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Neuronal Control of Heartbeat in the Medicinal Leech

I. Generation of the Vascular Constriction Rhythm by Heart Motor Neurons

Wesley J. Thompson* and Gunther S. Stent

Department of Molecular Biology, University of California, Berkeley, California 94720, USA

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Summary. The leech heartbeat consists of a constriction-dilation rhythm of two lateral heart tubes extending over the length of the body. The beats of the segmental sections of these two tubes are coordinated in such a manner that the heart tube of one body side produces a frontward peristaltic wave while the heart tube on the other body side produces nearly concerted constrictions. This rhythm is metastable, in that left and right heart tubes alternate between peristaltic and concerted constriction modes, with a given mode lasting for tens or hundreds of beat cycles.

The constriction-dilation cycles of the segmental heart tube sections are controlled by a set of rhythmically active motor neurons, the heart excitors, or HE cells. A bilateral pair of HE cells is located in all but the two frontmost and the two rearmost segmental ganglia of the ventral nerve cord. Each HE cell innervates via excitatory synapses the circular muscle fibers in the wall of the ipsilateral heart tube section. The activity cycle of the HE cells consists of an active phase, during which they are depolarized and produce a burst of impulses, and an inactive phase during which they are repolarized by a burst of inhibitory synaptic potentials. The intersegmentally coordinated activity cycles of the HE cell set are maintained in an isolated ventral nerve cord. Hence the generation of the heart excitor rhythm does not require sensory feedback.

Introduction

The blood of the medicinal leech, *Hirudo medicinalis*, circulates in a closed vascular network, whose major segmental components are shown diagrammatically in Figure 1. This segmental network is repeated throughout the 21 somatic segments of the leech (except for certain specializations in rostral and caudal segments, and in mid-body segments containing the intestine). There are 4

* Present address: Institute of Physiology, University of Oslo, Karl Johans Gate 47, Oslo, Norway

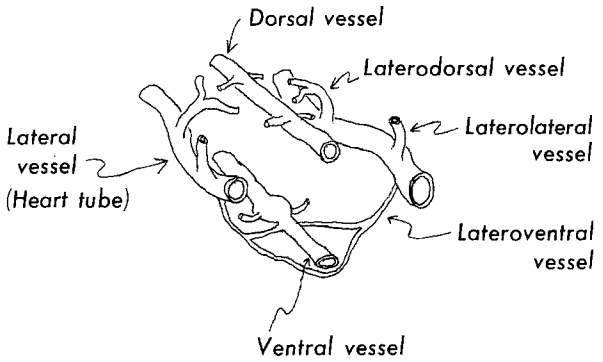

Fig. 1. The major circulatory vessels in a midbody segment of the medicinal leech (Mann, 1962)

major longitudinal vessels: a dorsal vessel; a ventral vessel (which encases the ventral nerve cord); and two lateral vessels, which are referred to as *heart tubes* in this and the following papers. The major vessels are joined to form a continuous conduit extending over the length of the body. Transverse branch vessels, not all of which are shown in Figure 1, issue from each of the major longitudinal vessels and eventually interconnect them after passing through the skin and muscle layers and the viscera in a highly stereotyped manner (Boroffka and Hamp, 1969). Circulation of blood in this vascular network is achieved by a pumping action of the heart tubes and of the laterolateral and laterodorsal branch vessels. The walls of the heart tubes and of the two branch vessels contain a layer of circular muscle fibers (Gaskell, 1914; Hammersen and Staudte, 1969). The pumping action consists of a constriction-dilation rhythm of these tubes and vessels generated by periodic contraction of their circular muscles.

The average period of the contractile rhythm of a segmental heart tube section and its branch vessels ranges from about 10 s at 25 ° to 30 s at 10 °. The rhythm is regular, in that for a given animal held at a constant temperature more than half the periods lie within 5% of the average. Previous investigations of the leech heartbeat (Boroffka and Hamp, 1969; Gaskell, 1914), have revealed that the cycle of each segmental heart tube section is comprised of three major phases. In the first phase, the laterolateral and laterodorsal branch vessels constrict approximately simultaneously. In the second phase, about 1 s after the beginning of the constriction of these branch vessels, the heart tube itself constricts. In the third and final phase, which begins about halfway into the cycle, all vessels begin to dilate. The constriction-dilation cycle, or beat, of each individual segmental heart tube section and its branch vessels is coordinated with the beat of other segmental sections so as to produce a peristaltic wave traveling frontward along the heart tube. This coordinated constriction pattern of heart tube sections and their branch vessels has been reported to produce a unidirectional blood circulation in the vascular network, consisting of a frontward flow in both heart tubes and a rearward flow in the dorsal and ventral vessels (Boroffka and Hamp, 1969). Furthermore, in the laterolateral and laterodorsal vessels, blood flows towards and, in the lateroventral vessel, away from the heart tubes.

The work reported in this and the following papers provides a neurophysiological account, in terms of identified cells in the leech ventral nerve cord and their synaptic connections, of how the contractile rhythm of the circular muscles in the segmental heart tube wall sections is generated and how it is coordinated intra- and intersegmentally.

Materials and Methods

Animals

Specimens of *Hirudo medicinalis* were purchased from a commercial supply house. The leeches were maintained for periods of up to 3 months in aquaria at 15 ° prior to experimental use.

Segmental Nomenclature

The system of Kristan et al. (1974) for the numbering of segments in the body of the leech was used in this study. According to this system, the 21 somatic segments which derive their innervation from the unfused ganglia of the ventral cord are numbered sequentially from anterior to posterior. The first six and last seven segments which derive their innervation from the fused ganglionic masses of the cerebral and caudal brains, are left unnumbered.

Preparations

The preparation used for monitoring the heartbeat patterns of the leech was made by opening the leech along the entire length of the dorsal midline and exposing its internal organs. The connective tissue and gut tissue overlying selected heart tube beats sections were removed so as to make these sections accessible for visual and electrical recordings of heart tube beats.

The "semi-intact" preparation used for recording the activity of neurons of the leech central nervous system while simultaneously monitoring the heart tube was similar to that described by Kristan et al. (1974). To make this preparation, five to seven segments in the mid-body of the leech were dissected and the rest of the animal was left intact. All of the opened segments, except for the central one, were denervated by severing the segmental nerve roots. In this manner, the central ganglion was left innervating a body wall flap containing the heart tube sections of its own segment. The ventral blood sinus which surrounds the ventral nerve cord was opened, dorsally and ventrally, to expose the central ganglion. Pieces of the blood sinus were left attached to the nerve roots of this ganglion to provide a means for pinning the ganglion. Finally, an incision was made along the ventral midline of the opened body wall to divide the body wall flap into right and left halves. The preparation was then pinned out in a glass dish whose bottom was covered with a transparent resin. The preparation was immersed in cold saline. During most experiments the preparation dish was maintained at a temperature of about 15 ° by a thermoelectric cooling device. Some experiments were carried out at somewhat higher temperatures, however, and the variations from experiment to experiment in the period of the muscular and neuronal activity cycles evident in the records presented in this and the subsequent two papers of this series are mainly attributable to differences in the temperature at which the preparations were maintained at various stages of this investigation.

