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NEURONAL CONTROL OF SWIMMING IN THE MEDICINAL LEECH

IV. IDENTIFICATION OF A NETWORK OF OSCILLATORY INTERNEURONES

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SUMMARY

Four oscillatory interneurones that appear to be the principal components of the central swim oscillator of *Hirudo medicinalis* have been identified on each side of the segmental ganglia of the ventral nerve cord. During 'swimming' episodes of an isolated nerve cord preparation each interneurone undergoes a polarization rhythm that is phase-locked with the impulse burst rhythm of the motor neurones known to drive the swimming movement. Passage of current into any of the interneurones can shift the phase of the swim rhythm. One of the interneurones projects its axon rearward to posterior ganglia and the other three project their axons frontward to anterior ganglia. The oscillatory interneurones are connected both intra- and interganglionically to form a topologically complex intersegmental network of concatenated ring circuits that possess the feature of recurrent cyclic inhibition. Theoretical analysis and electronic analogue models show that the network is inherently oscillatory and can produce both a cycle period and intra- and intersegmental phase relations of its elements that are appropriate for generating the body wave of the swimming movement.

INTRODUCTION

Leeches swim by undulating their extended and flattened body in the dorsoventral direction, to form a wave that travels backward along the animal. The troughs and crests of this body wave are produced by a metachronal rhythm of contraction-extension cycles of the dorsal and ventral longitudinal muscles in the body wall of each of the 21 abdominal body segments lying between the head and the tail sucker. The period of this rhythm varies from about 400 to 2000 ms. The preceding papers of this series (Kristan, Stent & Ort, 1974*a*, *b*; Ort, Kristan & Stent, 1974) have shown that the swimming rhythm of the medicinal leech *Hirudo medicinalis* is controlled by an

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ensemble of bilateral pairs of excitatory and inhibitory motor neurones in each ganglion of the ventral nerve cord. This neuronal ensemble includes excitors (cells 3, 5, 7 and 107) and inhibitors (cells 1 and 102) of the dorsal longitudinal muscles, as well as excitors (cells 4, 8 and 108) and an inhibitor (cell 2) of the ventral longitudinal muscles (Fig. 1). Central inhibitory synaptic connexions link the inhibitors with the excitors. During swimming the membrane potential of these motor neurones oscillates between a depolarized and a hyperpolarized state, with an impulse burst arising during the depolarized state. The phases of the rhythms of the motor neurone activity, as determined by the timing of the median impulse of each impulse burst, and with phase angle 0° arbitrarily assigned to the rhythm of cell 3, are approximately 90° for cell 1, 180° for cells 4 and 102, and 270° for cell 2. Furthermore, in accord with the rearward travel of the body wave, the impulse burst phase of each of these motor neurones leads that of its serial homologue in the next posterior ganglion. Execution of the swimming movement can therefore be accounted for by the activity pattern of the ensemble of identified motor neurones. The motor neurones are not, however, the source of their own activity pattern (Ort, Kristan & Stent, 1974). Instead, the swimming rhythm must be imposed on them by other oscillatory neural elements.

Kristan & Calabrese (1976) discovered recently that the motor neurones of an *isolated* leech ventral nerve cord can exhibit sustained episodes of the swimming rhythm. The motor neurones of an isolated ganglion do not, however, manifest that rhythm: a chain of at least six to eight ganglia appears to be required. Thus the central nervous system of the leech contains a *central swim oscillator*, whose intra- and interganglionic connexions can drive the motor neurones to produce periodic impulse bursts in the absence of any phasic sensory input. This report presents the identification of a set of interconnected interneurones that appears to make up the principal components of the central swim oscillator. A preliminary account of these findings has been published previously (Friesen, Poon & Stent, 1976).

MATERIALS AND METHODS

Specimens of *Hirudo medicinalis* were obtained from a commercial supplier and maintained in aquaria at 15 °C. The animals were allowed to feed on a bull frog or rabbit blood donor about once a month.

The nerve cord was isolated from the leech by a procedure similar to that previously described (Kristan & Calabrese, 1976). The preparation was pinned to the bottom of a chamber that provides for dark field illumination of three adjacent ganglia. The chamber was filled with leech physiological saline (Nicholls & Purves, 1970) and maintained at 15 °C by a cooling system.

The electrophysiological methods 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 passing current into nerve cells and electrical stimulation of nerves, and of numbering segments and designating the components of the segmental nerve system were those previously described (Kristan *et al.* 1974*a*; Ort *et al.* 1974; Kristan & Calabrese, 1976).

In isolated leech nerve cord preparations, there often occur spontaneous contractions of the muscle fibres imbedded in the connective tissue sheath enclosing the ganglia

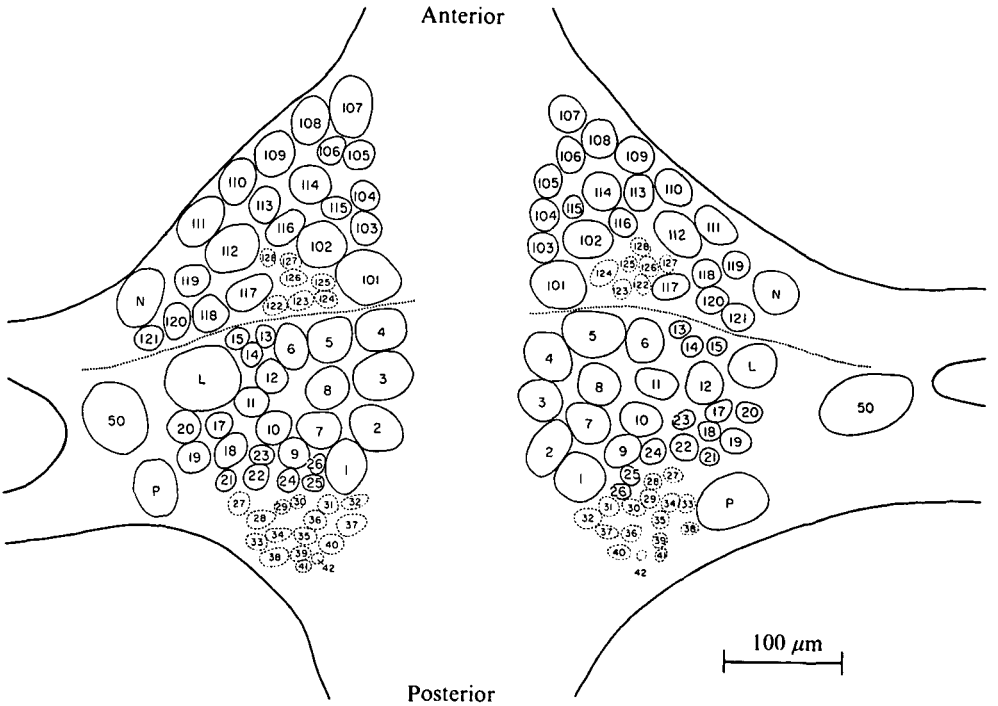

Fig. 1. Dorsal aspect of a segmental ganglion of the ventral nerve cord of the medicinal leech, *H. medicinalis*, showing the approximate location of cell bodies of identified motor neurones and interneurones taking part in the generation of the swimming rhythm. The cells are numbered according to the system of Ort *et al.* (1974). Cells with dashed outlines represent neurones whose location is subject to more variation than that of the cells with solid outlines. The dashed line traversing the ganglion is the packet margin.

These contractions dislodge the microelectrodes and make intracellular recording impossible. However, these contractions are abolished by bathing the preparation for 10 min in physiological saline containing 40 mM-Mg^{2+} and then returning the preparation to normal saline. Furthermore, such transient exposure to 40 mM-Mg^{2+} causes the isolated cord to produce a much more vigorous swimming rhythm. The reasons underlying these effects of transient exposure to high Mg^{2+} concentrations are as yet unexplained.

