bear a striking resemblance to the actual swimming rhythm of their real counterparts, as shown in Fig. 1. It should be noted, however, that in the model output the active phase of cell 2 lasts much longer than in the swimming rhythm. This is attributable to the model cell 2 receiving inhibition only from cell 28 of its own ganglion, whose active phase is the briefest of any interneurone. This makes it likely that cell 2 receives some additional, as yet unidentified synaptic inputs. However, the verisimilitude of the function of the analogue model of the motor neurone ensemble would make it appear that the identified output connexions summarized in Fig. 13 are very nearly adequate to allow the oscillatory interneurones to drive the motor neurones into the swimming rhythm.

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