The "isolated" preparation consisted of portions of the ventral nerve cord which had been removed completely from the animal. The extent of the cord removed varied from preparations consisting of a chain of 19 ganglia (referred to as the "isolated ventral cord preparation") to preparations consisting of only a single ganglion.

Bathing Fluids

During dissection and, unless otherwise noted, during the taking of electrophysiological recordings, the preparations were bathed in leech physiological saline (Nicholls and Baylor, 1968).

To abolish the spontaneous contractions of the muscle fibers embedded in the sheath surrounding the ganglia and connectives which occur in many isolated ventral cord preparations and which interfere with intracellular recordings, the preparations were usually bathed for 5 min after their dissection in leech saline to which either 20 mM Ca^{++} or 20 mM Mg^{++} was added. After return of the preparation to normal leech saline, the sheath contractions were almost always absent, whereas the neural activity of the heart system did not seem to be altered by this pretreatment.

Electrophysiological Procedures

The methods described previously (Kristan et al., 1974) were used for extracellular suction electrode recording and intracellular microelectrode recording, for amplifying and storing the electrophysiological signals received, and for stimulating nerves electrically and passing currents into nerve cell bodies. Neurons were repolarized by intracellular passage of current to counteract the depolarization following microelectrode penetration (Ort et al., 1974).

The heartbeat was monitored either visually or electrically. For visual monitoring, individual heart tube sections were observed under a microscope and the timing of their constrictions was recorded manually by pressing a key. For electrical monitoring, two different methods were used. One method was to place two wire electrodes on opposite sides of the heart tube section and to register changes in the impedance resulting from the constriction of the tube, using a Biocom Model 2991 Impedance Converter. The other method consisted of pressing a high-resistance glass microelectrode onto the heart tube surface and registering changes in the electrode tip potential resulting from movement of the tube.

Results

Dynamics of the Heartbeat

In order to study the dynamics of the leech heartbeat in detail, systematic observations were made of the constriction rhythm of the heart tube sections in different body segments. For this purpose the heart tubes were exposed by dissection and the constriction of two sections, one at the front and the other at the rear of the body, were monitored by means of electrodes that register the local impedance of the tube to flow of transverse electric current. A sample impedance record taken from the tube sections on the right side of the 4th and 17th segment is presented in part A of Figure 2. As can be seen, the period of the heartbeat cycle of this preparation was about 20 s, with the constrictions of the rear right segment preceding those of the front right segment by about half the cycle period. The concurrent constriction of sections of the right heart tube in segments between the 4th and 17th segment were observed visually. These visual observations showed that there was rear-to-front progression in the phase of the constriction cycle of successive sections of the right heart tube. In other words, the right heart tube maintained a frontward peristaltic wave that traveled from rear to front within half the beat cycle period.

The data presented in parts B and C show how the beat pattern of the right heart tube was coordinated with that of the left heart tube. In the 17th

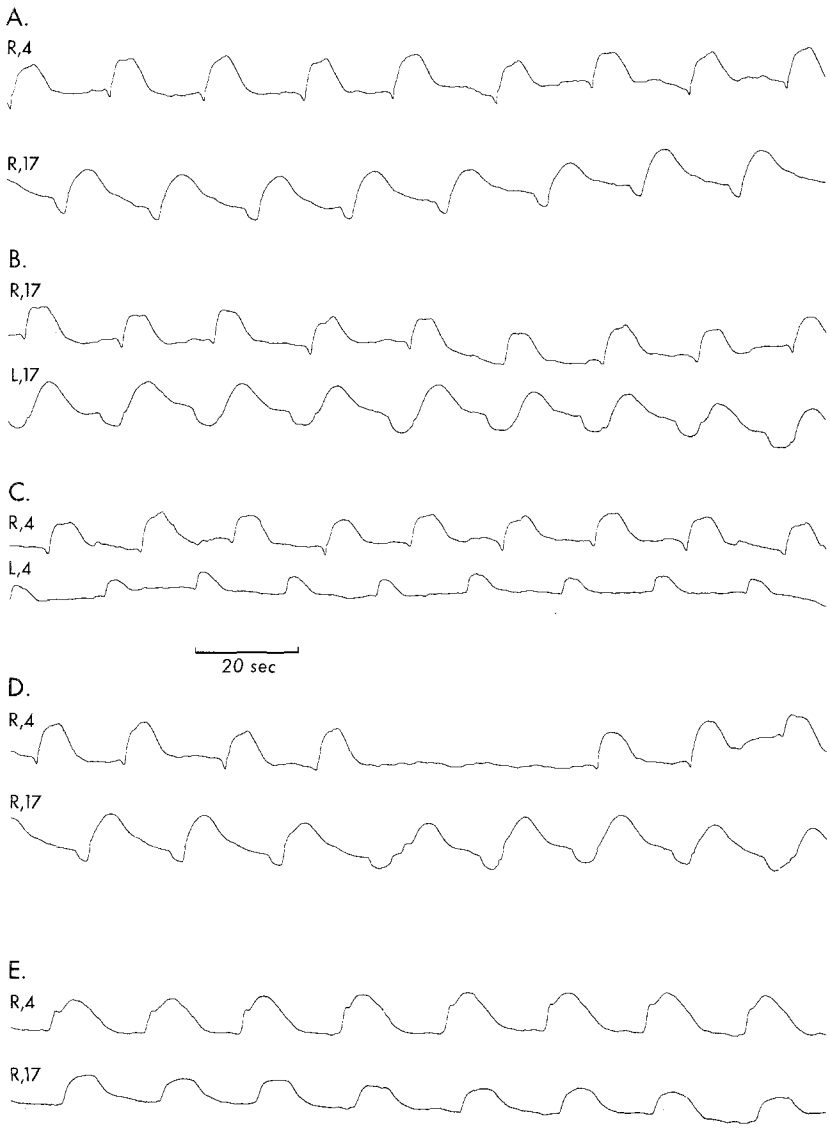

Fig. 2. Beats of the heart tube sections in two body segments recorded by monitoring trans-tube impedance changes. An upward deflection in each trace represents a heart tube constriction. The designations attached to each trace identify the particular heart tube section from which the record was taken. The letters *R* or *L* signify the right or left heart tube and the number signifies the body segment. The records of panels A, D and E were taken at progressively later times from the same preparation

segment the constriction cycles of right and left heart tubes were nearly in phase, whereas in the 4th segment the constrictions of the right and left heart tubes were separated by nearly half the cycle period. It follows that the heartbeat pattern is not bilaterally symmetric. Visual observation of the constrictions of intermediate sections of the left heart tube revealed the nature of this asymmet-