RESULTS

(1) *Identification of the oscillatory cells*

A search was carried out in segmental ganglia of the isolated ventral nerve cord of *H. medicinalis* for the component neurones of the central swim oscillator. A neurone was considered to be a candidate component if, during a swimming episode of the preparation, (1) its cell membrane underwent a polarization rhythm that was phase-locked with the rhythm of the bursts of motor neurone impulses; and (2) passage of current into the neurone shifted the phase of the latter rhythm. All previously identified neurones that meet the first of these two criteria fail the second (with the exception of the dorsal inhibitor, cell 1, to be considered further in the following

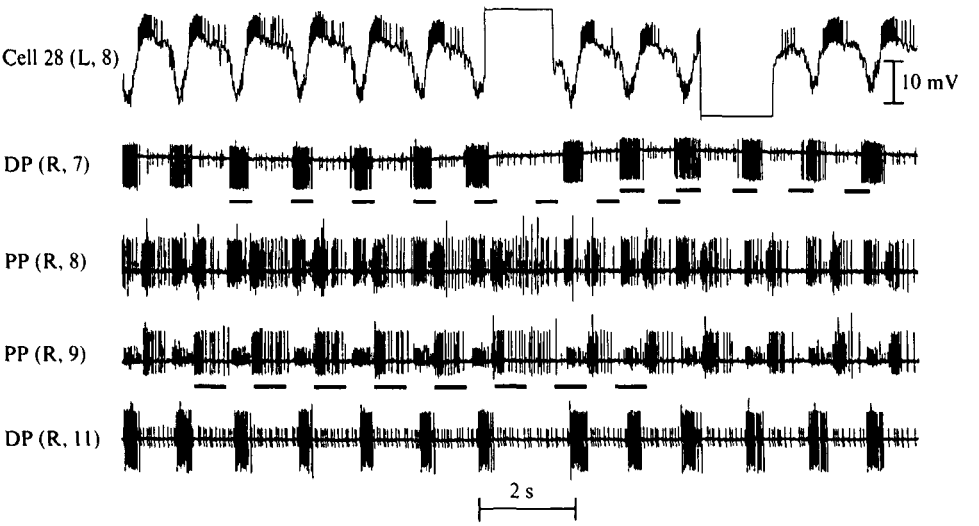

Fig. 2. Identification of the oscillator component, cell 28. Preparation swimming. Top trace: intracellular recording taken from cell 28. Traces labelled DP and PP: concurrent extracellular suction electrode recordings taken from the dorsal and posterior branches, respectively, of a posterior segmental nerve. In the DP traces the large amplitude spikes represent impulses of the dorsal excitor, cell 3. In the PP traces, the spikes whose bursts occur in phase with the cell 3 spike bursts of the DP records represent impulses of the dorsal excitors cells 5 and 7, whereas the spikes whose bursts occur out of phase with those of cell 3 represent impulses of the ventral excitors, cells 4 and 8. The bars drawn under the DP and PP traces indicate the times of occurrence of impulse bursts of cell 3 and of cells 4 and 8, respectively, to be expected if passage of current into the oscillator cell had *not* shifted the phase of the swimming rhythm. In this, and in all other electrophysiological records presented in this paper, the letters R and 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 the passage of depolarizing or hyperpolarizing current, respectively, of no more than 5×10^{-9} A into the cell.

paper of this series), and hence do not qualify as candidate components of the central oscillator. After an extensive survey of the segmental ganglia, which included most of the cells on both dorsal and ventral aspects, four bilateral pairs of neurones were found to meet both criteria. They are the right and left homologues of cells 123, 28, 33 and 27, all located on the dorsal aspect (Fig. 1).

Fig. 2 displays an intracellular recording from the left cell 28 of the 8th abdominal ganglion during a swimming episode of an isolated nerve cord preparation containing 18 ganglia. The figure presents also the concurrent output of suction electrodes attached to the dorsal and posterior branches of the posterior segmental nerve (DP and PP nerves, respectively) issuing from the 7th ,8th, 9th and 11th ganglia. The initial part of the record shows seven cycles of bursts of impulses characteristic of the swimming rhythm. The bursts recorded from the DP nerve represent the efferent activity of the dorsal excitor, cell 3, whereas the bursts recorded from the PP nerve represent the efferent activity of the dorsal excitors, cells 5 and 7, and of the ventral excitors, cells 4 and 8. The membrane potential of cell 28 oscillates in a rhythm that is phase-locked with the motor neurone bursts, with the cell producing a burst of impulses during its depolarized phase. Based on the arbitrary assignment of phas

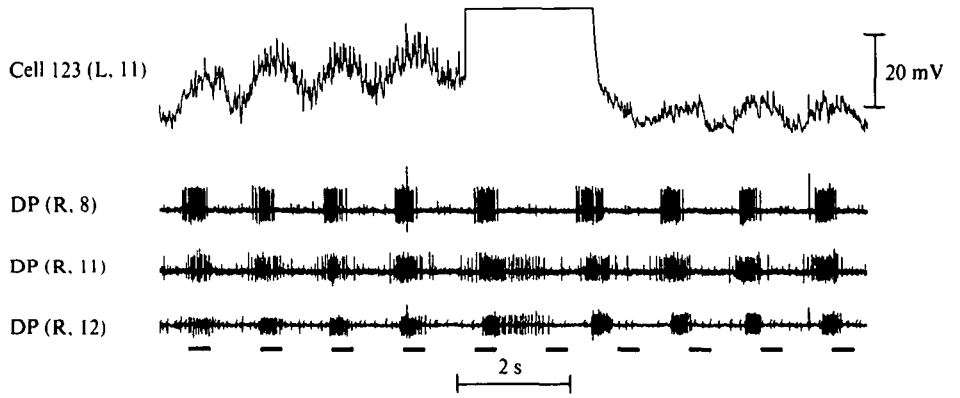

Fig. 3. Identification of the oscillator component, cell 123. Preparation swimming. Top trace: intracellular recording taken from cell 123. Traces labelled DP: as in Fig. 2.

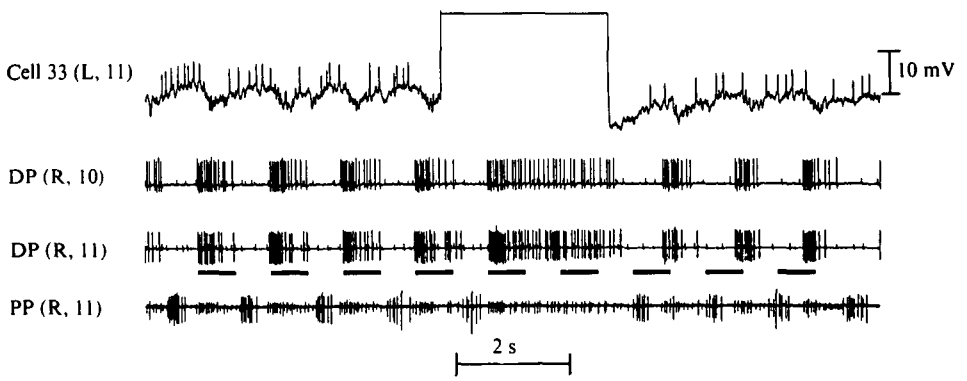

Fig. 4. Identification of the oscillatory component, cell 33. Preparation swimming. Top trace: intracellular recording taken from cell 33. Traces labelled DP and PP: as in Fig. 2.

angle 0° to the 'midpoint' (median impulse) of the burst of impulses of cell 3 in the swim cycle of any given segment, these records show that the midpoint for cell 28 occurs at a phase angle of approximately 90° in the swim cycle of its own segment. Transient passage of depolarizing current (less than 5 nA) into cell 28 of the 8th ganglion can be seen to affect the swimming rhythm in the 7th, 8th, 9th and 11th ganglia. It arrests the bursts of the dorsal excitors cell 3 (in the DP records) and cells 5 and 7 (in the PP records), while causing tonic activity of the ventral excitors cells 4 and 8 (in the PP records). After termination of current passage, the swimming rhythm resumes, but the phase of the motor neurone bursts has been shifted relative to the rhythm prior to current passage. Passage of hyperpolarizing current into cell 28 can be seen to have only a slight effect, if any, on the rhythm.