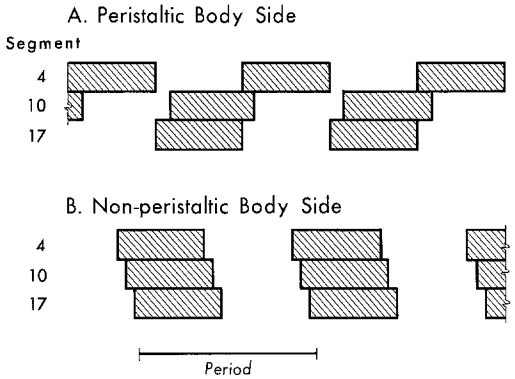

Fig. 3. Diagrammatic summary of the coordination of heart tube constrictions in segments 4, 10, and 17, on the peristaltic and non-peristaltic sides, based on records similar to those presented in Figure 2, taken from 4 different preparations, both while the right and while the left side was coordinated in the peristaltic mode. Each bar represents the phase angle during which a particular heart tube section is constricted. Time is running from left to right. The relative phase relations presented in this summary represent a best fit of the data, reached by trial and error adjustment of the average observed segmental contriction cycle phase differences, to satisfy the requirement of additivity. This requirement demands that if $I(4, 10)$, $I(10, 17)$ and $I(4, 17)$ designate the phase differences between the constriction cycles of the *ipsilateral* heart tube sections of the 4th, 10th and 17th segments and $C(4, 4)$, $C(4, 10)$, $C(10, 17)$ and $C(17, 17)$ the corresponding phase differences for the *contralateral* sections, then $I(4, 17) = I(4, 10) + I(10, 17)$; $C(4, 10) = C(4, 4) + I(4, 10)$; and $C(10,17) = I(10,17) + C(17, 17)$

ry: all sections of the left heart tube were beating nearly in concert, so that there was no peristalsis on that side.

As part D of Figure 2 shows, however, the heartbeat coordination in a given preparation is subject to sudden change, so that right and left sides exchange peristaltic and concerted, or non-peristaltic, coordination modes. Initially, the right front and rear sections were beating out of phase with each other, indicating that the right side was coordinated in the peristaltic mode. Suddenly the right front section missed two beats and then began beating in concert with the ipsilateral rear section, indicative of a transition of the right side to the non-peristaltic coordination mode. As shown in part E, this new non-peristaltic coordination continued to be maintained by the right heart tube. Concurrent observation of the left heart tube showed that it had meanwhile changed its coordination to the peristaltic mode. Hence, it appears that the heartbeat rhythm alternates between two metastable states, in which either the right or the left body side takes on a peristaltic constriction mode for tens or hundreds of heartbeats, while the opposite side carries out concerted contrictions in a non-peristaltic mode.

The results of a survey of the phase relations of the contriction pattern of the right and left heart tube sections observed in 4 different preparations are summarized diagrammatically in Figure 3. These results show that the intersegmental coordination of the heart tube constriction cycles is not only bilaterally asymmetric but also longitudinally non-uniform. Even on the peristaltic side the heart tube sections in the rear half of the body contrict nearly in concert,

so that the forward phase progression of the segmental constriction cycles responsible for the peristaltic wave is produced mainly in the anterior body half. On the non-peristaltic body side, the constrictions occur in nearly body-wide concert, but here there is nevertheless manifest a slight reverse, or front-to-rear, peristaltic phase progression amounting to less than 10% of the cycle period. Finally, the constriction cycles of the right and left sides are coordinated in a manner such that in the rear segments the cycle phase on the peristaltic side lags slightly behind the cycle phase on the non-peristaltic side. Thus the heartbeat pattern is more complicated than the previously reported pattern of a bilateral, antiphasic, frontward peristalsis (Gaskell, 1914; Gaskell, 1919; Boroffka and Hamp, 1969).

Central Nervous Control of the Heartbeat

The role played by the central nervous system in the generation and coordination of the heartbeat pattern was investigated by means of the semi-intact leech preparation described in Figure 4, in which the right and left heart tube sections of segment 11 were isolated from mechanical effects due to beats of tube sections in adjoining segments. As can be seen in Figure 4, the constriction rhythm of the mechanically isolated right heart tube section of segment 11 was coordinated in concert with the ipsilateral tube section in segment 13. This indicates that the right side was beating in the non-peristaltic mode. The right heart tube section of segment 11 also led in phase the cycle of the contralateral left tube on the peristaltic side of the same segment, in accord with the normal bilateral coordination pattern shown for segment 10 in Figure 3. Cutting the right anterior segmental nerve of segment 11, however, produced a dramatic change in the beat pattern. First, the period of the rhythm of the right tube section of segment 11 increased approximately twofold. Second, the rhythm of the right tube section was no longer phase-locked either with that of the contralateral tube section in the same segment or [as indicated by data not shown here] with that of the ipsilateral tube section in segment 13. Thus, the segmental constriction rhythm and its intersegmental coordination are under central nervous control. However, there does exist also a peripheral mechanism for generating phasic contractile activity of the vascular system, albeit with a longer period.

Identification of a Heart Excitor Motor Neuron

In order to elucidate the mechanism of central nervous control of the heartbeat, a search was made for neurons in the segmental ganglion with an activity pattern related to the heart tube constriction rhythm. For this purpose a preparation was used consisting of a single segmental ganglion attached by its segmental nerves to a body wall flap containing the segmental heart tube section. Individual nerve cell bodies in the ganglion and individual fibers of the circular muscle of the heart tube were simultaneously penetrated with microelectrodes. This search led to the identification of a bilateral pair of excitatory motor neurons to the circular muscles of the segmental heart tube section. These rhythmically