Fig. 3, 4 and 5 show similar data for cells 123, 33 and 27 of midbody ganglia of the nerve cord. As with cell 28, the membrane potentials of all these cells oscillate in a rhythm phase-locked with that of the motor neurone bursts, with the midpoint of the bursts from these cells occurring at a phase angle of about 0° for cell 123, 180° for

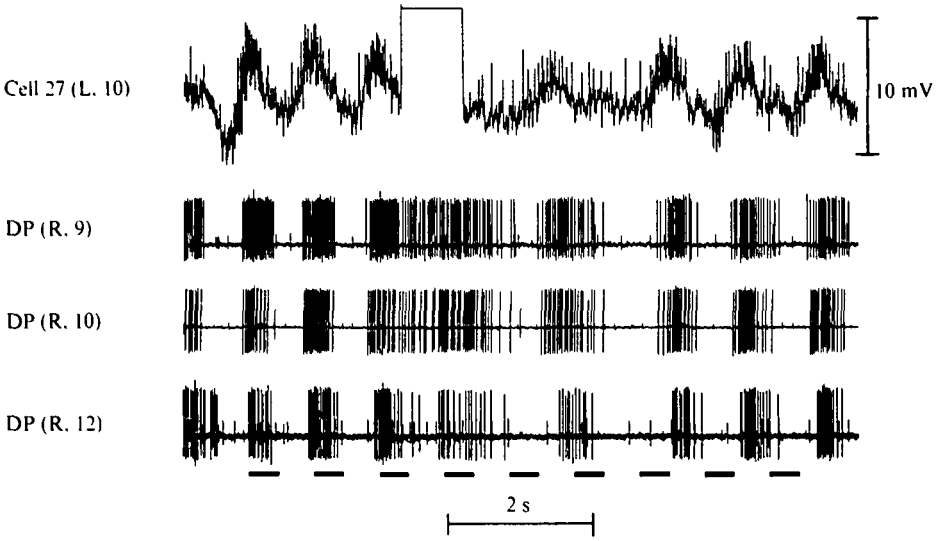

Fig. 5. Identification of the oscillator component, cell 27. Preparation swimming. Top trace: intracellular recording taken from cell 27. Traces labelled DP: as in Fig. 2.

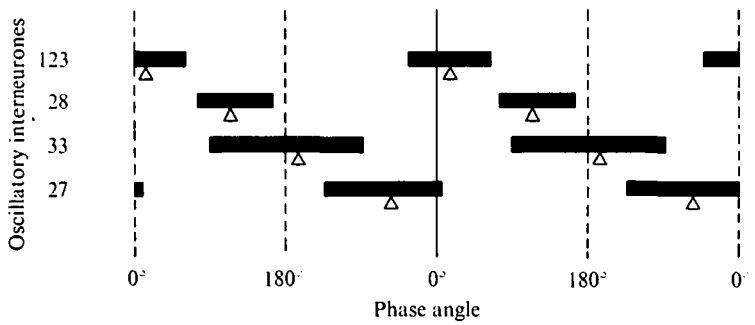

Fig. 6. Phase diagram of the swimming activity cycle of the identified swim oscillator cells. Each bar indicates the duration of the impulse burst of the cell, and the triangle under each bar points to the median spike, or burst 'midpoint'. The designated relative phase angles are based on the arbitrary assignment of the phase angle 0° to the burst midpoint of the dorsal excitor, cell 3.

cell 33 and 270° for cell 27. Also as for cell 28, transient passage of depolarizing current into any of these cells shifts the phase of the entire motor neurone rhythm. However, the transient effects caused by such current passage are not the same for all cells. Thus, evoked depolarization of cell 123 of the 11th ganglion arrests the bursts of the dorsal excitor, cell 3, only in the anterior, 8th ganglion, whereas it prolongs the cell 3 bursts in the 11th and 12th ganglia. This is evidently different from the result found for cell 28, whose depolarization arrests the bursts of dorsal excitors in both anterior and posterior ganglia. In contrast, evoked depolarization of cells 33 and 27 produces tonic activity in the dorsal excitors, or at least prolongs the burst in them, in both anterior and posterior ganglia. (The data for the effect of cell 33 on posterior ganglia are not presented here.) Data not presented here show that transient passage

Table 1. *Identification criteria of the oscillator cells*

Cell no.	Cell packet	Phase angle of impulse burst mid-point*	Impulse burst duration (fraction of total cycle)*	Impulse frequency during burst* (Hz)	Effect of passage of depolarizing current into cell on cell 3 impulse frequency† (non-swimming)	Connexion of cell to cell 1 in own ganglion‡
123	Anterior	0°	0·3	30	↑ Own ↑ Posterior	Unknown
28	Posterior	90°	0·25	40	↓ Own	Type A
33	Posterior	180°	0·5	20	↑ Own ↑ Anterior	Type A and type B
27	Posterior	270°	0·4	30	↑ Own ↑ Anterior	Type B and type C

* Approximate values derived from several records taken at swim cycle periods of 800–1000 ms.

† Upward and downward arrows signify increases and decreases in impulse frequency, respectively; 'own', 'posterior' and 'anterior' refer to the position of the ganglion in which the affected cell 3 is located, relative to the position of the ganglion of the oscillatory cell.

‡ Type A: rectifying electrical junction allowing passage of depolarizing current from oscillator cell to cell 1, and of hyperpolarizing current from cell 1 to oscillator cell. Type B: inhibitory connexion from oscillator cell to cell 1. Type C: inhibitory connexion from cell 1 to oscillator cell. The data substantiating these connexions are provided in the next paper of this series (Poon *et al.* 1978).

of hyperpolarizing current into any of these cells has little or no effect on the rhythm of the motor neurone bursts.

Cells 28, 123, 33 and 27 differ not only in the phase angle of the midpoints of their impulse bursts but also in the relative lengths of their bursts, or depolarized phases during the swim cycle, as shown summarily in Fig. 6. Evidently the longest burst is that of cell 33, which occupies about half the cycle period, whereas the shortest is that of cell 28, which occupies only about one quarter of the period. (The burst midpoint of cell 28 occurs well before the half-time of burst duration, because here the impulse frequency is much higher at the initial than at the final stages of the burst, as can be seen in the record of Fig. 2.)

(2) *Criteria for identifying individual oscillator cells*

It is much more difficult to find and identify a given oscillator cell than a given motor neurone: the four oscillator cells are among the smallest cells of the segmental ganglion, their cell bodies being only about 10 μm in diameter, and the position of these cell bodies, on the dorsal aspect of the ganglion, varies considerably from preparation to preparation (although it varies less from ganglion to ganglion of the same preparation). Hence it was necessary to develop a set of criteria by which each of the oscillator cells can be positively identified and distinguished from some other, nearby neurone that may resemble it in some properties. The criteria developed for the experiments presented in this and the following paper of this series are summarized in Table 1. The first three criteria, namely the phase angle of the midpoint of a burst, and the duration and frequency of a burst, are based on recordings taken during swim episodes of the preparation. The last two criteria, namely the effect of passage of depolarizing current into the cell on the burst frequency of the dorsal excitor, cell 3, and the nature of the connexion of the cell to the dorsal inhibitor, cell 1, are based on

recordings taken while the preparation is at rest. The documentation of the latter two criteria will be provided in the following paper of this series (Poon, Friesen & Stent, 1978). For positive identification, it is essential to take recordings during swim episodes *and* during rest: there are cells in the vicinity of the oscillator cells that pass the tests during the one but not the other condition.

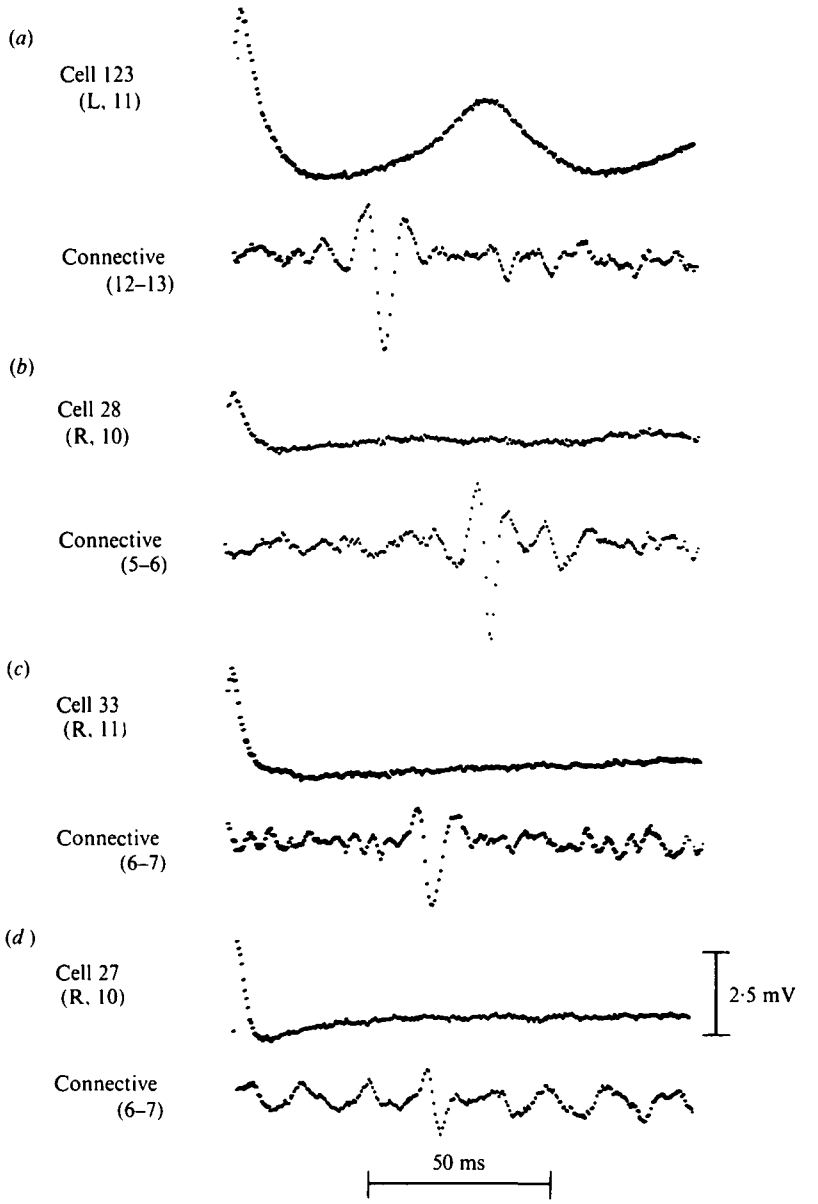

Fig. 7. Computer-averaged signal recorded intracellularly from the connective between the ganglia whose numbers are indicated in parentheses (lower trace of Panels *a–d*), triggered by impulses recorded intracellularly from an oscillator interneurone (upper trace of Panels *a–d*). Each record represents the averaged signal following 100 triggered impulses. These records are photographs of the CRT face of a Northern Scientific, Model 560 computer set for an averaging bin width of 0·5 ms.

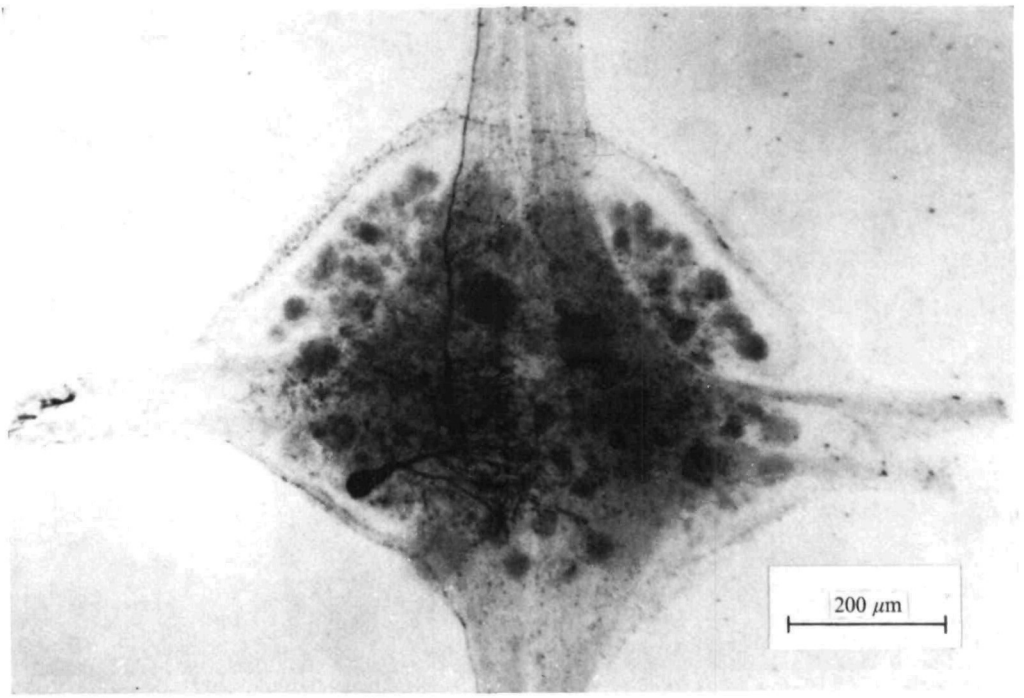

Fig. 8. Anatomy of oscillator interneurone cell 33. Photograph of the dorsal aspect of a ganglion in which the left cell 33 was stained by intracellular injection of horseradish peroxidase. The axon of the monopolar cell is seen to enter the anterior connective at the top of this picture.

(3) *Interneuronal character of the oscillator cells*

Electrophysiological and anatomical studies of the oscillator cells have shown that they are interneurones whose processes project intersegmentally to other ganglia of the nerve cord. First, these cells do not appear to send an axon to the periphery, since no trace of an impulse attributable to them can be found in the segmental nerves. Second, these cells do appear to send an axon into the interganglionic connective, since their intracellularly recorded action potentials can be matched after constant delay with a signal recorded extracellularly via suction electrodes attached to the connective. This is clearly seen after computer averaging of the extracellular records, as shown in Fig. 7. For instance, a signal was recorded between the 12th and the 13th ganglia with a mean delay of about 42 ms after intracellularly recorded impulses in cell 123 of the 11th ganglion. Thus the axon of cell 123 projects *rearward* for a distance of at least two segments. Since no comparable signal was found in similar recordings (not shown here) from the connective between the 10th and 11th ganglion, it can be inferred that the axon of cell 123 does not project frontward. The other records of Fig. 7 show that the axons of cells 28, 33 and 27 project *frontward* for a distance of at least five, five and four segments, respectively. Moreover, similar records not presented here show that the axons of cells 28, 33 and 27 do not project rearward.

Based on the delays between matching impulses evident in the records of Fig. 7, an intersegmental impulse conduction time of about 15 ms per segment can be estimated for the axons of cells 33, 28 and 27, and of about 20 ms per segment for the axon of cell 123. Since these conduction times can vary from preparation to preparation (and from segment to segment), a conduction time of 20 ms per segment will be taken as a representative value for the whole set of oscillator interneurones in subsequent discussions of the implication of these findings.

The anatomy of two identified oscillator cells was examined by means of intracellular injection of horseradish peroxidase, according to the technique of Muller & McMahan (1971, 1976). Stained in this fashion, cell 33 was shown to be monopolar and to have a dendritic tree lying mainly in the ipsilateral neuropile (Fig. 8). In agreement with the electrophysiological findings, the axon of cell 33 is seen to enter the anterior connective, without any trace of a branch entering the segmental nerve roots. A successful staining was also achieved for cell 28, which revealed anatomical features similar to those found for cell 33. No such staining has as yet been accomplished, however, for either of the other two oscillatory cells.