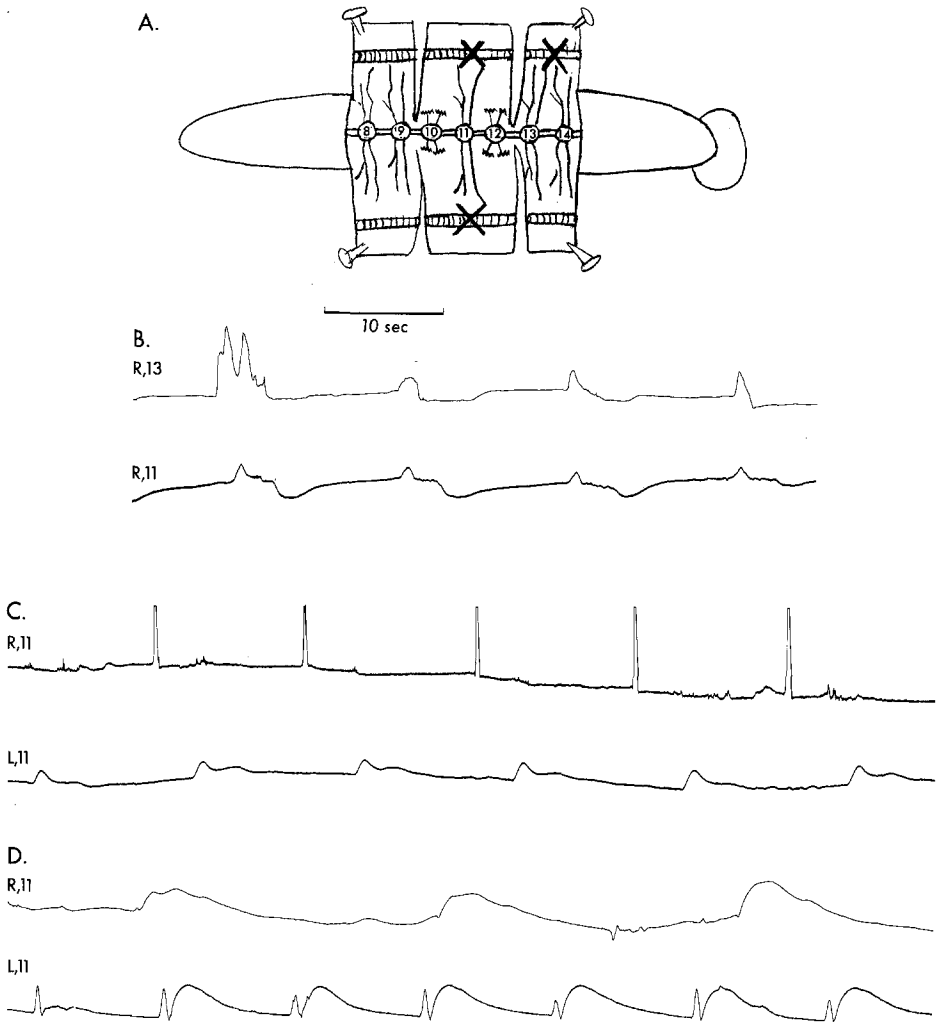

Fig. 4. Role of the central nervous system in the maintenance of the heartbeat. Panel A: Diagrammatic representation of the preparation used. Segments 8 to 14 of a leech were opened by a longitudinal incision along the dorsal midline; individual segments are identified in the diagram by a number placed inside the corresponding ganglion. The roots of ganglia 10 and 12 were severed on both sides and circumferential cuts were made in the exposed body wall extending from the dorsal midline to near the ventral midline between segments 9 and 10 and between segments 12 and 13. The symbol "x" in the diagram marks the heart tube sections whose beats were recorded. Panels B, C, and D show heart tube beats recorded by monitoring the tip potential of a microelectrode pressed on the heart tube surface. The upward deflection in each trace represents a heart tube constriction. The records in Panel D were taken after cutting the right anterior segmental nerve root of ganglion 11. The slight decrease in the heartbeat period in the second trace of panel D was the result of an increase in temperature of the preparation

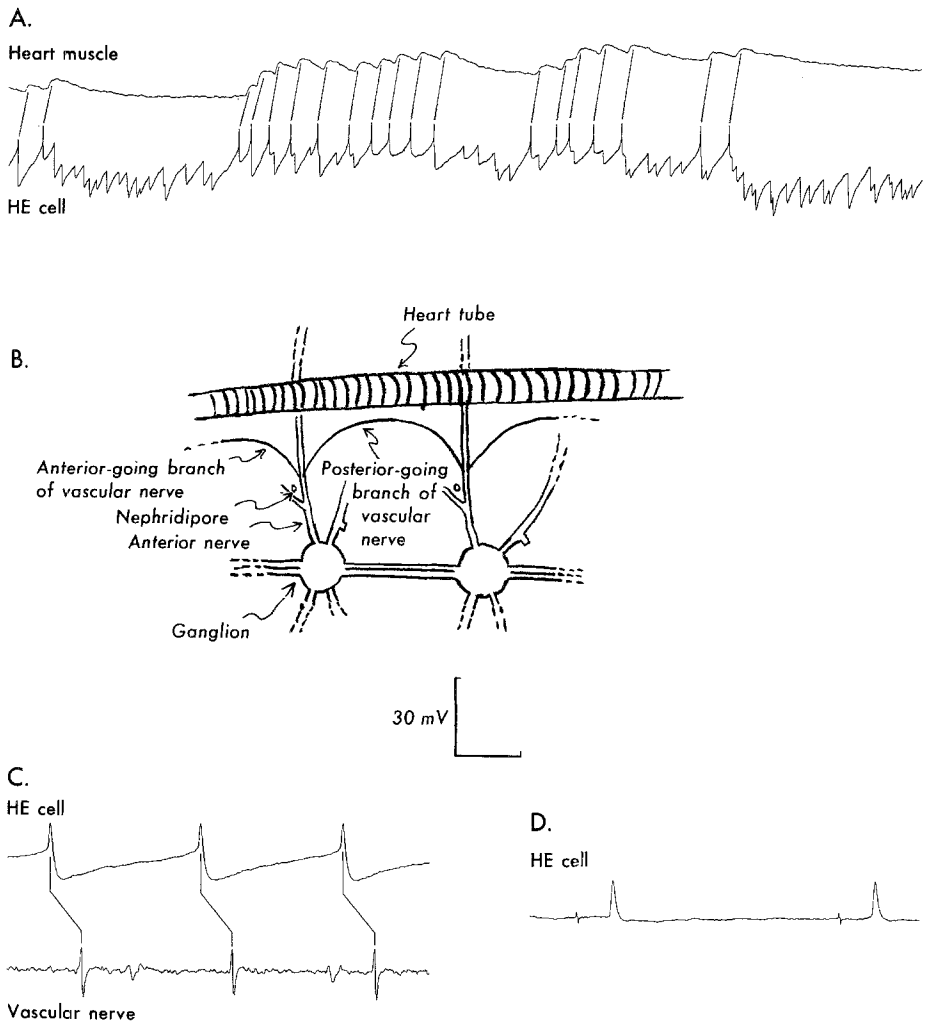

Fig. 5. Identification of a heart tube motor neuron. Panel A: Intracellularly recorded activities of an HE cell (bottom trace) and of a circular muscle fiber in the ipsilateral heart tube of the same segment (top trace). The preparation was bathed in a 20 mM Ca^{++} saline to increase the size of junctional potentials. Panel B: Innervation of the heart tube. The diagram shows two segments of body wall from ventral midline to lateral edge with their ganglia and nerve roots. The heart tube is innervated by two small nerve branches (the anterior- and posterior-going branches of the vascular nerve) arising from the medial branch of the anterior segmental nerve. Panel C: Matching orthodromic action potentials recorded intracellularly from an HE cell and extracellularly from the proximal end of the cut ipsilateral anterior-going vascular nerve. Panel D: Antidromic action potentials recorded from the HE cell body, evoked by stimulation of the anterior-going vascular nerve. The HE cell is the same as that from which the record of panel C was taken. Calibration marks, vertical: 30 mV for all intracellular records; horizontal: 1 s in panel A, and 62.5 ms in panels C and D

active neurons were heart excitors, or HE cells. HE cell bodies have been identified in the same location in all ganglia of the ventral cord, except in ganglia 1, 2, 20 and 21, where despite repeated searches, no HE cells have been found. As shown in Figure 5, the HE cell manifests a phasic activity pattern in which trains of action potentials alternate with trains of inhibitory synaptic potentials (ISP's). Each HE cell action potential is followed after a constant delay of about 100 ms by an excitatory junction potential in the muscle fiber of the ipsilateral heart tube, demonstrating that the HE cell is an excitatory motor neuron to the circular heart tube muscles. By way of further support of that identification, passage of hyperpolarizing current of sufficient strength to block generation of action potentials into one of the HE cells was found to cause an arrest of beating in the ipsilateral heart tube (cf. Fig. 7).