(4) *Connexions of the interneurones*

To ascertain the source of the activity rhythm of the oscillatory interneurones, experiments were designed to reveal the manner in which these cells are interconnected, both *intra-* and *inter*ganglionically. Simultaneous intracellular recordings were taken from pairs of interneurones after both cells had been identified according to the criteria of Table 1. The two cells were inferred to be connected if passage of current into one of them via the inserted microelectrode evoked a change in membrane potential, or impulse frequency, in the other. This test does not, of course, reveal whether a particular connexion is direct or indirect, i.e. whether it is mono- or polysynaptic. It was also difficult to penetrate a given interneuronal cell pair at will,

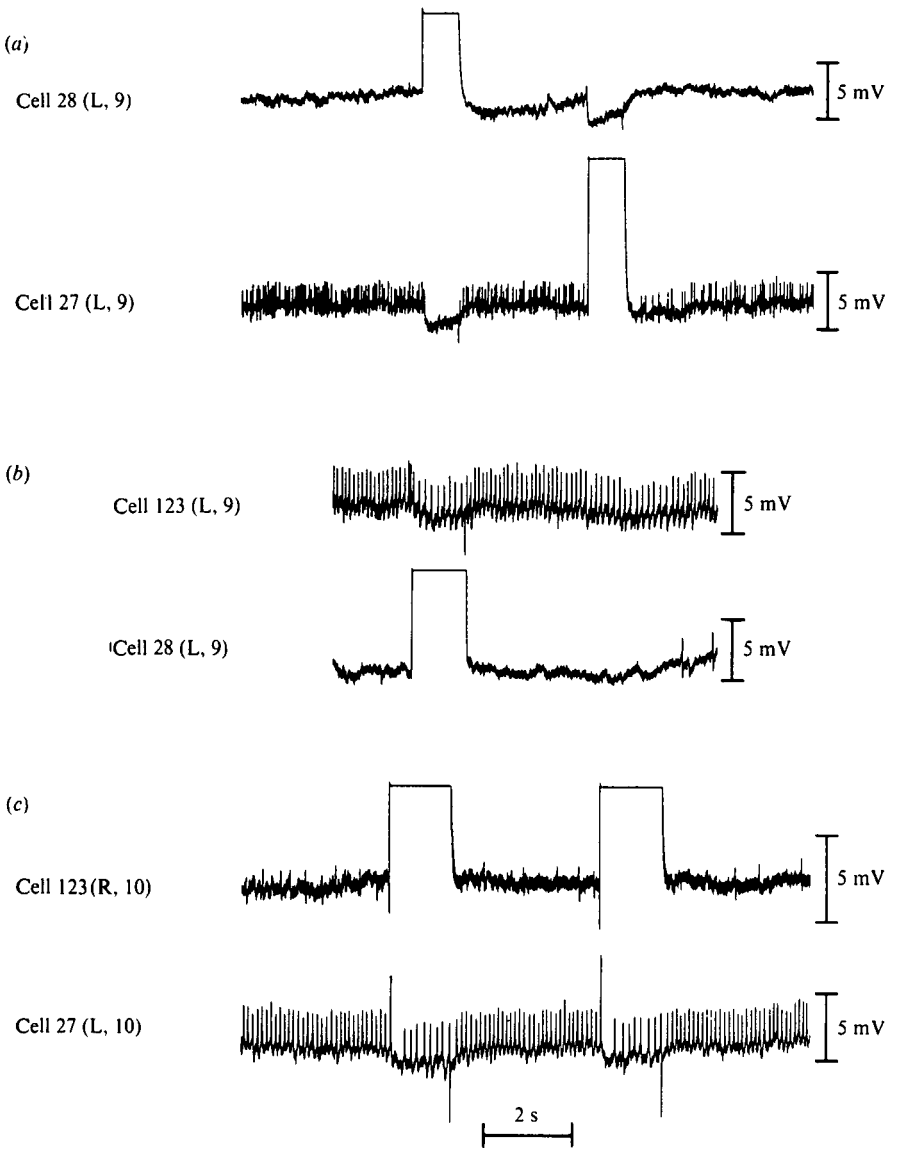

Fig. 9. Intraganglionic connexions between oscillator interneurones. Records taken while preparation is not swimming. (*a*) Reciprocal inhibitory connexion between cell 28 and cell 27. Passage of depolarizing current into either member of the cell pair hyperpolarizes the other member. (*b*) Inhibitory connexion from cell 28 to cell 123. Passage of depolarizing current into cell 28 hyperpolarizes and decreases impulse frequency of cell 123. (*c*) Inhibitory connexion from cell 123 to cell 27. Passage of depolarizing current into cell 123 hyperpolarizes and reduces the impulse frequency of cell 27.

particularly if the pair consisted of two adjacent cells in the same ganglion; so many of the connexions of the network reported here are each based on observations made with a single pairwise penetration. However, replicate observations were made for the reported connexions between cells 27, 28 and 33. The characteristics of our electrophysiological apparatus made it difficult to secure simultaneous intracellular recordings

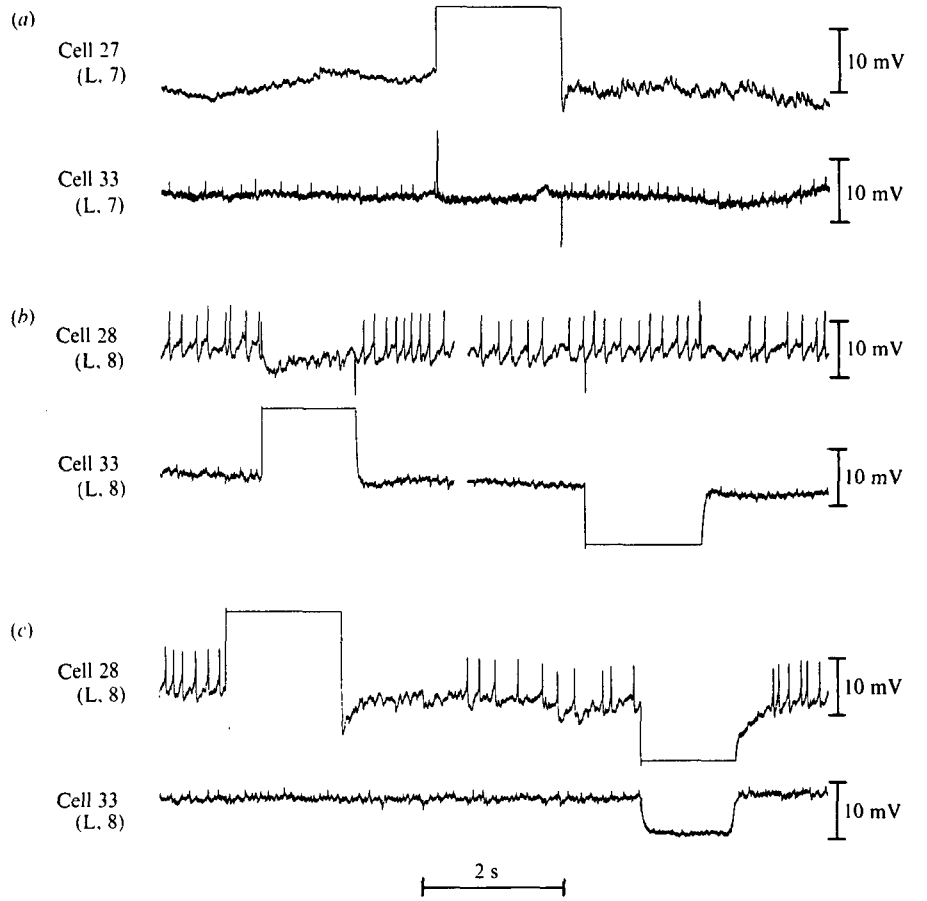

Fig. 10. Intraganglionic connexions between oscillatory interneurones. Records taken while preparation is not swimming. (*a*) Intraganglionic inhibitory connexion from cell 27 to cell 33. Passage of depolarizing current into cell 27 arrests impulse activity in cell 33. The large amplitude spikes in the cell 33 record are stimulus artifacts. (*b*) Intraganglionic inhibitory connexion from cell 33 to cell 28. Passage of depolarizing current into cell 33 hyperpolarizes cell 28, and postinhibitory rebound of cell 33 upon the termination of passage of hyperpolarizing current causes transient arrest of impulses in cell 28. (*c*) Rectifying electrical junction linking cell 33 and cell 28. Passage of depolarizing current into cell 28 has no effect on cell 33. Passage of hyperpolarizing current into cell 28 strongly hyperpolarizes cell 33.

from cells in different ganglia, other than adjacent ganglia. Hence most of the interganglionic connexions were established by this method only for a distance of a single segment, and the remainder for a distance of two segments.

Intraganglionic connexions were as follows. There were reciprocal inhibitory connexions between cells 28 and 27 (Fig. 9*a*), and an inhibitory connexion from cell 28 to cell 123 (Fig. 9*b*) with no reciprocal connexion (as indicated by an unshown continuation of the record of Fig. 9*b*). There was an inhibitory connexion from cell 123 to cell 27. (Fig. 9*c*; a record taken from a *contralateral* cell pair. The possible significance will be considered below.) Fig. 10*a* demonstrates the existence of an inhibitory connexion from cell 27 to cell 33. According to records not shown here, passage of

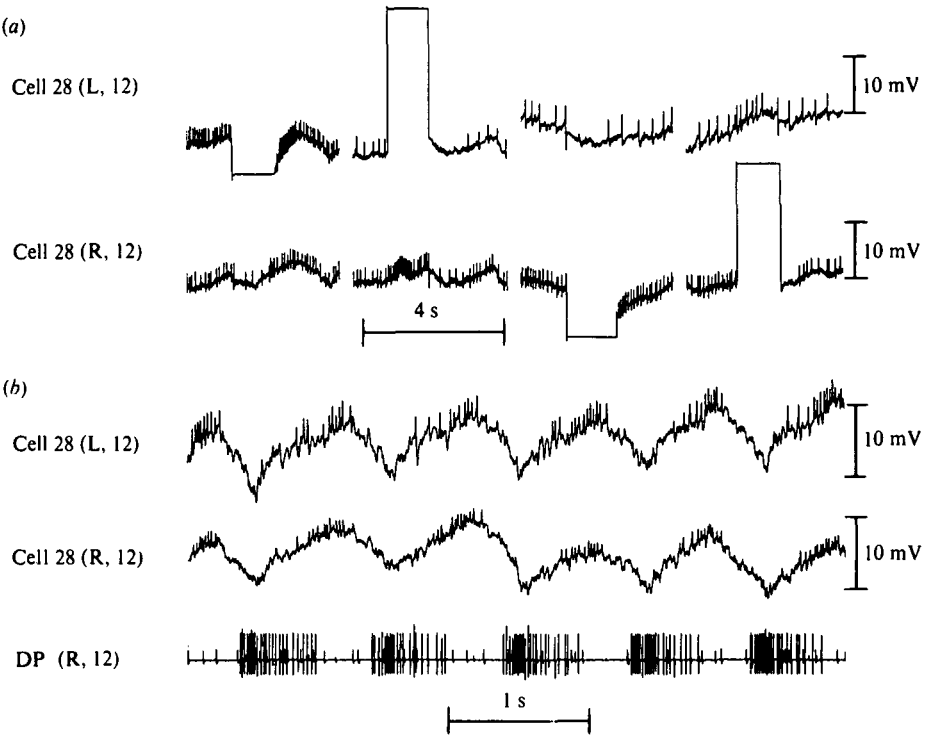

Fig. 11. Electrical junction linking the bilateral cell 28 homologues. (*a*) Passage of depolarizing and hyperpolarizing current into cell 28 of one side of the ganglion, respectively, depolarizes and hyperpolarizes the contralateral homologue. Preparation not swimming. (*b*) In-phase polarization rhythm of the bilaterally homologous cell 28 pair during swimming of the preparation.

current into cell 33 has no noticeable effect on cell 27. There was also a one-way inhibitory connexion from cell 33 to cell 28 (Fig. 9*b*, *c*). However, since passage of hyperpolarizing current into cell 28 hyperpolarizes cell 33, it can be inferred that these two cells are additionally linked via a rectifying electrical junction that permits passage of hyperpolarizing, but not of depolarizing current, from cell 28 to cell 33. The inference of such a double link is supported also by the delayed recovery of cell 28 after the end of passage of hyperpolarizing current (Fig. 10*c*). The delayed recovery of cell 28 is evidently due to its receipt of a burst of inhibitory input upon transient post-inhibitory rebound of cell 33.

The intraganglionic connexions presented thus far link *ipsilateral* pairs of oscillatory interneurones (except for the inhibitory link from cell 123 to cell 27). The records of Fig. 11 now show that the bilateral pair of cell 28 homologues, whose polarization rhythms can be seen to occur in the same phase of 90°, are linked by a non-rectifying electrical junction. Similar results (not presented here) have been found for the bilateral pair of cell 33 homologues. The existence of these electrical junctions is, of course, in accord with the obvious need to phase-lock the swim cycles of the oscillatory interneurones on the right and left sides of the leech. Thus the inhibitory effect of depolarizing cell 123 on the contralateral cell 27 seen in Fig. 9*c* could be an indirect

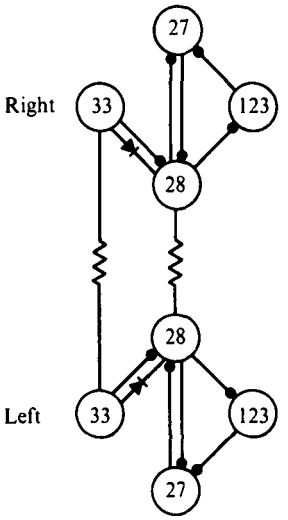

Fig. 12. Summary circuit diagram of identified intraganglionic connexions between oscillatory interneurones. Meaning of symbols: filled circle = inhibitory synapse; resistor = electrical junction; diode = rectifying electrical junction.

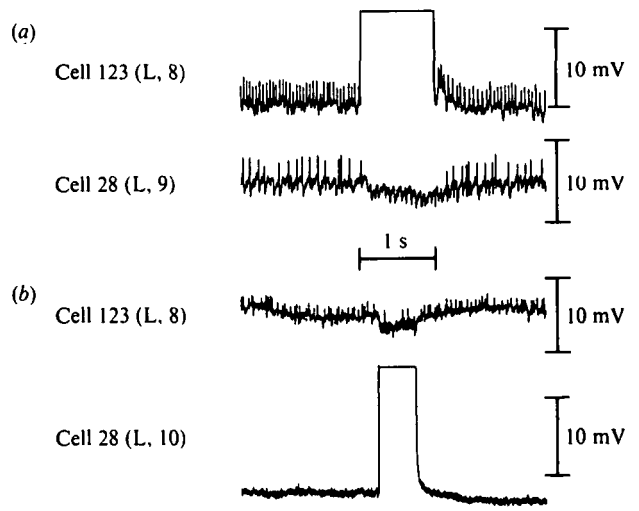

Fig. 13. Interganglionic reciprocal inhibitory connexion between cell 123 and cell 28. Preparation not swimming. (*a*) Passage of depolarizing current into cell 123 hyperpolarizes cell 28 of the next posterior ganglion. (*b*) Passage of depolarizing current into cell 28 hyperpolarizes cell 123 of a ganglion two segments more anterior.

one, mediated via a connexion from cell 123 to its ipsilateral cell 27 and an electrical junction between the contralateral pair of cell 27 homologues. It has been shown previously (Ort *et al.* 1974) that electrical junctions exist also between right and left homologous motor neurones, to provide for a bilaterally symmetric swimming movement.

The *intraganglionic* connexions between oscillatory interneurones revealed by these

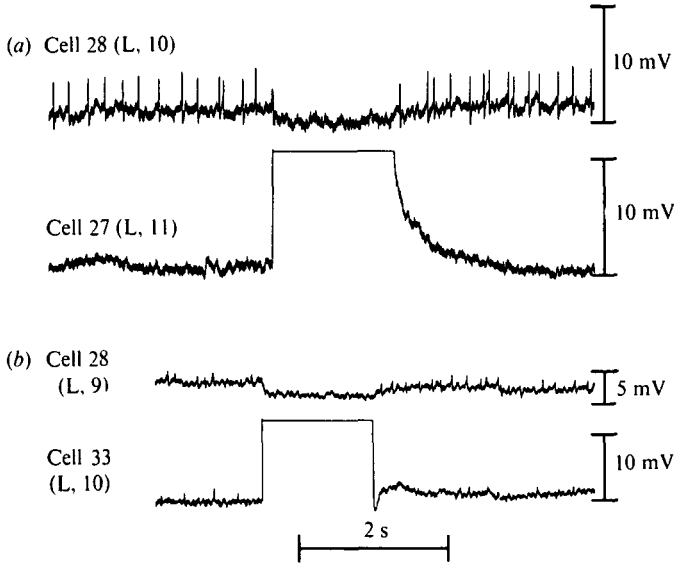

Fig. 14. Interganglionic inhibitory connexions leading from cell 27 and from cell 33 to cell 28. Preparation not swimming. (*a*) Passage of depolarizing current into cell 27 hyperpolarizes cell 28 of next anterior ganglion. (*b*) Passage of depolarizing current into cell 33 hyperpolarizes cell 28 of the next anterior ganglion.

experiments are summarized schematically in Fig. 12. A direct inhibitory connexion from cell 27 to cell 33 has been omitted from this diagram because the data of Fig. 10(*a*), which show an inhibitory connexion, are compatible also with an *indirect* inhibitory pathway leading from cell 27 to cell 33 via cell 28 and its rectifying electrical connexion to cell 33. Moreover, theoretical analysis of the function of the interneuronal network has shown that the existence of a direct connexion from cell 27 to cell 33 is not required for rhythm generation (Friesen & Stent, 1977). Nevertheless, the present data are compatible also with the existence of such a direct connexion.