Figure 5 presents also the pattern of innervation of the heart tube by the vascular nerves of the segmental nerve system, first described by Gaskell (1914). The vascular nerves are very fine superficial nerve branches originating from the medial branch of the anterior nerve at a point just distal to the nephridiopore. One vascular nerve courses anteriorly and the other posteriorly. Both nerves lie next to the heart tube, running parallel to the longitudinal body axis. The posterior-going nerve of one segment is anatomically continuous with the anterior-going nerve of the next posterior segment. Simultaneous records presented in Figure 5 taken from a suction electrode attached to the proximal cut end of the anterior-going vascular nerve and from a microelectrode inserted into the cell body of the ipsilateral HE cell show that every HE cell action potential is followed after a delay of 33 ms by a characteristic spike in the vascular nerve. Since similar results were obtained with a suction electrode attached to the posterior vascular nerve, the axon of the HE motor neuron must project into both anterior- and posterior-going vascular nerves of its segment. In accord with this inference, electrical stimulation of the vascular nerve through the suction electrode resulted in antidromic action potentials in the HE cell body after the same 33 ms delay as that found between the orthodromic HE cell action potential and the vascular nerve spike. This experimental arrangement allowed also the observation of the collision of orthodromic and antidromic HE cell spikes, lending further support to the inference that the HE cell axon projects into the vascular nerves.

A photomicrograph of an HE cell stained by the intracellular injection of horseradish peroxidase (Muller and McMahan, 1975) is shown in Figure 6. The HE cell is a monopolar neuron with an extensive dendritic tree in the ipsilateral neuropile and a cell body located on the ventral aspect of the anterior lateral cell packet of the segmental ganglion. In agreement with the electrophysiological findings, it can be seen that the axon of the HE cell exits from the ganglion via the ipsilateral anterior root. Staining of HE cells in various ganglia of the cord has shown essentially similar morphological features.

Activity Pattern of the Heart Excitors

The participation of the HE cells in the control of the heartbeat was studied in a semi-intact leech preparation. Here the contractile rhythm of exposed heart

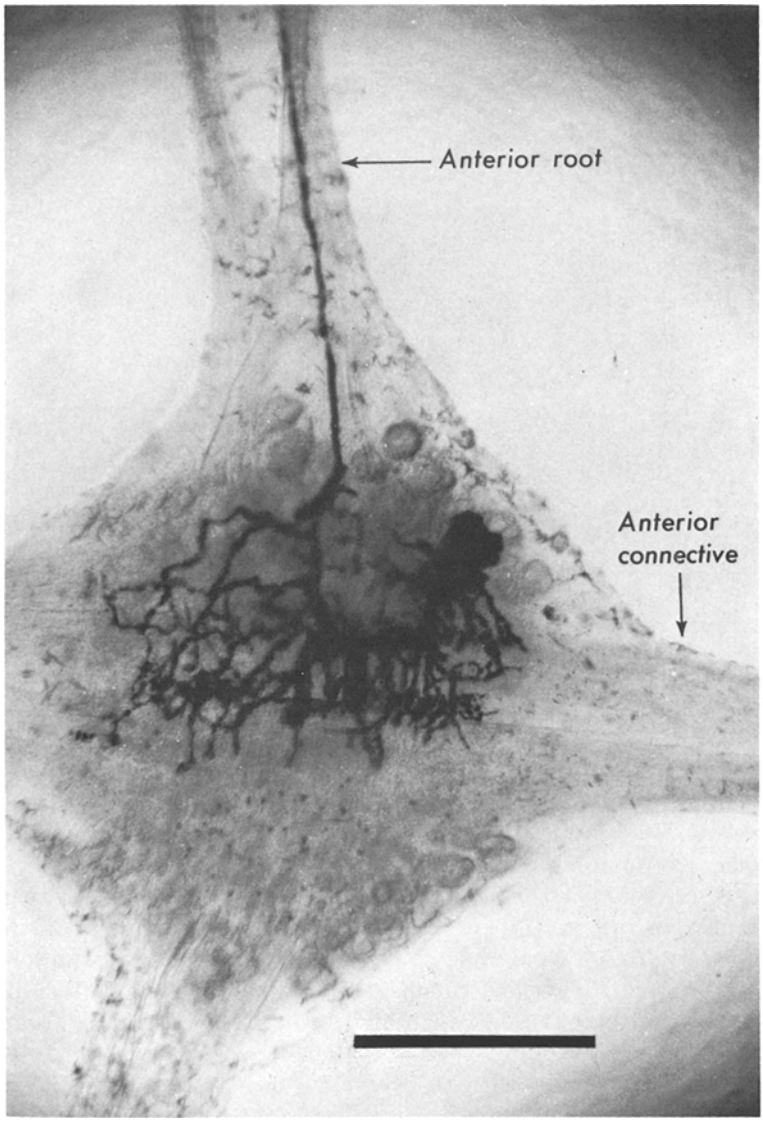

Fig. 6. Anatomy of the heart excitor neuron. Photograph taken from the dorsal aspect of a midbody ganglion in which one of its two HE cells was stained by intracellular injection of horseradish peroxidase. Distance marker about 200 μm

tube sections can be observed visually while the electrical activity of the corresponding HE cells is monitored with intracellular microelectrodes in the exposed segmental ganglion. As can be seen in Figure 7, the membrane potential of the HE cell pair varies rhythmically between a depolarized phase with an action potential burst, and a repolarized phase with an ISP burst. The polarization cycles of the right and left HE cells have the same period, which is also the

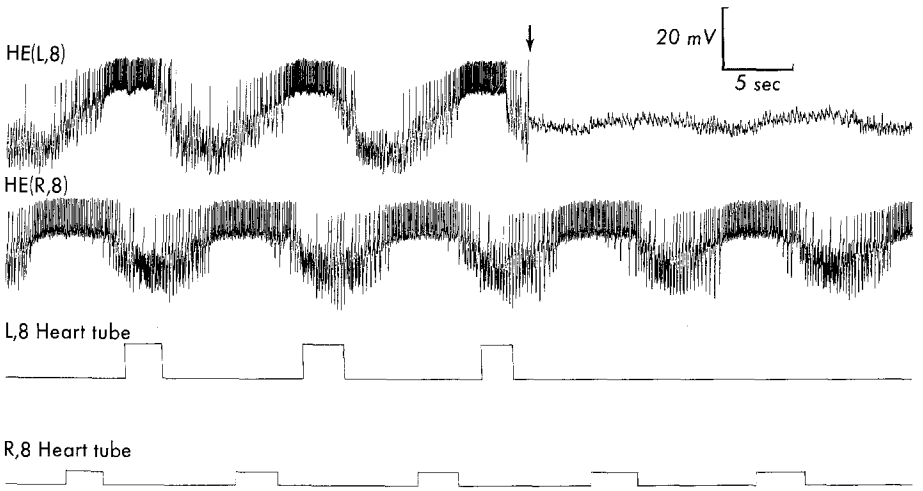

Fig 7. Activity rhythm of the HE cells in a semi-intact leech preparation. The first trace is an intracellular record from the left HE cell of ganglion 8, HE(L,8); the second trace is an intracellular record of the right HE cell of ganglion 8, HE(R,8); the third and fourth traces present the visually monitored beats of the left and right heart tubes, respectively. Upward deflections in the third and fourth traces designate constrictions of the heart tube. The arrow above the first trace indicates the beginning of passage of hyperpolarizing current into the HE(L,8) cell body

period of the constriction cycles of the right and left heart tube sections. Furthermore, the depolarized phase of the HE cell cycle coincides with the constricted phase of the ipsilateral heart tube, in agreement with the identification of the HE cell as an excitor of the circular muscles of the ipsilateral heart tube section.