The following interganglionic connexions were found. There was a rearward inhibitory connexion from cell 123 to cell 28 of a posterior ganglion, and a reciprocal frontward inhibitory connexion from cell 28 to cell 123 of an anterior ganglion (Fig. 13). Frontward inhibitory connexions were also found from cell 27 and from cell 33 to cell 28 of an anterior ganglion (Fig. 14). Records not presented here also show a frontward inhibitory connexion from cell 27 to cell 33 of an anterior ganglion. However, as is the case with the intraganglionic connexion from cell 27 to cell 33, this connexion could take (and will be assumed to take, for the time being) an indirect path via the frontward inhibitory connexion leading from cell 27 to cell 28 and the rectifying electrical junction of cell 28 with cell 33.

The hemilateral network of identified intra- and interganglionic connexions is presented in summary form in Fig. 15. As can be seen, the three frontward interganglionic inhibitory links, namely those reaching cell 28 of an anterior ganglion from cell 27 and 33, and the link reaching cell 123 of an anterior ganglion from cell 28 are homologues of *intra*ganglionic connexions. However, the rearward interganglionic connexion from cell 123 to cell 28 of a posterior ganglion has no intraganglionic

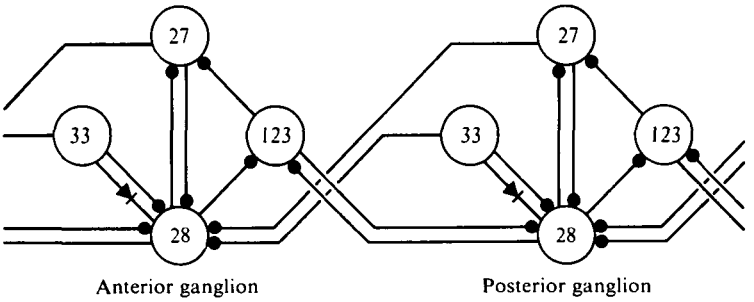

Fig. 15. Hemilateral circuit diagram of the identified *intra-* and *inter*ganglionic synaptic connexions between oscillatory interneurones in the leech swimming oscillatory network. Meaning of symbols as in Fig. 12.

homologue, and no interganglionic homologue has been found for the intraganglionic link from cell 28 to cell 27. It is to be noted that the frontward direction of the interganglionic links provided by cells 27, 33 and 28 is fully consonant with the previous conclusions that the axon of each of these interneurones projects into the anterior connective. Similarly, the rearward direction of the interganglionic link provided by cell 123 is fully consonant with the previously inferred projection of its axon into the posterior connective.

DISCUSSION

It would appear that the neural system responsible for generating the leech swimming rhythm is iterated metamerically over most of the ventral nerve cord. Earlier studies have indicated that motor neurones driving the leech swimming rhythm are present as far frontward as the 2nd and as far rearward as the 18th ganglion (Kristan *et al.* 1974*b*), and in the present study oscillatory interneurones were found in the 2nd to the 17th ganglion, other ganglia not having been examined. Although the inter- and intraganglionic connexions linking the oscillatory interneurones were identified mainly by recordings taken from midbody ganglia 7 to 12 inclusive, some homologous network connexions were found also in more rostral and more caudal segments. The nature of the leech central swim generator is thus different from that of the leech heartbeat generator, in which a segmentally highly differentiated, non-metameric network produces a body-wide contractile wave (Thompson & Stent, 1976*a*, *b*).

Despite its richness of iterated elements and connexions the identified network of oscillatory interneurones presented in Fig. 15 is unlikely to represent the complete central swim oscillator of the leech. First of all, there exists an additional oscillatory interneurone, cell 42, whose very small cell body is located in the posterior cell packet, in the vicinity of cells 27, 28 and 33. During swim episodes, the membrane potential of cell 42 oscillates in a rhythm phase-locked with that of the motor neurones, with the midpoint of its impulse burst occurring at a phase angle of about 145°. Cell 42 qualifies as a candidate component of the central swim oscillator, since passage of depolarizing current into it can shift the phase of the swimming rhythm (Poon, 1976). Unfortunately, cell 42 is very difficult to locate, and no reliable data regarding its connexions to other oscillatory cells are so far available. Moreover, it is possible that one or more

additional oscillatory interneurones are located in the anterior cell packet, in the vicinity of cell 123. In attempts to penetrate cell 123 for recording purposes, a rhythmically active cell was occasionally encountered in the anterior packet whose properties resembled those of an oscillatory interneurone but which was clearly not cell 123. Second, some hitherto unidentified connexions between the oscillatory interneurones are likely to exist. Indeed, the intracellular record of Fig. 4 provides evidence for the existence of at least one such unidentified connexion. As can be seen, during its swim cycle cell 33 sustains a burst of inhibitory synaptic potentials that reaches its maximum intensity during the midpoint of the cell 3 impulse burst, or at a phase angle of 0°. As a few successful simultaneous penetrations of cell 33 and cell 123 have shown, cell 123, the only oscillatory interneurone producing its impulse burst at a phase angle of 0°, does not form a direct inhibitory link to cell 33 of its own ganglion. No successful simultaneous electrode penetrations of cell 123 and of cell 33 of a posterior ganglion have as yet been achieved, so that it is possible that there exists a rearward *inter*ganglionic inhibitory input to cell 33 from cell 123 of an anterior ganglion. Alternatively, it is possible that cell 33 receives direct inhibitory input from an as yet unidentified oscillatory cell, whose impulse burst midpoint occurs at a phase angle of 0°.

It should be noted in this connexion that simultaneous recordings from cell 33 and cell 123 of the same ganglion also failed to reveal a reverse inhibitory connexion from cell 33 to cell 123. Such an intraganglionic connexion had been previously inferred to exist on the basis of indirect evidence and was included in the swim oscillatory network diagram presented in the preliminary account of this work (Friesen *et al.* 1976). In accord with these more recent negative data this connexion has been eliminated from the diagrams of Figs. 12 and 15.

To identify the ultimate source of the activity rhythm of the oscillatory interneurones two alternative possibilities can be considered. First, the interneurones may be individually capable of undergoing an endogenous membrane polarization rhythm, as is known to be the case for some rhythmically active neurones of invertebrates (Alving, 1968). In this event, the role of the identified network interconnexions of Fig. 15 would be to lock the endogenous polarization rhythms of the interneuronal ensemble into an appropriate phase relation, as with the set of heart interneurones, or HN cells, that drive the leech heartbeat (Thompson & Stent, 1976*a*, *b*). However, it seems rather unlikely that the swim oscillatory interneurones do possess an endogenous polarization rhythm. In contrast to the HN cells, the rhythm of the swim oscillatory interneurones is episodic, being envoked only by stimuli, such as delivery of electric shocks to the segmental nerves (Kristan & Calabrese, 1976) that initiate swimming in the entire cord. Moreover, the absence of an activity rhythm of individual swim oscillatory interneurones during non-swimming cannot be attributed to the absence of tonic excitatory or presence of tonic inhibitory input, since direct passage of increasing strengths of depolarizing current into a quiescent interneurone of a non-swimming preparation merely evokes a progressive increase in the level of tonic impulse activity of the cell, but never brings forth oscillations of its membrane potential. Similarly, replacement of chloride by sulphate ions in the physiological saline in which the preparation is bathed, a condition under which leech neurones are relieved of most of their inhibitory synaptic input and which allows the HN cells to

continue their chronic endogenous activity rhythm (R. L. Calabrese, personal communication), merely evokes tonic but not rhythmic activity of the swim oscillatory interneurones (Poon, 1976).