In order to determine whether sensory input is necessary for generation of the HE cell rhythm, the activity of the HE cells was examined also in a completely isolated nerve cord (consisting of all ganglia except the head and tail brains and the first and last segmental ganglia). The activity pattern of the HE cells of this isolated cord preparations was found to be indistinguishable from that of the semi-intact preparation; hence, the motor neuron activity pattern responsible for the coordinated heartbeat rhythm is generated by a central nervous oscillator which does not require sensory input for its phasic output.

HE Cell Activity and Heart Tube Constriction Dynamics

The activity rhythms of HE cells in various segmental ganglia of isolated ventral cord preparations were compared with the heart tube constriction rhythm. Figure 8 shows the patterns of activities recorded from pairs of right and left HE cells in ganglia 4, 10, and 17. In Panel A the phase of the activity cycle of the left HE cell in ganglion 4, HE(L,4), can be seen to lead that of the right HE cell in the same ganglion, HE(R,4). Using the first impulse in each

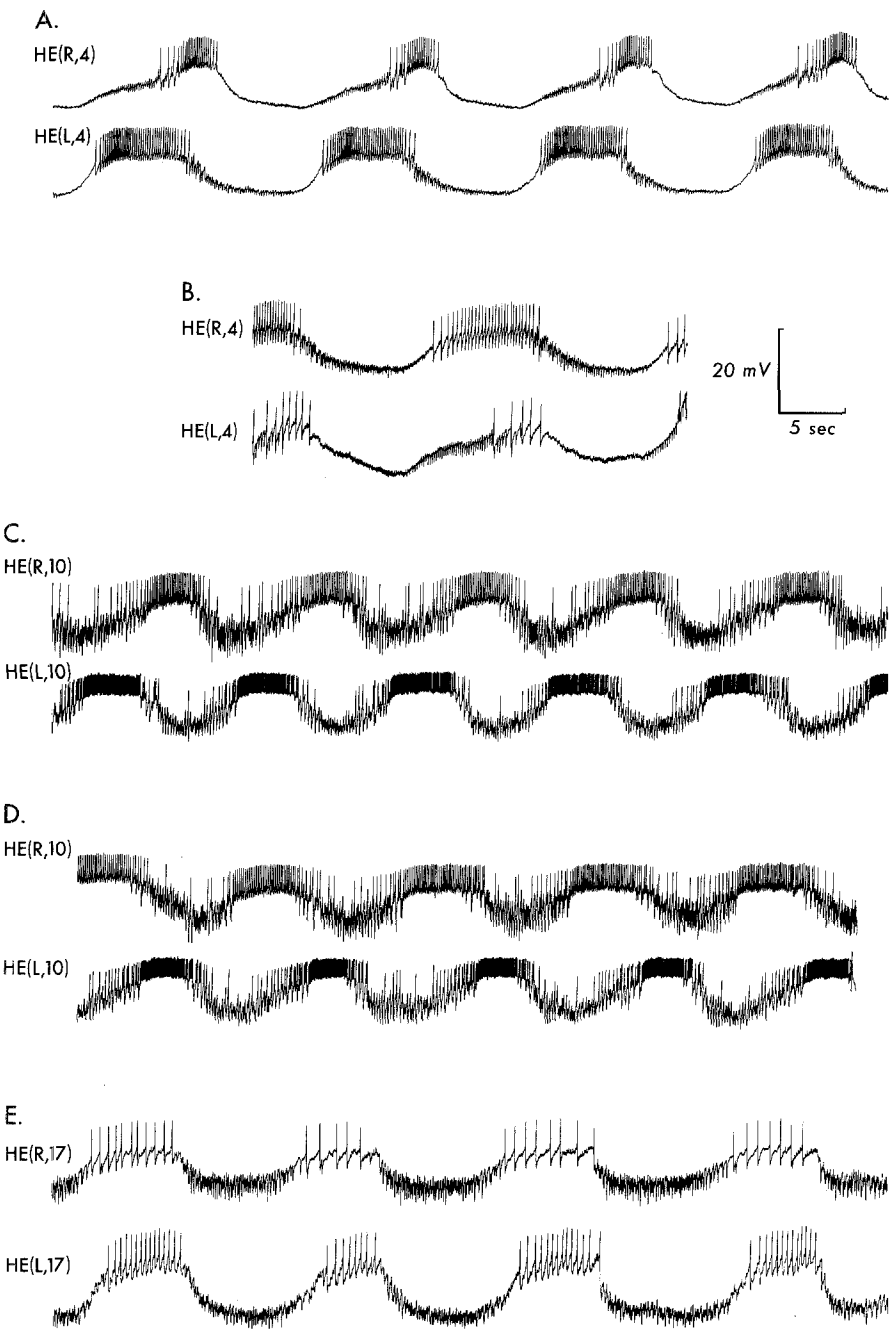

Fig. 8. Intrasegmental right-left coordination of the HE cell activity rhythms. The second of the two pairs of records presented for the HE(4) and HE(10) cell pairs was taken after a spontaneous change in coordination mode had occurred. Panels A and D: left side coordinated in peristaltic and right side in non-peristaltic mode. Panels B, C, and E: right side coordinated in peristaltic and left side in non-peristaltic mode

action potential burst as the point of reference for timing both the period and the cycle lead, the left-right phase lead can be estimated to be about 100 °. In the record taken later from the same HE(4) cell pair (Panel B), however, the phase relation of the two activity cycles has been reversed, so that now the phase of the activity cycle of the right cell HE(R,4) leads that of the left cell HE(L,4) by about 100 °. Comparison of these HE cell activity cycle phase relations with the data of Figure 3 for the constriction cycle phases shows that the two HE cell coordination modes correspond to the two alternative states of heart tube constrictions in segment 4. The earlier left-right HE(4) cell phase lead of 100 ° corresponds to the state in which the left heart tube beats in the peristaltic and the right heart tube in the non-peristaltic mode, whereas the later right-left HE(4) cell phase lead of 100 ° corresponds to the right heart tube beating in the peristaltic and the left heart tube in the non-peristaltic mode.

Similarly, two alternative coordination states are manifest for the left-right phase relation of the activity cycles of the HE cells of ganglion 10. In the earlier record (Panel C), the activity cycle of cell HE(R,10) led that of HE(L,10) by about 200 ° whereas in the later record (Panel D), that relation was reversed. Comparison with the constriction data of Figure 3 indicates that the phase relation of the earlier HE(10) cell record is appropriate for the right side and of the later record appropriate for the left side beating in the peristaltic mode. Finally, the activity cycles of the HE cell pair of ganglion 17 can be seen to be very nearly in phase. In the record presented in Panel E, the cycle of cell HE(R,17) had a slight phase lead over that of cell HE(L,17) which, according to Figure 3, would be appropriate for the left side beating in the peristaltic mode. In a later record not presented here a reversal of this small right-left phase lead has been observed, indicative of a switch to the other coordination mode of the HE(17) cell pair.

The intersegmental coordination of HE cell activity was investigated by simultaneous intracellular recording from HE cells in different ganglia of an isolated ventral cord preparation (Fig. 9). The records of panels A and B show that the phase of the activity cycles of cell HE(R,10) and HE(R,17) led that of cell HE(R,4) by about 145 ° and 170 ° respectively. According to the data of Figure 3, these phase leads correspond to the beat coordination on the peristaltic body side in segments 17, 10 and 4. The records of panels C and D of Figure 9 show a rear-to-front activity pattern of ipsilateral HE cells corresponding to the non-peristaltic beat mode. In this set the cycle of cell HE(4) actually had a small phase lead over the cycles of cells HE(10) and HE(17), indicative of a slight reverse, i.e. front-to-rear, peristalsis.

A sudden shift in the coordination of activity cycles of ipsilateral HE cells between the two states has been observed while intracellular records were being taken. This shift, just as the shift in the heartbeat dynamics, occurs suddenly rather than as a gradual phase drift of the activity cycles. At the beginning of the record presented in Figure 10 the activity cycles of the two HE cells were coordinated in the peristaltic mode, with the phase of the HE(L,17) cell cycle leading that of the HE(L,4) cycle by about 170 °. Toward the center of the record a shift in the coordination occurred to the non-peristaltic mode,

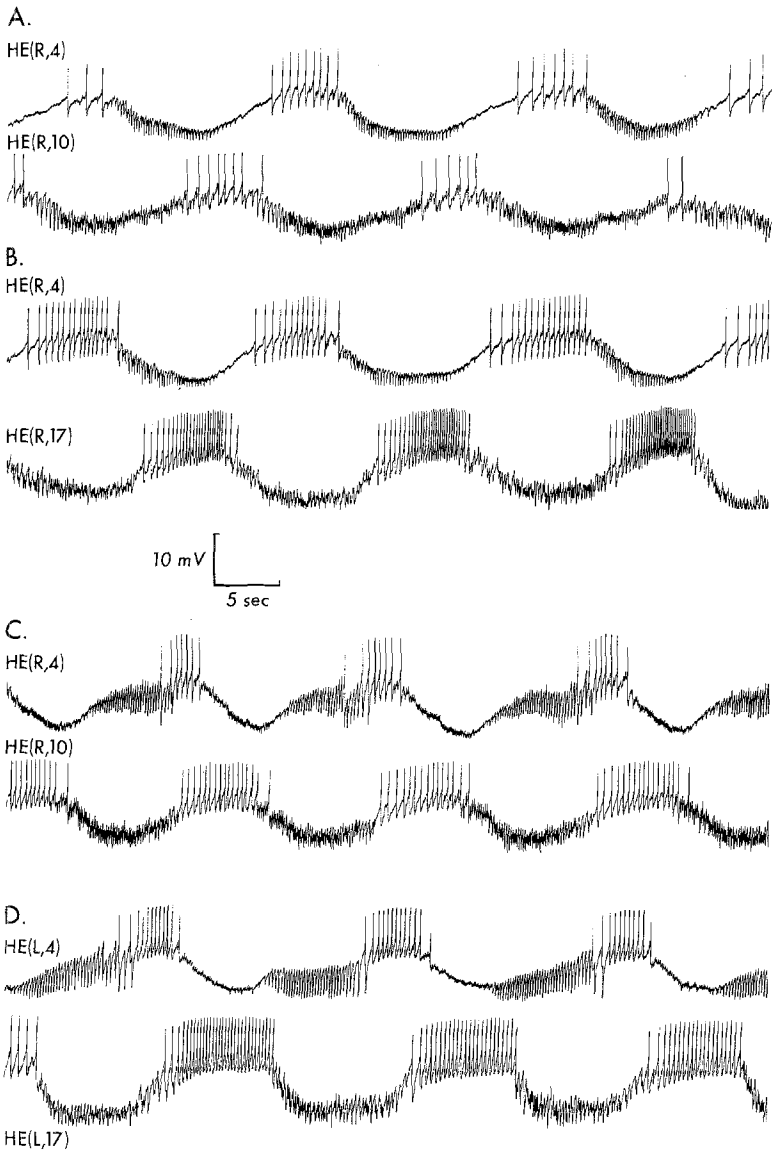

Fig. 9. Intersegmental front-rear coordination of the HE cell activity rhythms. Side from which records were taken was coordinated in peristaltic mode in Panels A and B and in non-peristaltic mode in Panels C and D

so that the activity cycles of cells HE(L,17) and HE(L,4) came to be more nearly in phase, with the HE(L,4) cycle having a slight lead over that of cell HE(L,17).

In order to investigate in more detail the phase progression of HE cell activity cycles in the anterior segments where, according to the dynamic data, most of the peristalsis actually takes place, recordings were taken from

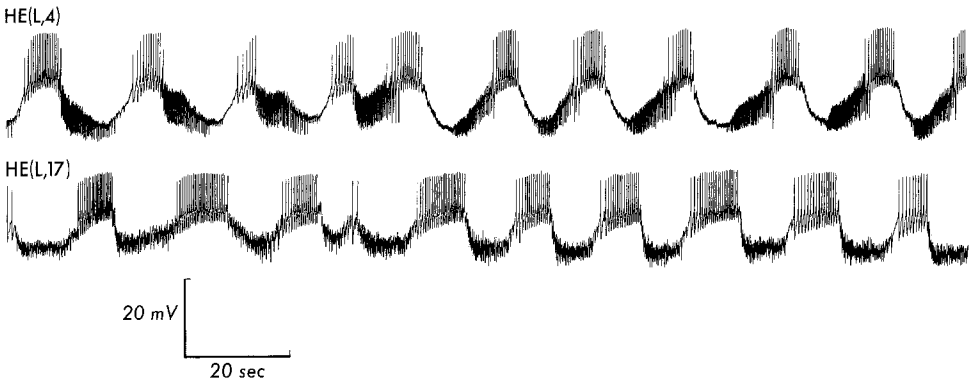

Fig. 10. Records of a shift in coordination of HE cell activity. At the beginning of these records the left side was coordinated in the peristaltic mode. The fourth ISP burst was abbreviated in cell HE(L,4) and extended in cell HE(L,17), and thereafter the left side was coordinated in the non-peristaltic mode

HE cells between ganglia 4 and 10 on the side which happened to beat in the peristaltic mode. The result of these recordings was that in the case of every cell pair, the cycle of the posterior HE cell led in phase that of the ipsilateral anterior HE cell. A quantitative estimate of the phase leads based on the time of occurrence of the first impulse in individual HE cell action potential bursts in these records indicated that in regard to the phases of their activity cycles, cell HE(4) leads cell HE(3) by 14 °, cell HE(5) leads cell HE(3) by 18 °, cell HE(6) leads cell HE(5) by 43 °, cell HE(7) leads cell HE(6) by 54 °, and cell HE(9) leads cell HE(7) by 32 °. The sum of the phase leads between HE(9) and HE(3) is 18 °+43 °+54 °+32 °=147 °, or approximately equal to the 145 ° phase lead between cell HE(10) and cell HE(4) estimated from the record of Figure 9. It may be concluded therefore that there is a monotonic phase progression in the activity cycles of the HE cells on the peristaltic side between ganglia 10 and 3.