It would appear, therefore, that the second of the two possible sources of the interneuronal activity rhythm is likely to obtain, namely that the network of Fig. 15 is inherently oscillatory. That this is indeed the case has been shown by a detailed analysis of the functional properties of this network (Friesen & Stent, 1977). The fundamental principle to which the network owes its oscillatory character is the *recurrent cyclic inhibition* of neuronal loops, to whose possible existence and generative properties Székely (1965) first drew attention. According to this analysis, the basic oscillatory loop of the central swim oscillatory is inter- rather than intrasegmental. It consists of the five-membered, recurrent cyclic inhibition ring formed by cells 28 and 123 of an anterior ganglion and cells 28, 123 and 27 of a posterior ganglion. Moreover, since the intersegmental inhibitory connexions formed by cells 27 and 123 are repeated in several anterior and posterior ganglia respectively, the basic system consists of a series of intersegmentally concatenated, five-membered rings. As long as the cells are provided with a source of *tonic* excitation, this basic network is capable of producing a crude version of the swimming rhythm. Under this rhythm cells 28 and 123 of a given ganglion would produce antiphasic oscillations and the impulse burst midpoint of either cell would lead in phase that of its homologous interneurones in a posterior ganglion. The cycle period of the basic rhythm would take realistic values in the range of 400–1000 ms, as long as the intersegmental axons of cells 27 and 123 incorporate the (observed) impulse conduction delays of about 20 ms per segment and the time required for each interneurone to recover from inhibition falls into the realistic range from about 30 to 150 ms (Friesen & Stent, 1977). The topologically more complex network of Fig. 15 can be considered a refinement of the basic five-membered intersegmental ring, in the sense that the additions of cell 33, of reciprocal inhibitory connexions between cells 27 and 28, and of frontward intersegmental inhibitory connexions of cells 28 and 33 create a set of subsidiary rings.

To test the theoretically predicted oscillatory properties of this complex cyclic network an electronic analogue model of the interneurones and their intra- and interganglionic connexions was constructed, according to the schema shown in the insert of Fig. 16. This model consists of eight interconnected electronic 'neuromime' elements (Lewis, 1968). Each such element mimics an excitable nerve cell membrane, in that it gives rise to an electrical impulse once membrane polarization has fallen below threshold level. The cell membrane analogues also provide for the simulation of both excitatory and inhibitory synaptic currents, whose summed effects determine whether the membrane is polarized above or below threshold level. The details of the mode of employ of these neuromimes in the modelling of the swim oscillator network is described elsewhere (Friesen & Stent, 1977). The model of Fig. 16 represents the four oscillatory interneurones, cells 123, 28, 33 and 27 of one ganglion (ganglion X) embedded in a chain of 13 ganglia, of which ganglia W and Z are the front- and rearmost and ganglia X and Y are the fifth and ninth in the chain, respectively. The model includes also cells 123 and 28 of ganglion W and cells 28 and 33 of ganglion Y. The output of this analogue model is presented in Fig. 16. As can be seen, the model oscillator runs with a realistic swim period of about 840 ms, reproduces for the four

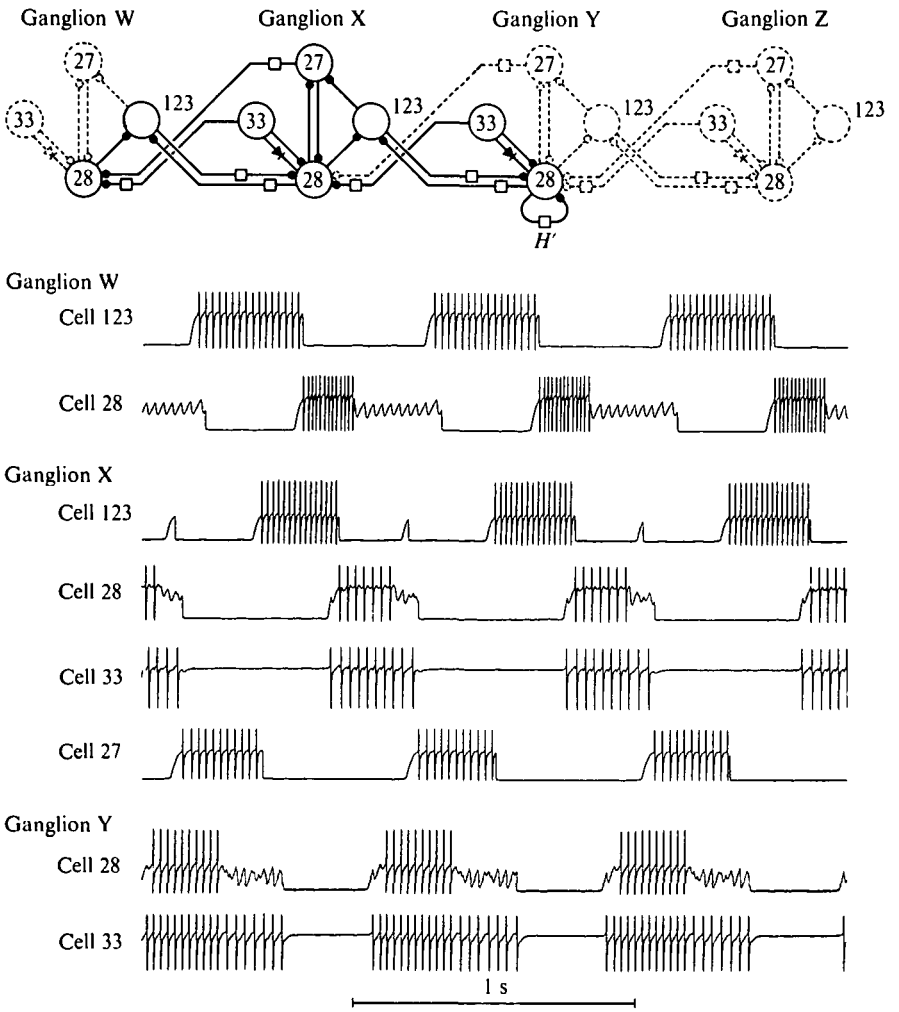

Fig. 16. Oscillations of a partial electronic analogue model of the network of oscillatory interneurones of Fig. 15. Impulse burst generated by eight neuromimes connected according to the circuit shown in the insert. The insert schematizes the oscillatory interneurones of four ganglia W, X, Y, Z, representing the 1st, 5th ,9th and last of an isolated chain of 13 ganglia. Cells represented by neuromimes and their modelled connexions are shown in solid lines, cells and connexions omitted from the model circuit are shown in dashed outline. The square boxes designate lines with an impulse transmission delay of length H. The self-inhibitory 'phantom' connexion of cell 28 of ganglion Y incorporating a transmission delay of length H' replaces the presence of cells 123 of ganglion Y and of cells 33 and 28 of ganglion Z (Friesen & Stent, 1977). Impulse transmission delays of H = 80 ms and H' = 250 ms were modelled by means of shift registers. Sufficient tonic excitation was provided to each interneurone analogue to produce an impulse frequency of about 80 Hz at the height of its active phase.

interneurones of ganglion X a good approximation of the observed interneuronal impulse burst relations shown in Fig. 6 and gives rise to an appropriate rostro-caudal phase progression of the cycle phases of the homologues of cells 123 and 28 in ganglia W, X and Y. The only obvious departure of the model output from the actual operation of the swim oscillator is presented by the somewhat abbreviated impulse burst

duration of cell 33 in ganglion X. This unrealistic feature is most likely due to the failure of the model to incorporate the as yet unidentified source of direct inhibitory synaptic input to cell 33. But the otherwise high degree of verisimilitude of the model output make it appear that the interneuronal network of Fig. 15 constitutes the major components of the central swim oscillator of the leech. This conclusion will be strengthened further by data presented in the following paper of this series (Poon *et al.* 1978), in which the output connexions from the oscillatory interneurones to the motor neurones are identified.

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