Source of the Inhibitory Synaptic Input to the HE Cells

In order to search for the source of the intersegmental coordination of the HE cell activity cycles in the chain of ventral cord ganglia, a detailed examination was made of concurrent ISP bursts in homolateral HE cells of adjacent segments. These are shown for cells HE(R,5) and HE(R,6) in Figure 11, where it can be seen that every ISP of the burst in the anterior HE cell can be matched with a later ISP in the posterior HE cell. These matching ISP's follow each other with either of two fixed delays, namely 28 and 32 ms. It therefore appears that HE cells receive their inhibitory inputs from two or more cells whose axons carry impulses rearward through the connectives with different impulse conduction velocities.

Further support for the inference that the HE cell ISP's are produced by impulses traveling in interganglionic axons was provided by direct electrical

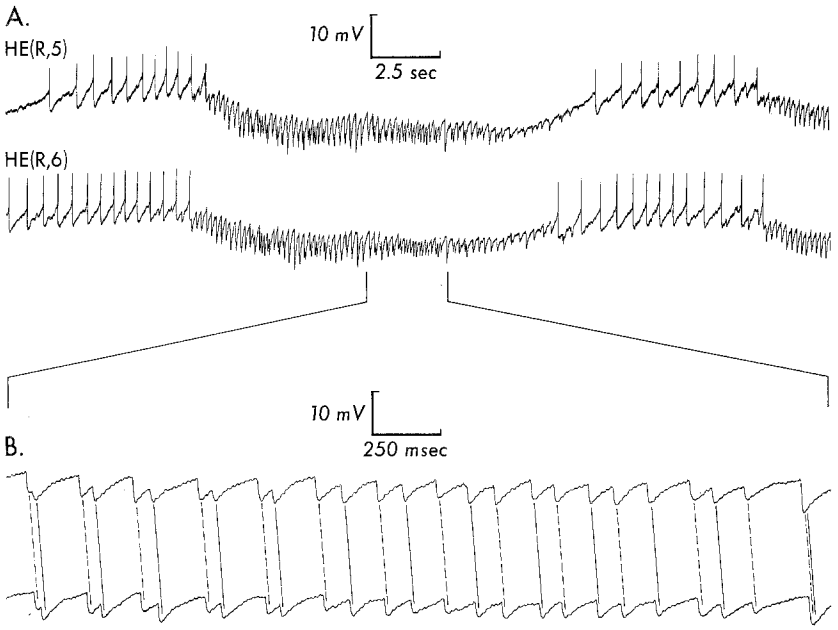

Fig. 11. Interganglionic matching of individual HE cell ISP's. Panel B is an expansion of a portion of the record of panel A. Every ISP in cell HE(R,5) precedes an ISP in cell HE(R,6) with one of two different lead times: 28 ms (dashed connecting lines) and 32 ms (solid connecting lines)

stimulation of the connectives. Each stimulus delivered to the right or left connective via a suction electrode gave rise to an ISP in the ipsilateral HE cells, regardless of whether the stimulation site was anterior or posterior to the ganglion from which the HE cell recording was taken. That the ISP's evoked by stimulation of the connective anterior or posterior to the ganglion were produced by impulses traveling in opposite directions in the same axon was shown by occluding these impulses by their collision. For this purpose, the connective was stimulated both anteriorly and posteriorly to the ganglion of record, with various time intervals between the stimuli. When the interstimulus interval was less than the inter-site conduction time, then the two stimuli gave rise to only a single ISP. The cell bodies of these interganglionic axons will be identified in the following paper of this series (Thompson and Stent, 1976).

Discussion

A detailed analysis of the implications of the constriction rhythm of the heart tubes for the fluid dynamics of blood circulation through the leech vascular system is beyond the scope of this study. But one aspect of the functional significance of the rhythm can be readily appreciated. It is apparent that a bilaterally symmetric rhythm of alternating forward peristaltic constrictions of right and left heart tubes (Gaskell, 1914; Boroffka and Hamp, 1969) might

not accomplish the main function of the circulating blood, namely to flow through the fine transverse capillary system and provide gas exchange and nourishment to the body tissues. Since the major longitudinal vessels of the leech circulatory system are not only interconnected by the transverse capillaries but also joined directly at their anterior and posterior ends (Boroffka and Hamp, 1969), the major vessels could form a circuit of much lower resistance to blood flow than the capillary system. Hence a bilaterally antiphasic, frontward peristalsis, under which the heart tube on one body side begins to constrict only after the tube on the other side has dilated, might merely push blood back and forth between the heart tubes via their direct joints at the body ends without flushing the capillary system. With the constriction rhythm diagrammed in Figure 3, however, under which at the very time that the one heart tube begins its peristalsis the other heart tube is constricted along its entire length, the blood pushed forward on the peristaltic side could not enter the tube on the non-peristaltic side and would thus be forced into the transverse capillaries.

Although no evidence for sensory feedback to the heart motor neuron system was found in the present study, it is probable that some sensory input to the system does exist. For it would be surprising if the leech could not adjust its heartbeat rate in accord with its physiological needs. In any case, there is evidence that the periphery plays some role in the generation of the heartbeat rhythm. As the experiment presented in Figure 4 showed, there must exist a peripheral heartbeat generator in addition to the central nervous oscillator, since the heart tubes continue to constrict rhythmically, albeit with a longer period, after they are disconnected from the ventral nerve cord. Although the peripheral source of the slow heartbeat has not yet been identified, it was found that after scission of the segmental nerves or prolonged passage of hyperpolarizing current into the HE cell body bursts of antidromic impulses arise in the peripheral reaches of the HE cell axons near their termini in the vascular nerves. Their bursts are concurrent with constrictions of the corresponding heart tube section.

These observations can be explained by envisaging that the circular muscle fibers of the heart tubes undergo a slow endogenous depolarization-repolarization rhythm and that the HE cell axon terminals are linked to their muscle fibers via both excitatory chemical and electrical junctions. Thus during the depolarized phase of the myogenic polarization rhythm, the HE cell terminals would become depolarized by current flowing into them from the muscle fibers via the electrical junctions, and an antidromic impulse burst would arise at peripheral HE cell impulse initiation sites. The effect of this peripheral positive feedback loop would be to amplify the myogenic polarization rhythm, since the endogenous depolarization of the muscle fibers would be reinforced by the excitatory synaptic potentials delivered to them by the HE cell axon responding to that depolarization. In support of this proposal, it was found that the circular muscles do exhibit an appreciable polarization rhythm whose period matches that of the heartbeat and under which the membrane potential of the fibers becomes depolarized during the constriction phase by about 30 mV from the repolarized potential that obtains during the dilation phase. According

to this explanation, in the innervated heart tube preparation the slow myogenic rhythm would be entrained by the faster, centrally generated HE cell rhythm conveyed to the periphery by impulse bursts traveling outward in the vascular nerves.

These observations are compatible also with other, more complicated explanations, which do not invoke an endogenous, or myogenic, polarization rhythm of the heart tube muscles. For instance, it is possible that the peripheral heartbeat generator consists of a rhythmically active peripheral neural element which courses axially across the circular muscle fibers along the wall of the heart tube and which provides phasic synaptic input to the impulse initiation sites of the HE cell axon termini in the vascular nerves. The anatomical finding of a polyaxonal nerve which actually takes such a course (Hammersen and Staudte, 1969) can be cited in support of this alternative proposal.

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